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CATION FLUX IN THE EHRLICH ASCITES TUMOR CELL
EVIDENCE FOR Na^+-FOR-Na^+ AND K^+-FOR-K^+ EXCHANGE DIFFUSION

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SUMMARY

In a previous study, evidence was presented for an external Na^+-dependent, ouabain-insensitive component of Na^+ efflux and an external K^+-dependent component of K^+ efflux in the Ehrlich ascites tumor cell. Evidence is now presented that these components are inhibited by the diuretic furosemide and that under conditions of normal extracellular Na^+ and K^+ they represent Na^+-for-Na^+ and K-$^+$for-K^+ exchange mechanisms. Using ^{86}Rb to monitor K^+ movements, furosemide is shown to inhibit an ouabain-insensitive component of Rb^+ influx and a component of Rb^+ efflux, both representing approx. 30 % of the total flux. Inhibition of Rb^+ efflux is greatly reduced by removal of extracellular K^+. Furosemide does not alter steady-state levels of intracellular K^+ and it does not prevent cells depleted of K^+ by incubation in the cold from regaining K^+ upon warming. Using ^{22}Na to monitor Na^+ movements, furosemide is shown to inhibit an ouabain-insensitive component of unidirectional Na^+ efflux which represents approx. 22 % of total Na^+ efflux. Furosemide does not alter steady-state levels of intracellular Na^+ and does not prevent removal of intracellular Na^+ upon warming from cells loaded with Na^+ by preincubation in the cold. The ability of furosemide to affect unidirectional Na^+ and K^+ fluxes but not net fluxes is consistent with the conclusion that these components of cation movement across the cell membrane represent one-for-one exchange mechanisms. Data are also presented which demonstrate that the uptake of α-aminoisobutyrate is not affected by furosemide. This indicates that these components of cation flux are not directly involved in the Na^+-dependent amino acid transport system *A*.

INTRODUCTION

In a previous study, data were presented for the existence of an ouabain-insensitive, external Na-dependent component of Na efflux in the Ehrlich ascites cell [1]. A component of K^+ efflux dependent on the presence of external K^+ was also identified. The external dependency of a component of efflux for the same element is consistent with the presence of a one-for-one exchange component, as originally envisioned by Ussing [2]. If it can be demonstrated that the alteration in the uni-

directional efflux of the ion in question cannot be accounted for by changes in concentration and/or membrane potential resulting from its removal externally, then such changes in cation flux may be attributable to inhibition of such exchange mechanisms. Our previous study suggested this was the case for the external K^+-dependent component of K^+ efflux. The nature of the external Na^+-dependent component of Na^+ efflux was not clear since, based on values for membrane permeability to Na^+, cell membrane potential and Na^+ distribution, it was estimated that the steady-state influx of Na^+ could be entirely accounted for by a passive diffusional Na^+ influx. This implied that the ouabain-insensitive Na^+-dependent component of Na^+ efflux represented a net Na^+ efflux.

Our interest in a further characterization of these components of Na^+ and K^+ flux in the Ehrlich ascites cell stemmed from two considerations. First, in the human red cell it has been demonstrated that an ouabain-insensitive component of Na^+ efflux dependent on the presence of external Na^+ is capable of achieving net Na^+ efflux under conditions of reduced extracellular Na^+ [4]. At elevated extracellular Na^+ it represents an Na^+-for-Na^+ exchange [5]. This component is inhibited by ethacrynic acid [5–7] and furosemide [4, 5]. Our initial observations in the ascites cell indicated that an ouabain-insensitive, external Na^+-dependent component of Na^+ efflux was present [1]. Furthermore, they suggested that this component might represent a net efflux of Na^+. Our interest turned therefore to investigating whether this component of Na^+ efflux was also furosemide sensitive and, if so, would furosemide inhibition lead to a reduction in net Na^+ efflux, thus suggesting an alternate Na^+ pump mechanism. The results indicate that the component is furosemide sensitive but, under conditions of normal extracellular Na^+, it represents an Na^+-for-Na^+ exchange.

The second consideration of interest dealt with the relationship of these components of cation flux to amino acid transport in the ascites cell. There is a large body of evidence supporting the hypothesis that certain amino acids are actively transported into the ascites cell at the expense of the passive Na^+ gradient (e.g. see review by Christensen [8]). It has also been suggested that cation exchange processes may be involved in amino acid transport [9]. The ability to specifically inhibit the exchange components with furosemide allowed us to investigate their involvement in the Na^+-dependent amino acid transport system *A* [10]. Using the transport of α-aminoisobutyrate as representative of system *A*, we have observed its uptake in the presence of furosemide. These data indicate that the uptake of α-aminoisobutyrate is not affected and therefore not directly linked to the furosemide-sensitive fluxes.

MATERIALS AND METHODS

Ehrlich Lettre (ELD) cells were grown in female Ha/ICR mice. Cells were isolated as described previously [1]. All experiments were run at 26–28 °C in a solution consisting of 147 mM NaCl, 6 mM KCl, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, 46 mM Tris · OH^- brought to pH 7.4 with H_3PO_4. Modification of this medium for specific experiments is described in the figure legends. Ouabain was obtained from Sigma Chemical Co., St. Louis, Mo. Furosemide was a gift of Hoechst Pharmaceuticals, Somerville, N. J. Addition of furosemide at 10^{-3} M or greater led to acidification of the incubation medium and the pH was readjusted to 7.4 with small amounts of NaOH.

Unidirectional influx or efflux of Rb^+ was measured from the kinetics of $^{86}Rb^+$ uptake or loss. $^{86}Rb^+$ serves as an analog of K^+ movements in these cells (e.g. ref. 1). For unidirectional influx measurements a trace of $^{86}Rb^+$ was added to cell suspensions at zero time. At intervals 1- or 2-ml aliquots were removed and centrifuged through 12 ml of cold isotonic choline chloride (200 mM choline chloride, 10 mM Tris · OH^-; pH 7.4 with HCl). Isotope trapped in the extracellular space was corrected for by subtraction of zero time points taken immediately after addition of the tracer to the medium. The pelleted cells were resuspended and lysed in distilled water. The lysate was then transferred to a counting vial and isotope content was determined from measurements of Cerenkov radiation in a liquid scintillation counter. In the presence of cell lysate counting efficiency is reduced 3–5 % for both $^{22}Na^+$ and $^{86}Rb^+$. In certain experiments, subsequent to counting, the same samples were analyzed for internal Na^+ and K^+ using an atomic absorption spectrometer. From these data any changes in net Na^+ and K^+ fluxes were monitored.

Unidirectional Na^+ and Rb^+ efflux were determined from the kinetics of $^{86}Rb^+$ or $^{22}Na^+$ loss. Cells were preloaded with tracer by incubation for at least 60 min in the case of $^{86}Rb^+$ or at least 30 min in the case of $^{22}Na^+$. These times are sufficient for the bulk of the intracellular cation to exchange with the external isotope. At the termination of the loading period, the cells were centrifuged, washed twice in the incubation solution or the appropriate modification of it, and resuspended. In the case of $^{22}Na^+$, the original medium was removed, passed through a 0.2 μM filter and reused in subsequent experiments. Loss of isotope was monitored by removal of cell aliquots at specific intervals following resuspension in isotope free medium. The cells were processed as for the isotope influx experiments. It should be noted that efflux experiments in K-free medium required that the ratio of cell volume to medium volume be 0.01 or less. At higher cell densities, K^+ loss from the cells led to significant K^+ content in the external medium. The kinetic analysis of the isotope flux experiments has been previously described [1].

To determine the kinetics of α-aminoisobutyrate uptake, cells were suspended in the normal incubation solution with 1 mM α-aminoisobutyrate plus a trace of ^{14}C-labeled α-aminoisobutyrate with or without 1 mM furosemide. 1-ml cell aliquots were taken at zero time and subsequent intervals. The cells were centrifuged in 14 ml of isotope-free incubation medium. The cells were then resuspended in 1 ml of 5 % trichloroacetic acid, 1 mM α-aminoisobutyrate. After incubation for approx. 30 min, the precipitate was centrifuged and 200-μl samples were removed from the supernatant and added to 10 ml of Bray's counting solution. Isotope content was determined in a liquid scintillation counter. Intracellular versus extracellular α-aminoisobutyrate ratios were determined knowing the specific activity of the extracellular medium (cpm/mol). 50-μl samples of the external medium were counted in 10 ml of Bray's plus 200 μl of 5 % trichloroacetic acid to compensate for quenching of the cell samples. Pellet volume was determined from average cell diameter and cell numbers, as determined in a cell counting chamber under phase optics.

RESULTS

Furosemide effects on Rb^+ flux

Approx. 31 % of the total unidirectional Rb^+ influx of the ascites cell is inhibited

TABLE I

FUROSEMIDE AND OUABAIN INHIBITION OF CATION FLUX

The fluxes under the various conditions are presented as mean ± S.E. The number in parenthesis represents the number of separate experiments on different cell populations. The magnitudes of the unidirectional fluxes of Na^+ and Rb^+ were determined from the kinetics of uptake or loss of $^{22}Na^+$ and $^{86}Rb^+$. Kinetic data from individual experiments is presented in Figs 1 and 3. For both Na^+ and Rb^+ fluxes, the sum of the inhibitions resulting from furosemide or ouabain alone is within 7% of the inhibition resulting from their presence simultaneously at 1 mM concentrations. The magnitude of the effect of furosemide on Rb^+ influx versus efflux is nearly similar and removal of external K^+ causes a 3-fold reduction in the magnitude of the inhibition of furosemide on Rb^+ efflux. The magnitudes of the furosemide-sensitive components of Rb^+ influx and Na^+ efflux are comparable to the external K^+-dependent component of K^+ efflux and the external Na^+-dependent component of Na^+ efflux previously identified [1].

Conditions	Rb^+ influx (pmol/s per 10^5 cell)	Inhibition relative to control (%)	Rb^+ efflux (pmol/s per 10^5 cell)	Inhibition relative to control (%)	Na^+ efflux (pmol/s per 10^5 cell)	Inhibition relative to control (%)
Control (6 mM $[K]_o$)	19.2 ± 2.2 (7)	0	17.0 ± 1.3 (5)	0	16.5 ± 3.0 (6)	0
1 mM ouabain	9.5 ± 1.3 (7)	51	—	—	7.4 ± 1.6 (6)	55
1 mM furosemide	13.2 ± 1.2 (7)	31	11.2 ± 1.7 (5)	34	12.9 ± 2.0 (6)	22
1 mM ouabain plus 1 mM furosemide	2.3 ± 1.1 (7)	88	—	—	4.4 ± 1.1 (6)	73
Control (zero $[K]_o$)			10.2 ± 1.3 (5)	0		
1 mM furosemide	—	—	9.0 ± 1.4 (5)	12		

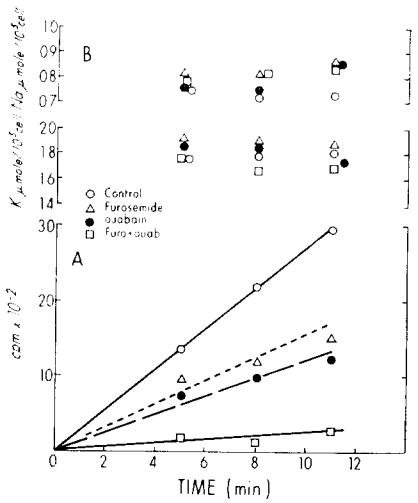

Fig. 1. Unidirectional influx of Rb^+ as determined from $^{86}Rb^+$ uptake in the presence of 1 mM furosemide, 1 mM ouabain or 1 mM furosedime plus 1 mM ouabain. (A) cpm $^{86}Rb^+$ accumulated versus time under the conditions indicated. The symbols correspond to the legend in the figure. For this particular experiment the unidirectional flux values are (pmol/s per 10^5 cell): control, 23; furosemide, 11; ouabain, 9; furosemide plus ouabain, 2. (B) The Na^+ and K^+ content of the same cells over the duration of the influx experiment. The maximal change in Na^+ or K^+ content over the 11-min interval in which the isotope uptake was measured is 15% (conditions in which ouabain is present). This indicates that the flux measurements were carried out under near steady-state conditions. Seven such experiments are summarized in Table I.

TABLE II

EFFECT OF INCREASING CONCENTRATIONS OF OUABAIN AND FUROSEMIDE ON CATION FLUX

The effect of furosemide and ouabain on Rb^+ influx was measured by $^{86}Rb^+$ uptake. The data were obtained from cells isolated from one mouse. Their effect on Na^+ efflux, as monitored by $^{22}Na^+$ loss, was obtained on cells from a separate mouse. The magnitudes of the fluxes were determined from experiments similar to those presented in Figs 1 and 3. The results are typical of two such experiments on Rb^+ influx and Na^+ efflux. The inhibition by both ouabain and furosemide becomes maximal at concentrations between 0.5 and 1 mM.

Conditions	Rb^+ influx (pmol/s per 10^5 cell)	Inhibition relative to control (%)	Na^+ efflux (pmol/s per 10^5 cell)	Inhibition relative to control (%)
Control	18.4	0	16.8	0
0.01 mM ouabain	—	—	12.9	23
0.1 mM ouabain	13.1	28	10.1	40
0.5 mM ouabain	8.6	53	7.4	56
1.0 mM ouabain	7.8	58	6.9	59
2.0 mM ouabain	7.7	58	6.7	60
0.01 mM furosemide	16.0	13	—	—
0.1 mM furosemide	14.4	22	15.5	8
0.5 mM furosemide	12.9	30	15.0	11
1.0 mM furosemide	12.4	33	11.8	30
2.0 mM furosemide	12.6	32	11.9	29

by furosemide (Fig. 1, Table I). The concentration of furosemide at which this inhibition is maximal lies between 0.5 and 1 mM (Table II). The inhibition by furosemide is distinct from the ouabain-sensitive component of unidirectional Rb^+ influx since addition of furosemide in the presence of ouabain leads to further inhibition of unidirectional Rb^+ influx (Fig. 1, Table I) The ouabain-sensitive component is maximally inhibited between 0.5 and 1 mM ouabain (Table II) and it represents approx. 51 % of the total unidirectional influx (Table I). The addition of 1 mM ouabain plus 1 mM furosemide leads to 88 % reduction in unidirectional influx, which is nearly equivalent to the sum (82 %) of the reductions observed in ouabain or furosemide alone. The concentration of ouabain required to achieve a maximum inhibition of the Na^+, K^+ pump is somewhat higher than observed in other systems, suggesting a low affinity of ouabain for the transport mechanism (e.g. see also ref. 3). This requirement could lead to the supposition that ouabain and furosemide act on the same component of Rb^+ influx but that the action of ouabain is incomplete, as has been suggested for the effect of ethacrynic acid in the presence of ouabain on Na^+ efflux in toad oocytes [11]. However, the present data indicate this is not the case in the ascites cell. In experiments discussed below, furosemide has been shown not to alter net Na^+ or K^+ movements whereas ouabain does. Furthermore, furosemide reduces Rb^+ efflux to a near equal extent as Rb^+ influx. Ouabain has little or no effect on Rb efflux [1].

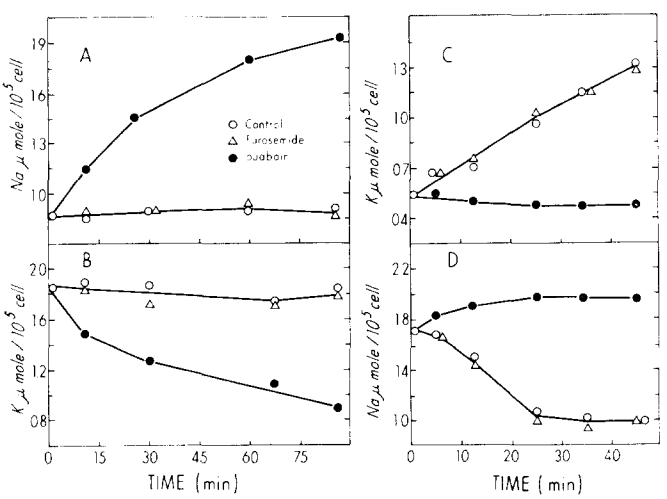

Fig. 2. (A, B) The effect of furosemide or ouabain on intracellular Na^+ and K^+ content. The legend in (A) applies to all points in the figure. At zero time equal aliquots of cells were suspended in 1 mM furosemide medium or 1 mM ouabain medium or normal medium at 28 °C. The zero time point represents an aliquot taken from the control cells immediately upon resuspension. The cells were all obtained from the same animal. The results are typical of four such experiments. (C, D) The effect of furosemide and ouabain on net Na^+ extrusion and K^+ uptake in cells depleted of K^+ and loaded with Na^+ by preincubation in the cold. Cells from a single animal were incubated with gentle stirring for approx. 1 h at 2–4 °C to induce Na^+ gain and K^+ loss. The cells were then centrifuged and equal aliquots were resuspended at zero time in 28 °C medium containing 1 mM furosemide or 1 mM ouabain or no additions. The zero time point was taken from the control cells immediately upon resuspension. The results are typical of four such experiments.

Removal of external K^+ reduces the inhibition of furosemide on unidirectional Rb^+ efflux to approx. 12 % of the total efflux (Table I). This residual effect may represent an incomplete removal of external K^+ from the cell surface or a second effect of furosemide on Rb^+ efflux which is independent of external K^+. The nearly equivalent inhibition of influx and efflux in the presence of external K^+ and the reduction of the inhibition of efflux by removal of external K^+ are consistent with the conclusion that furosemide inhibits a one-for-one K^+-for-K^+ exchange. This is further substantiated by the observation that furosemide does not alter the steady-state levels of intracellular K^+ (Fig. 2A), as would be expected upon inhibition of a component of flux which does not contribute to net flux. The effect of furosemide on net flux of K^+ was also investigated in another manner. Ascites cells were incubated at 2–4 °C for approx. 1 h. This treatment reduces intracellular K^+ levels and elevates intracellular Na^+ due to the inhibition of the ATP-dependent Na^+, K^+ pump. Upon return to elevated temperatures, intracellular K^+ levels are regained and intracellular Na^+ is reduced. The gain of K^+ and the loss of Na^+ are not altered by furosemide. In the same cell populations ouabain inhibits this process (e.g. Fig. 2B).

Furosemide effects on Na^+ flux

Approx. 22 % of the unidirectional Na^+ efflux is inhibited by furosemide (Fig. 3, Table I). This inhibition is maximal at a concentration of furosemide between 0.5 and 1 mM (Table II). The furosemide-sensitive component is distinct from the ouabain-sensitive component of Na^+ efflux (Table I). At a concentration of 1 mM, ouabain maximally inhibits the ouabain-sensitive component of Na^+ efflux (Table II). This component represents approx. 55 % of the unidirectional Na^+ efflux. The addition of 1 mM furosemide further inhibits unidirectional Na^+ efflux and the sum

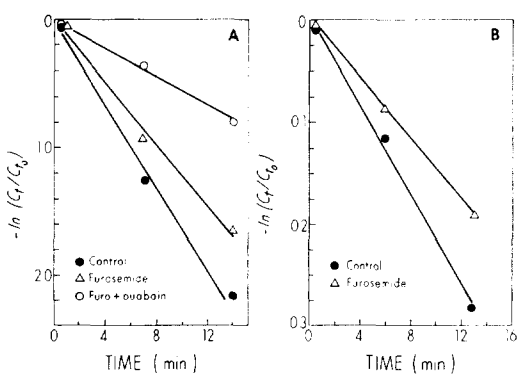

Fig. 3. (A) Unidirectional Na^+ efflux in the presence of 1 mM furosemide or 1 mM furosemide plus 1 mM ouabain. Na^+ efflux was monitored as the loss of $^{22}Na^+$ from cells preloaded with the tracer. After loading, the cells were washed twice in isotope-free medium and equal aliquots were then resuspended in the appropriate medium. Points were then taken and these are designated as zero time. The data are plotted as the log of the counts in the cells at time t (C_t) over the counts at zero time (C_{t0}) versus t. The slope of the line represents the unidirectional efflux rate coefficient (t^{-1}). Six such experiments are summarized in Table I. (B) Unidirectional Rb^+ efflux in the presence or absence of 1 mM furosemide. Rb^+ efflux was monitored as $^{86}Rb^+$ loss from preloaded cells. The cells were handled and the data is plotted as in (A) above. Five such experiments are summarized in Table I.

of the individual effects of ouabain and furosemide (77 % reduction) is nearly equivalent to the reduction observed when both inhibitors are present (73 %; Table II).

As observed with K^+, furosemide does not alter steady-state levels of intracellular Na^+ (Fig. 2A). Furthermore, in cells loaded with Na^+ by incubation at 2–4 °C, furosemide does not prevent the net loss of Na^+ in these cells upon incubation at elevated temperatures. Ouabain prevents the net Na^+ loss (Fig. 2B).

The observation that furosemide does not alter steady-state Na^+ levels and does not alter net Na^+ efflux coupled with the observation that it inhibits approx. 22 % of the unidirectional Na^+ efflux indicates that, in the presence of external Na^+, furosemide inhibits a one-for-one Na^+-for-Na^+ exchange. The absence of an effect on net Na^+ flux is also consistent with the conclusion that the furosemide-sensitive component is distinct from the ouabain-sensitive component.

Based on the conclusion that furosemide inhibits Na^+-for-Na^+ exchange, it would be expected that a furosemide-sensitive component of unidirectional Na^+ influx of approximately the same magnitude as the efflux component should exist. However, in our hands, measurements of unidirectional Na^+ influx by the tracer technique have been highly variable. This stems from the rapid accumulation of labeled Na^+ and the relatively high amount of label trapped in the extracellular space after washing as compared to e.g. K^+. However, the results of the Na^+ efflux experiments, coupled with observations on net Na^+ flux, are consistent with the presence of a furosemide-sensitive component of Na^+ influx.

Furosemide and amino acid transport system A

The uptake of α-aminoisobutyrate is due to the Na^+-dependent system *A* in the Ehrlich ascites cell [10]. The ability of furosemide to alter its uptake was investigated. In four experiments, 1 mM furosemide had no effect on the accumulation of α-aminoisobutyrate (intracellular/extracellular ratio: control, 8.6±0.9; furosemide, 8.1±1.2, S.E.; 40 min incubation). In parallel experiments it was observed that the cation fluxes in these cell populations were reduced in a manner similar to our previous experiments. In two experiments involving the amino acid uptake, $^{86}Rb^+$ uptake was reduced by a mean value of 41 % in the same cell populations. In two other experiments, $^{22}Na^+$ efflux was reduced by a mean value of 29 %.

DISCUSSION

In previous studies, ethacrynic acid and furosemide have been used to characterize ouabain-insensitive, non-diffusional components of cation flux. Components of Na^+ flux sensitive to ethacrynic acid have been obtained in frog muscle [16], toad oocyte [11] and red blood cells [5–7]. K^+ influx has also been shown to be sensitive to ethacrynic acid in human red cells [6]. Furosemide has been shown to inhibit components of Na^+ flux in the human red blood cell [4, 5]. Our data indicate the presence of furosemide-sensitive components for both Na^+ and K^+ in the Ehrlich ascites cell. At normal extracellular levels of Na^+ and K^+ they represent one-for-one exchange fluxes. It should be emphasized that these exchange components may be capable of mediating net fluxes under certain experimental conditions. For instance, Dunn [5] has demonstrated in the human red cell that the ouabain-insensitive,

furosemide-sensitive component of Na^+ efflux represents an Na^+-for-Na^+ exchange at high levels of extracellular Na^+. This conclusion was based on the observation that furosemide does not inhibit a net efflux of Na^+ and that the inhibition of furosemide on unidirectional Na^+ influx and efflux was nearly equal. Lubowitz and Whittam [7] also concluded that the ouabain-insensitive, external Na^+-dependent component of Na^+ efflux does not represent a net Na^+ efflux in the human red cell, but rather an Na^+-for-Na^+ exchange. However, Hoffman and Kregenow [6] using the inhibitor ethacrynic acid demonstrated an ouabain-insensitive, ethacrynic acid-sensitive, external Na^+-dependent component of Na^+ efflux and a component of K^+ influx sensitive to ethacrynic acid. (In the presence of ouabain, ethacrynic acid appears to inhibit the same component of Na^+ efflux as furosemide [5].) Based on the observations that ethacrynic acid had no effect on Na^+ influx and that depleted cells gained more Na^+ than fresh cells when extracellular Na^+ was high as opposed to when it was low, Hoffman and Kregenow [6] concluded that the external Na^+-dependent, ethacrynic acid-sensitive component of Na^+ efflux represented a net Na^+ efflux which they defined as pump II. Sachs [4] has subsequently demonstrated that the furosemide-sensitive component of Na^+ efflux in the human red cell is capable of net Na^+ efflux at reduced extracellular Na^+, but at elevated extracellular Na^+ it represents an Na^+-for-Na^+ exchange. Our present data in the ascites cell also indicate that the furosemide-sensitive component of Na^+ efflux represents an exchange mechanism at high extracellular Na^+. The nature of this flux at reduced extracellular Na^+ is not known. Direct experimental test of this is hampered since, unlike the red cell, changes in extracellular Na^+ or K^+ result in rapid alterations in intracellular Na^+ and K^+. Experiments to assess net cation fluxes under these conditions must therefore account for changes independent of the furosemide-sensitive fluxes and, in turn, these changes may overshadow any net flux contributed by these components. Experiments are in progress to determine whether suitable conditions can be achieved to further characterize the nature of the furosemide-sensitive components.

Our previous study [1] identified the presence of both ouabain-insensitive, external Na^+-dependent Na^+ efflux and external K^+-dependent K^+ efflux. The data indicated that, under physiological conditions, the external K^+-dependent K^+ efflux represented approx. 22 % of the total K^+ efflux and the external Na^+-dependent component represented approx. 31 % of the total Na^+ efflux. The furosemide-sensitive components of Rb^+ and Na^+ efflux represent 34 and 22 %, respectively (Table 1). The components identified in the two studies are in reasonable agreement, thus indicating that the external K^+- or Na^+-dependent components and the furosemide-sensitive components are one and the same. In our previous study we suggested that the external Na^+-dependent component of Na^+ efflux could represent a net Na^+ efflux. This suggestion was based on the observation that our measurements of membrane potential, membrane permeability to Na^+ (P_{Na}, cm/s) and Na^+ distribution yielded a diffusional Na^+ influx which could account for the total unidirectional Na^+ influx, based on the steady-state assumption. Our present data do not support this previous concept since unidirectional Na^+ efflux is partially inhibited by furosemide but net Na^+ fluxes are not altered. Thus, of the total unidirectional Na^+ influx, approx. 22 % must represent an exchange flux.

It is still unclear as to whether the Na^+-dependent transport of amino acids by the *A* system in the Ehrlich cell can be accounted for entirely by the passive

electrochemical gradient of Na^+ or Na^+ plus K^+ (e.g. ref. 12) or whether a direct coupling to metabolism is involved (e.g. ref. 13). In the former case it is conceivable that exchange fluxes could participate in the amino acid movements. This is based on the consequence that the magnitudes of the exchange fluxes are sensitive to alterations in the Na^+ and K^+ distributions [1]. Thus, an observed inhibition of amino acid transport by reduction of external Na^+ could be attributed to a reduction in the magnitude of the passive Na^+ gradient but it could also be attributed to an alteration in the magnitude of an Na^+-for-Na^+ exchange flux. This possibility was further substantiated by observations in the pigeon erythrocyte. It has been observed in this system that both inward and outward fluxes of Na^+ are stimulated by the uptake of alanine through the *ASC* system. No net change in Na^+ flux occurred and the increased Na^+ fluxes were ouabain insensitive [14]. These results are consistent with the involvement of an Na^+-for-Na^+ exchange in alanine uptake. In contrast, our results with the Ehrlich cell are consistent with the conclusion that the exchange fluxes identified through the use of furosemide are not directly involved in the process of Na^+-dependent amino acid transport since α-aminoisobutyrate uptake is insensitive to furosemide. These results are consistent with certain previous observations in the Ehrlich cell. Riggs et al. [15] have observed that the uptake of amino acids by system *A* is accompanied by a gain in internal Na^+ and loss of internal K^+, thus indicating that the amino acid fluxes are associated with net cation fluxes and not one-for-one exchange fluxes.

The exchange fluxes identified through the use of furosemide represent a substantial portion of the total cation flux in the Ehrlich cell. Their elimination as factors in the accumulation of α-aminoisobutyrate and possibly all amino acids associated with system *A* may serve to reduce somewhat the complexities that need to be dealt with in characterizing this amino acid transport system. Furthermore, it remains to be determined what the functional significance of cation exchange diffusion is in the growth and metabolism of these cells. In experiments to be presented in detail elsewhere, we observe that the magnitude of these exchange fluxes is cell cycle dependent.

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REFERENCES

1 Mills, B. and Tupper, J. T. (1975) J. Membrane Biol. 20, 75
2 Ussing, H. H. (1949) Physiol. Rev. 29, 127
3 Maizels, M., Remington, M. and Truscoe, R. (1958) J. Physiol. Lond. 140, 61
4 Sachs, J. R. (1971) J. Gen. Physiol. 57, 259
5 Dunn, M. J. (1970) J. Clin. Invest. 49, 1804
6 Hoffman, J. F. and Kregenow, F. M. (1966) Ann. N.Y. Acad. Sci. 137, 566
7 Lubowitz, H. and Whittam, R. (1969) J. Physiol. Lond. 202, 111
8 Christensen, H. N. (1970) Membranes and Ion Transport (Bittar, E. E., ed.), pp. 365, Wiley Interscience, New York

9 Hempling, H. G. (1972) Transport and Accumulation in Biological Systems (Harris, E. p. 271, Butterworth, London
0 Oxender, D. L. and Christensen, H. N. (1963) J. Biol. Chem. 238, 3686
1 Bittar, E. E., Dick, D. A. T. and Fry, D. J. (1968) J. Physiol. Lond. 196, 693
2 Morville, M., Reid, M. and Eddy, A. A. (1973) Biochem. J. 134, 11
3 Geck, P., Heinz, E. and Pfeiffer, B. (1972) Biochim. Biophys. Acta 288, 486
4 Wheeler, K. P. and Christensen, H. N. (1967) J. Biol. Chem. 242, 3782
5 Riggs, T. R., Coyne, B. A. and Christensen, H. N. (1954) J. Biol. Chem. 209, 395
6 Erlij, D. and Leblanc, G. (1971) J. Physiol. Lond. 214, 327