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Regulatory volume increase in Ehrlich ascites tumor cells

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Ehrlich ascites tumor cells, shrunken as a result of KCl-depletion and Na^+ loading, re-establish normal ionic concentrations by the combined activity of the Na^+/K^+ pump and the ($2Cl^- + K^+ + Na^+$) cotransport system. Restoration of cell volume, however, correlates only with the increase in intracellular Cl^-. This along with the finding that the equilibrium volume is linearly related to the steady state [Cl^-] suggests that the extent to which cell volume increases is determined by Cl^- transport. Net Cl^- uptake, which is mediated almost exclusively by the cotransport system, is ultimately responsible for establishing the steady-state intracellular Cl^- concentration. Transport mediated by this pathway ceases when the sum of the chemical potentials for Na^+, K^+ and Cl^- approaches zero and corresponds with the establishment of a steady state for Cl^-. These findings suggest that Cl^- plays a key role in the regulation of net cotransport activity and thereby cell volume.

Introduction

It is now well established that many types of animal cells [1–3], including the Ehrlich ascites tumor cell [4], when suspended in hypotonic media rapidly swell, subsequently lose KCl along with osmotically obligated water and return to isotonic volume. This sequence is commonly referred to as regulatory volume decrease (RVD). Subsequent resuspension of Ehrlich cells in hypertonic media (i.e., 300 mosmolar) results first in shrinkage which is then followed by restoration of the cell volume to its normal isotonic value. This progression of events termed regulatory volume increase (RVI), is due to the activation of a diuretic-sensitive, NaCl cotransport with subsequent uptake of K^+ and extrusion of Na^+ mediated by the K^+/Na^+ pump [5]. A similar phenomenon is observed when KCl-depleted, Na^+-enriched cells are incubated in a K^+-containing NaCl medium. The return of cell volume in this case is due to the activation of a diuretic-sensitive ($K^+ + Na^+ + 2Cl^-$) cotransport with the resultant re-accumulation of electrolytes and water [6,7]. The reason for the differences between these studies is largely unknown but may reflect a single cotransport system operating in different modes [4].

The regulation of RVD and RVI is incompletely understood. There is some evidence suggesting that intracellular Ca^{2+} plays an important role in RVD. For example, in the *Amphiuma* red blood cell the net loss of KCl induced by either cell swelling or by the Ca^{2+} ionophore, A23187, occurs mainly by way of a Ca^{2+} activated K^+/H^+ exchange functionally coupled to Cl^-/HCO_3^- exchange [8]. In contrast, separate conductive pathways for K^+ and Cl^- appear to be activated during RVD in lymphocytes and Ehrlich cells [9,10]. In the Ehrlich cell there is evidence that Ca^{2+} and calmodulin are involved in the activation of K^+ and Cl^- channels, while the Cl^- channel in lymphocytes appears to be Ca^{2+} independent. Although the release of intracellular Ca^{2+} appears to play a role, no change in cytosolic Ca^{2+} could be detected in human lymphocytes during RVD [11]. Measurements of changes in the internal Ca^{2+} during RVD have not been reported for the Ehrlich cell.

Activation and subsequent regulation of transport during RVI are largely unknown. Although there are indications that some cotransport systems are influenced by changes in certain biochemical pathways, particularly those involving inositol phospholipid metabolism [1], the limited data suggest that this is probably not the case in the Ehrlich cell [12]. It is also

Abbreviations: RVD, regulatory volume decrease; RVI, regulatory volume increase; PCA, perchloric acid.

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unlikely that the hydrolysis of ATP plays a role since there is no demonstrable stimulation of ATP turnover during net cotransport activity [6]. In a previous study we found that in KCl-depleted, Na^+-enriched cells the re-establishment of normal ionic gradients, appeared to serve as a major regulatory factor in RVI [7]. The present experiments were undertaken to further characterize the Cl^--dependent cation cotransport system. The results indicate that net ion transport during RVI is driven by the energy contained within the sum of the chemical potential gradients for Na^+, K^+, and Cl^-. Restoration of cell volume which is directly related to the change in the intracellular [Cl^-], occurs when this gradient declines to zero.

Materials and Methods

Reagents

Bumetanide was kindly supplied by Hoffman-La Roche, Inc., Nutley, NJ. Ouabain was a product of Sigma Chemical Co., St. Louis, MO, while [^{3}H]mannitol (19.1 Ci/mmol) was purchased from New England Nuclear, Boston, MA. All other reagents were of the highest quality obtainable.

Cell suspension

Experiments were performed with Ehrlich Lettre' ascites tumor cells (hyperdiploid strain) maintained in Ha/ICR white male mice by weekly transplantation. Tumor bearing animals with growths between 8 and 10 days were used. Cells were removed from the peritoneal cavity by aspiration and washed free of ascitic fluid [13]. The wash solution had the following composition (mM): 150 NaCl, 6 KCl, 10 Hepes-NaOH (pH 7.3–7.45; 290–300 mosM). This, as well as all other media, was filtered (Gelman Metricel; 0.45 μm) prior to use. Cells were washed twice in this solution and subsequently incubated at a density of 18–20 mg dry weight/ml under an air atmosphere for 30 min at 21–23° C. In order to deplete Ehrlich cells of KCl and enrich with Na^+, aliquots of cell suspension were washed twice and subsequently resuspended in ice-cold, K^+-free sodium gluconate (140 mM; buffered to pH 7.35 with 10 mM Hepes-NaOH) at a density of 4–7 mg dry weight/ml.

Ion fluxes

The K^+ and Cl^- depleted, Na^+-enriched cells were then resuspended in 1 to 2 ml of either K^+-free sodium gluconate or K^+-free NaCl and at time zero added to 10–15 ml of media (O_2 equilibrated, 37° C, pH 7.35–7.45) whose composition varied depending on the experiment. In most studies the medium contained 15 mM KCl + 130 mM NaCl. In some cases as indicated in the figures, the media also contained bumetanide or ouabain. Periodically during the next 15 to 25 min, 0.5 ml aliquots of cell suspension were removed and added to pre-weighed 1.5 ml microcentrifuge tubes containing 0.8 ml ice-cold choline dihydrogen citrate solution (CDHC; [14]) plus 0.4 μCi of [^{3}H]mannitol. The samples were immediately centrifuged 15 s at 15 000 × g, the supernatant removed and the tubes weighed before the addition of 1 ml of 1% (v/v) ice-cold perchloric acid (PCA). Net fluxes of K^+, Na^+ and Cl^- were estimated as the slopes of the initial time-dependent change in cellular electrolyte content and are expressed as: mequiv./(kg dry wt. per min). In general, the slopes were constant during the first 5 min. Errors are reported as ± standard error (S.E.) of the mean.

Analytical methods

The cell pellets were extracted with PCA in an ice-bath for 60 min and subsequently centrifuged 2 min at 15 000 × g to remove the PCA-insoluble residue. Aliquots of the PCA extracts and medium were used to determine Na^+ and K^+ by emission flame photometry using Li^+ as an internal standard. Cl^- was assayed with an autotitrator [15]. Aliquots of the PCA extract and the CDHC solution were assayed for [^{3}H]mannitol with a liquid scintillation spectrometer. Correction for Na^+, K^+ and Cl^- trapped in the extracellular space was determined in each experiment from distribution of the [^{3}H]mannitol. Since mannitol is an impermeant solute ([12]; unpublished observations), the radioactivity associated with the cell pellet served as a measure of the trapped fluid. In over 300 determinations conducted at varying cellular and extracellular ionic compositions, as well as, cell volumes, the extracellular water content of the cellular layer (ECS) ranged between 19 and 22% with a mean of 19.9 ± 1.8% of the weight of the cell pellet. Cell volume, which is reported in terms of kg cell water (corrected for ECS) per kg dry cell weight, was measured as described previously [16]. During the normal physiological steady state (290–300 mosM; pH 7.3–7.4) cell volume was equivalent to 3.55 ± 0.35 (n = 35) kg water/kg dry weight.

Results

Incubation of Ehrlich cells in cold K^+-free sodium gluconate medium results in the time-dependent loss of osmotically active solute and therefore cell shrinkage (approx. 25%). Although the loss of K^+ content (mequiv./kg dry wt.) exceeds the gain in Na^+, electroneutrality is maintained by the concomitant loss of Cl^- as well as by the increase in the concentration of non-diffusible cellular anions (A^-). This is shown in Table I.

The suspension of K^+ and Cl^--depleted, Na^+-enriched cells in a physiological medium results in a rapid reaccumulation of K^+ and Cl^-, extrusion of Na^+ and return of cell volume to its normal steady-state value. These changes are brought about by the combined

TABLE I

Water and electrolyte composition of Ehrlich tumor cells incubated in sodium gluconate

Cells were washed twice and subsequently incubated in ice-cold isosmotic, K^+-free sodium gluconate. At the times indicated aliquots of cell suspension were removed for the analysis of K^+, Na^+, Cl^- and water. Zero refers to cells incubated in the standard medium (6 mM KCl, 150 mM NaCl buffered to 7.4 with 10 mM Hepes-NaOH) at 23°C for between 60 and 120 min. Values represent means ± S.E. of at least six determinations.

Time (min)	mequiv./kg cell water			H_2O (kg/kg dry wt.)
	K^+	Na^+	Cl^-	
Zero	160±20	28±8	68±7	3.55±0.4
4–10	138± 6	45±6	50±8	3.41±0.4
25–35	110± 6	70±7	40±6	3.05±0.6
55–65	89± 9	91±7	30±5	2.85±0.4
80–90	80±11	108±5	24±4	2.72±0.6
105–120	76± 6	110±4	20±2	2.68±0.5
140–155	65± 9	125±9	22±5	2.65±0.3

activity of the Na^+/K^+ pump and the $K^+/Na^+/Cl^-$ cotransport system. In this as well as past studies [7] standard functional definitions of transport activity have been adopted. Activity of the $K^+/Na^+/Cl^-$ cotransport system is that which persists in the presence of 1 mM ouabain but is inhibited by 25–50 μM bumetanide. Na^+/K^+ pump activity is operationally defined as that persisting in the presence of bumetanide but is inhibited by 1 mM ouabain.

Fig. 1 shows the results of a typical experiment. Ehrlich cells were incubated 60 min in cold K^+-free, sodium gluconate prior to resuspension in 15 mM KCl/130 mM NaCl medium. Within 8–10 min the physiological steady state had been restored, that is, intracellular $[K^+]$ (mequiv./kg water) increased from 90 to 155, $[Na^+]$ decreased from 90 to 25 while $[Cl^-]$ increased from 25 to 70. Cell water content increased by 21% thereby returning volume to its normal level. Note that Fig. 1 also shows that bumetanide when present in the resuspending medium completely inhibited net Cl^- uptake as well as the restoration of cell volume but was without effect on the activity of the Na^+/K^+ pump. Fig. 2 shows the effect of Na^+/K^+ pump inhibition on the restoration of electrolytes and water. Although the cellular concentrations of Na^+ and K^+ remain constant during the first 6 min of incubation, the $[Cl^-]$ increases and attains its steady-state value by 6 to 8 min. The time course of the change in cell volume parallels that of Cl^- suggesting a relationship between the two. The observation that both the $[K^+]$ and $[Na^+]$ remain constant during volume restoration does not signify the absence of net uptake. Rather, this indicates that the concentration of each of these ions in the 'transported solution' is approximately the same as that within the cell water (about 90 mM). Since the transport is bumetanide sensitive (Fig. 1), it can be assumed that the cotransport system is responsible for RVI. Accepting a coupling stoichiometry of $1K^+ : 1Na^+ : 2Cl^-$ [6,17], the theoretical osmolality of the solution entering the cells over the range of $[Cl^-]$ 160 to 180 mM is 320–360 mosmol/kg water. This is in good agreement with our previous value [7] as well as that reported by others [12].

The influence of the starting cellular concentrations of Na^+, K^+ and Cl^- on the re-establishment of the steady state is shown in Figs. 3–5 (Table I). Fig. 3 illustrates a typical experiment in which Ehrlich cells, incubated for 2 h in cold sodium gluconate, were subsequently resuspended in 15 mM K^+/130 mM NaCl medium containing ouabain. The initial intracellular $[Na^+]$ was 125 but decreased to 105 mequiv./kg water

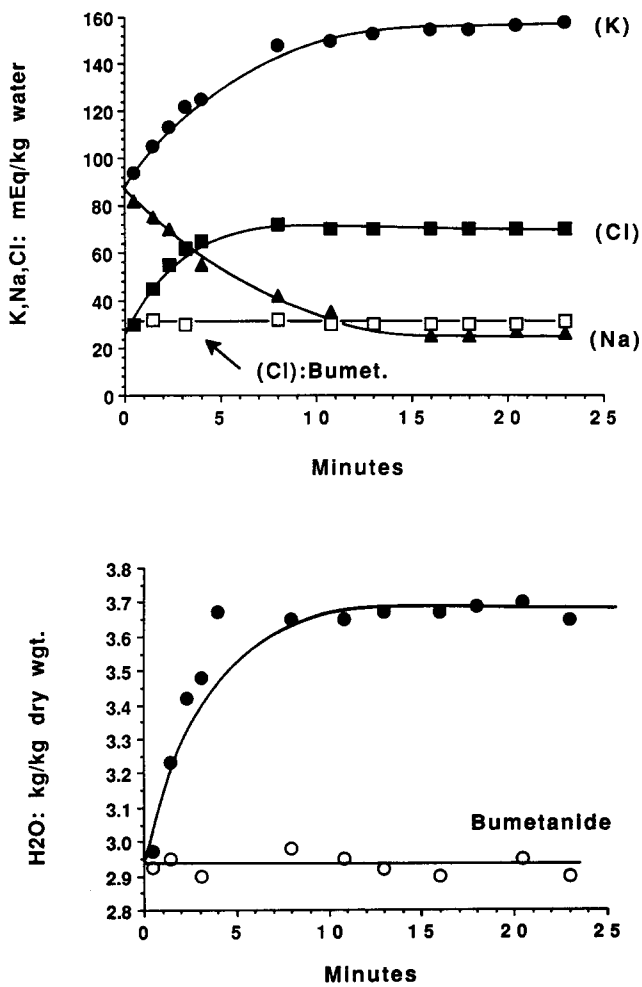

Fig. 1. Time-dependent changes in cellular Na^+, K^+, Cl^- and water in Ehrlich ascites tumor cells. Upper panel: Na^+, K^+, Cl^- concentrations expressed as mequiv./kg cell water; lower panel: Cell water content in kg/kg dry cell wt. Tumor cells were incubated 60 min in cold K^+-free sodium gluconate to deplete K^+ and Cl^- and to load with Na^+. After centrifugation the cells were suspended in medium containing 15 mM KCl+130 mM NaCl±50 μM bumetanide and cellular electrolytes/water content measured as a function of time. A single representative experiment is shown. Ten (control) and six (bumetanide) others gave similar results.

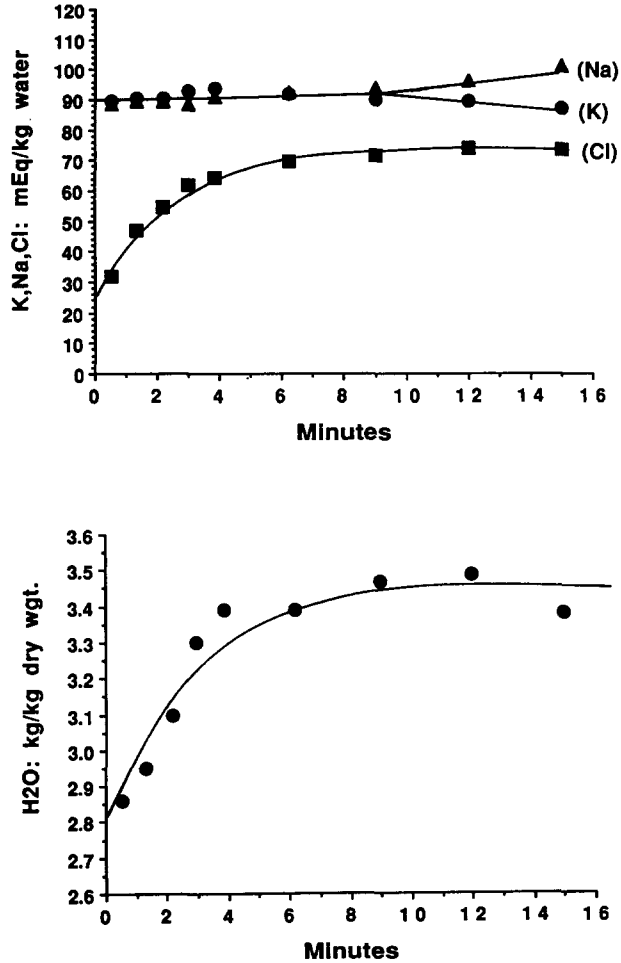

Fig. 2. The effect of ouabain on the time-dependent changes in cellular Na^+, K^+, Cl^- and water in Ehrlich ascites tumor cells. Upper panel: Na^+, K^+, Cl^- concentrations expressed as mequiv./kg cell water; lower panel: Cell water content in kg/kg dry cell wt. Cells were prepared as described in Fig. 1. [Ouabain] = 1 mM. A single representative experiment is shown. Five others gave similar results.

by 6 min while the $[K^+]$ started at 65 and increased to 85 mequiv./kg water. Since the Na^+/K^+ pump was inhibited, these changes reflect activity of the cotransport system. In as much as both Na^+ and K^+ enter the cell at approx. 90 mM, the decrease in $[Na^+]$ is presumably due to dilution while the increase in $[K^+]$ is due to entry of a solution containing greater than 65 mequiv. K^+/kg water. Since cell volume attains equilibrium by about 7 min and thereby signals termination of net cotransport activity, subsequent changes in $[Na^+]$ and $[K^+]$ reflect movement down their respective electrochemical gradients. Note that although little correlation exists between changes in either Na^+ or K^+ and cell volume, the time course of water uptake closely follows that of Cl^-. Fig. 4 shows results of a similar type of experiment in which Ehrlich cells were incubated for only 10 min in cold sodium gluconate before resuspension in 15 mM K^+ medium containing ouabain. Because the exposure to cold K^+-free, sodium gluconate was so brief there were only small changes in the concentrations of K^+, Na^+ and Cl^-. Intracellular $[Na^+]$ increased throughout the course of the experiment while the $[K^+]$ decreased. Once again the time course of volume restoration closely paralleled only that of net Cl^- uptake.

The data shown in Fig. 5 demonstrate that even when the starting $[K^+]$ and $[Na^+]$ are close to their physiological levels, cell volume increases so long as there is a net uptake of Cl^-. In this type of experiment K^+ and Cl^-- depleted, Na^+-loaded cells were incubated for 30 min in 15 mM potassium gluconate/130 mM sodium gluconate. Activation of the Na^+/K^+ pump resulted in the reaccumulation of K^+ and the extrusion of Na^+, but because Cl^- was absent the cotransport system was inoperative. At 'time zero' these cells were suspended in 15 mM KCl/130 mM NaCl medium and changes in the electrolytes and volume were measured. The sequential changes in $[Na^+]$ and $[K^+]$ appear to be

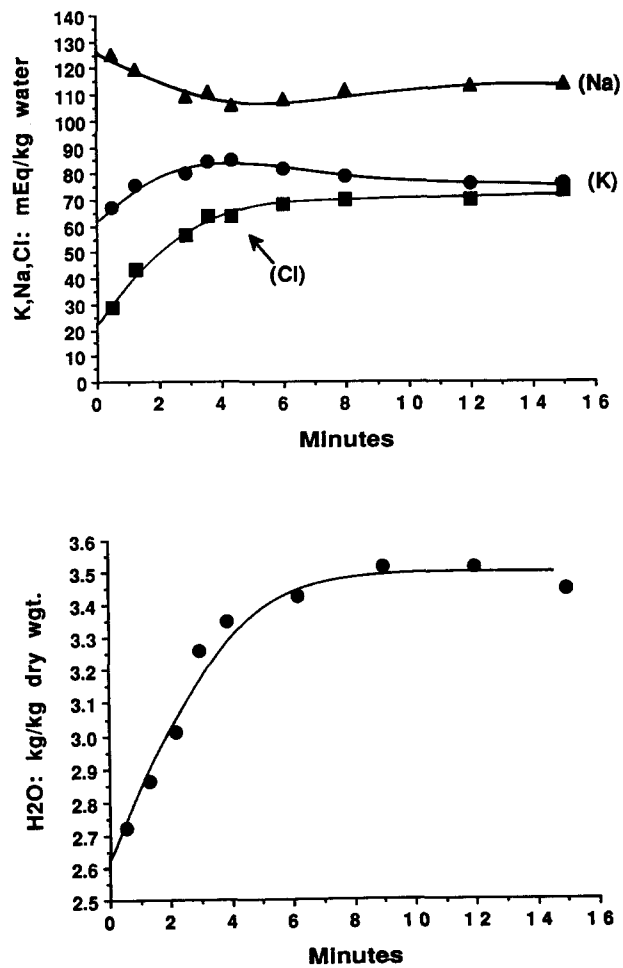

Fig. 3. Ouabain-insensitive changes in cellular Na^+, K^+, Cl^- and water in Ehrlich ascites tumor cells. Upper panel: Na^+, K^+, Cl^- concentrations expressed as mequiv./kg cell water; lower panel: Cell water content in kg/kg dry cell wt. Cells were prepared as described in Fig. 1 except they were incubated 2 h in cold K^+-free sodium gluconate. [Ouabain] = 1 mM. A single representative experiment is shown. Three others gave similar results.

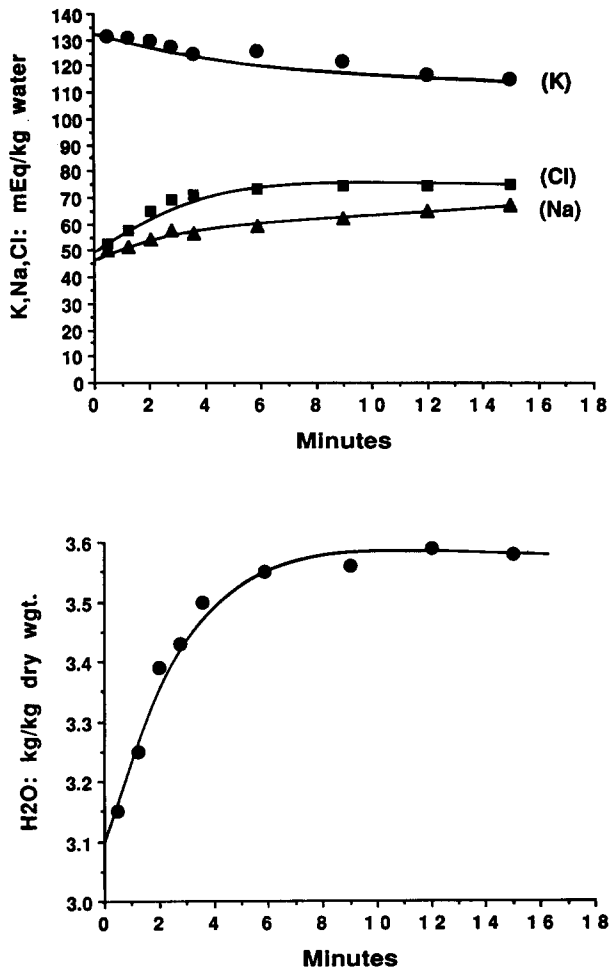

Fig. 4. Ouabain-insensitive changes in cellular Na^+, K^+, Cl^- and water in Ehrlich ascites tumor cells. Upper panel: Na^+, K^+, Cl^- concentrations expressed as mequiv./kg cell water; lower panel: Cell water content in kg/kg dry cell wt. Cells were prepared as described in Fig. 1 except they were incubated 10 min in cold K^+-free sodium gluconate. [Ouabain] = 1 mM. A single representative experiment is shown. Three others gave similar results.

due to activation of the $K^+/Na^+/Cl^-$ cotransport system resulting first in net uptake followed by or concomitant with adjustment in the final concentrations by the Na^+/K^+ pump. As before, termination of net Cl^- uptake marked the end of the volume change. In other words, it appears as if net cotransport activity ceases when intracellular $[Cl^-]$ attains its normal physiological level.

Although the relationship between the restoration of cell volume and net Cl^- transport points to an important role for Cl^-, only a single extracellular $[Cl^-]$ was used. Therefore, it was important to determine whether the same relationship held over a range of extracellular $[Cl^-]$. In the next series of experiments K^+ and Cl^--depleted, Na^+-enriched cells were incubated in isosmotic medium containing 15 mM KCl and a mixture of NaCl/sodium gluconate, providing extracellular $[Cl^-]$ of between 20 and 160 mM. Fig. 6 shows that the equilibrium or steady-state $[Cl^-]$ increased linearly in response to increases in the extracellular $[Cl^-]$ concentration. Similarly, the equilibrium cell volume and intracellular Cl^- in these cells are linearly related (Fig. 7). This result strongly suggests that the steady-state volume is set by the level of intracellular Cl^-, at least under these experimental conditions. Furthermore, as noted above, when the intracellular $[Cl^-]$ reaches its steady-state or equilibrium value net cotransport activity ceases. This suggests that at the outset of the experiment there is a substantial inward driving force favoring net ion uptake that decreases to zero as Cl^- is re-accumulated.

Bumetanide sensitivity of the ion and volume restorative process strongly supports a primary role for the cotransport system in RVI. In this case the magnitude of the passive driving force for net salt transport

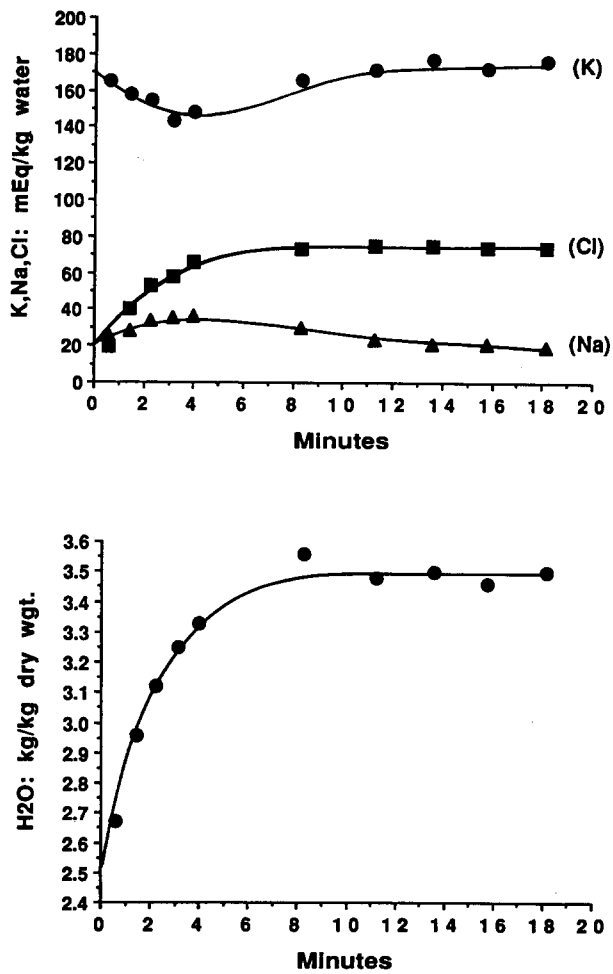

Fig. 5. Activation of net cotransport in cells containing normal $[K^+]$ and $[Na^+]$. KCl-depleted, Na^+-loaded cells were centrifuged and suspended in medium containing 15 mM potassium gluconate + 130 mM sodium gluconate. Following incubation at 37 °C for 30 min the cells were centrifuged and suspended in medium containing 15 mM KCl + 130 mM NaCl and cellular electrolytes/water content measured as a function of time. A single representative experiment is shown. Four others gave similar results.

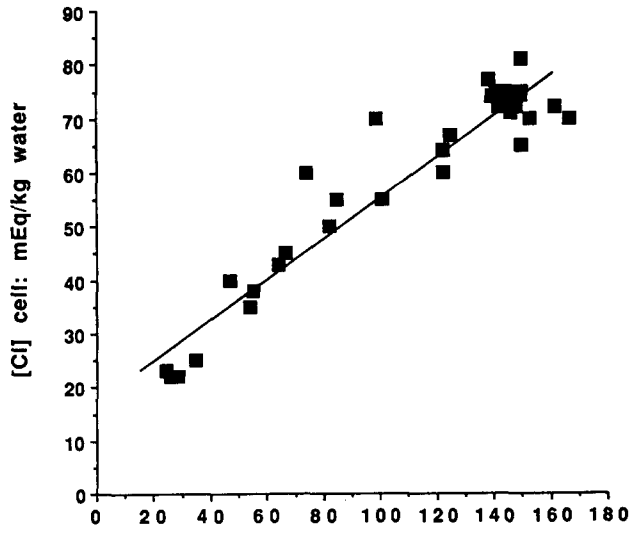

Fig. 6. Relationship between the extracellular [Cl^-] (mequiv./l) and the intracellular [Cl^-] (mequiv./kg water) at equilibrium. K^+ and Cl^--depleted, Na^+-loaded cells were centrifuged and suspended in media containing 15 mM KCl + 130 mM NaCl/sodium gluconate. Cellular electrolytes and water were then measured as a function of time. Equilibrium [Cl^-] represents the intracellular [Cl^-] after the attainment of the steady state. The line represent the best least-squares fit. Regression equation: $y = 18.1 + 0.38x$ ($r = 0.95$). Results from seven separate experiments.

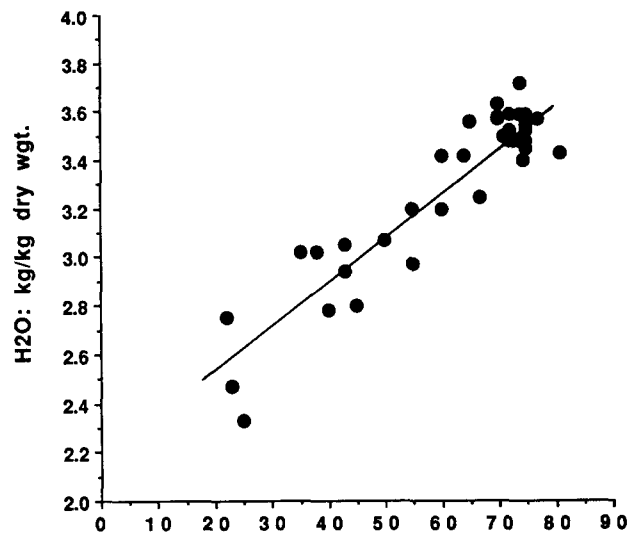

Fig. 7. Relationship between the intracellular [Cl^-] (mequiv./kg water) and the cell water content (kg/kg dry wt.) at equilibrium. K^+ and Cl^- depleted, Na^+-loaded cells were centrifuged and suspended in isosmotic media containing 15 mM KCl + 130 mM NaCl/sodium gluconate. Cellular electrolytes and water were then measured as a function of time. Equilibrium water content and [Cl^-] represents steady-state values. The line represents the best least-squares fit. Regression equation: $y = 2.19 + 0.0182x$ ($r = 0.93$). Results from seven separate experiments.

can be estimated from the difference in the sum of the chemical potentials of Na^+, K^+ and Cl^- between intracellular and extracellular phases. Assuming a stoichiometry of $1K^+ : 1Na^+ : 2Cl^-$ and electroneutrality [6,17], this is given by [18]:

$$\Delta\mu_{net} = \Delta\mu_K + \Delta\mu_{Na} + 2\Delta\mu_{Cl}$$

$$= RT \ln \{[K_o][Na_o][Cl_o]^2/[K_i][Na_i][Cl_i]^2$$

where o and i represent extra- and intracellular concentrations, respectively; R and T have their usual meanings. Application of this expression to the data shown in Figs. 1–5 is summarized in Table II. Note that in each case, at the initiation of net transport, $\Delta\mu_{net}$ is a large positive value indicating a net inwardly directed driving force. When the Na^+/K^+ pump is inhibited and thereby unable to contribute to the regeneration of the Na^+ and K^+ gradients (Figs. 2–4), $\Delta\mu_{net}$ approaches

TABLE II

Net chemical driving force on ($Na^+ + K^+ + Cl^-$) for the experiments shown in Figs. 1–5

For each Figure the $\Delta\mu_{net}$ was calculated at the start of the experiment, that is, when KCl-depleted, Na^+-enriched cells were added to 15.1 ± 2 mM KCl/132 ± 7 mM NaCl medium ± ouabain (1 mM) and again after cell volume had been restored to its steady-state level (equilibrium), usually within about 8 min. The [Cl^-], [Na^+] and [K^+] are those measured at the initiation of net transport (start of the experiment) and after volume restoration.

Fig.	Ouabain		mequiv./kg cell H_2O			$\Delta\mu_{net}$ (kcal/mol)
			Cl^-	Na^+	K^+	
1	no	start	25 ± 4	90 ± 9	90 ± 7	1.27 ±0.18
		equil.	72 ± 9	25 ± 4	160 ± 11	0.38 ±0.06
2	yes	start	25 ± 2	90 ± 9	90 ± 11	1.24 ±0.16
		equil.	75 ± 7	90 ± 8	90 ± 12	−0.023 ± 0.005
3	yes	start	23 ± 3	128 ± 17	62 ± 8	1.37 ±0.21
		equil.	70 ± 9	110 ± 11	80 ± 9	−0.027 ± 0.009
4	yes	start	50 ± 8	45 ± 8	133 ± 17	0.58 ±0.08
		equil.	72 ± 10	60 ± 5	120 ± 15	0.005 ± 0.003
5	no	start	20 ± 4	20 ± 3	170 ± 20	2.05 ±0.34
		equil.	70 ± 9	30 ± 4	165 ± 18	0.33 ±0.05

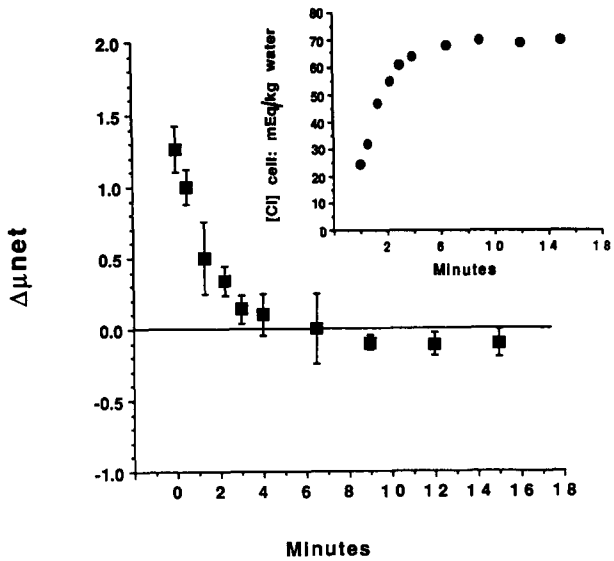

Fig. 8. Time-dependent changes in $\Delta\mu_{net}$ (sum of the chemical driving forces on Na^+, K^+ and Cl^-). $\Delta\mu_{net}$ was calculated at each time point using the data from the experiments described in Fig. 2. Extracellular medium (mM): $K^+ = 15.8 \pm 2$, $Na^+ = 132 \pm 5$, $Cl^- = 140 \pm 6$; cellular (mequiv./kg water): $K^+ = 90 \pm 3$, $Na^+ = 88 \pm 4$, $Cl^- = 25 \pm 2$ and increased to 75 ± 7. Inset displays the corresponding time course of Cl^- uptake.

zero as Cl^- is restored to its normal physiological concentration by the cotransporter. It is clear that there is no consistent relationship linking either Na^+ or K^+

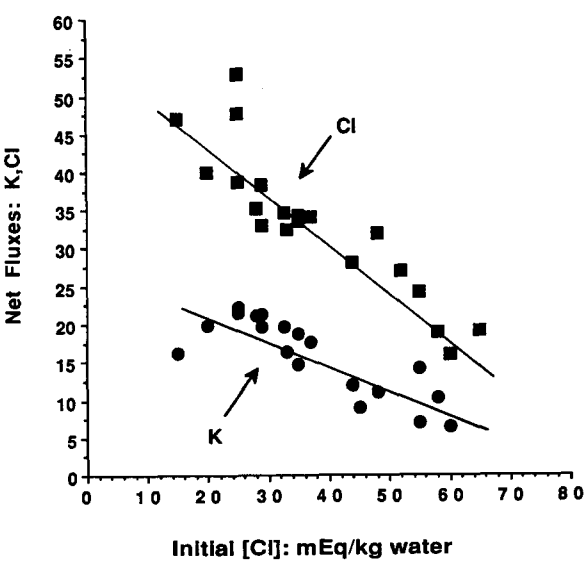

Fig. 9. Bumetanide-sensitive net fluxes of Cl^- and K^+ (mequiv./kg dry wt per min) as a function of the initial intracellular [Cl^-]. Tumor cells were incubated in cold K^+-free sodium gluconate for 10 min to 2.5 h (Table I), centrifuged and resuspended in medium containing 15 mM KCl+130 mM NaCl±50 μM bumetanide. The time-dependent uptake of K^+, Na^+, Cl^- and water was measured and the bumetanide-sensitive net fluxes calculated from the initial, linear portion of the uptake curves. The lines represent the best least-squares fit. Regression equations: K^+: $y = 27.3 - 0.32x$ ($r = 0.89$); Cl^-: $y = 55.8 - 0.68x$ ($r = 0.90$). Results from six separate experiments.

with the steady state that is achieved. For example, compare the distribution of Na^+ and K^+ in Fig. 2 and Fig. 5. The close relationship between the decrease in $\Delta\mu_{net}$ and Cl^- uptake is shown most clearly in Fig. 8 which is derived from the data of Fig. 2. These results along with those shown in Fig. 9 are consistent with the idea that net transport stops when [Cl^-] attains equilibrium. The experiments summarized in Fig. 9 display the bumetanide-sensitive net fluxes of K^+ and Cl^- measured in cells that contained varying concentrations of K^+ and Cl^-. Note that there is an inverse relationship between the bumetanide-sensitive net fluxes and the initial intracellular [Cl^-], that is, net fluxes decrease with an increase in the starting [Cl^-]. Cessation of transport occurs when net ion fluxes mediated by this pathway go to zero. Extrapolation of these data to zero net flux indicates that both K^+ and Cl^- fluxes become zero at same [Cl^-]. When the slopes of the regression lines relating net K^+ and Cl^- transport to the intracellular [Cl^-] are compared, a ratio of 2.1 (0.68 Cl^-/0.32 K^+) is found. This provides additional evidence that the stoichiometric coupling of the cotransport system is $2Cl^- : 1K^+$ over a wide range of intracellular [Cl^-].

Discussion

In an earlier study we established that Ehrlich tumor cells, shrunken as a result of KCl depletion and Na^+ loading, recovered ionic content by the combined activity of the Na^+/K^+ pump and the bumetanide-sensitive ($2Cl^- + K^+Na^+$) cotransport system [7]. These transporters operate in unison to affect net K^+ uptake while at the same time move Na^+ in opposite directions across the cell membrane. Return of cellular Cl^- is mediated exclusively by the cotransport system which serves as the major pathway for Cl^- entry (Fig. 1). The net result, therefore, is the uptake of four osmotically active particles ($2K^+ + 2Cl^-$) followed by osmotically obligated water. Therefore, the increase in cell volume (RVI) is dependent on net accumulation of K^+ and Cl^- and approaches zero as the net ion fluxes go to zero. An alternate view is that the restoration of cell volume, per se, in some fashion limits net ion transport and thereby reestablishes the physiological steady state [12,19–21]. This is not the case in Ehrlich cells, however, since the activity of the cotransport system seems to be independent of cell volume [7].

The results of the present investigation extend our earlier studies and show that while the redistribution of Na^+ and K^+ is important for the re-establishment of normal ionic concentrations (Fig. 1), cell swelling during RVI correlates only with the increase in the intracellular [Cl^-] (Figs. 2–5). This is shown most clearly in the studies where cells were prepared containing a wide range of Na^+ (20–125 mequiv./kg cell water) and K^+ (60–170 mequiv./kg cell water). When these cells were

subsequently incubated at 37°C in an iso-osmotic medium containing Na^+, K^+ and Cl^- net salt uptake along with osmotically obligated water could be demonstrated. However, there is no predictable relationship between the change in cell volume and corresponding changes in cellular [Na^+] or [K^+], or in equilibrium cell volume and [Na^+] or [K^+] (Table II). Only with Cl^- could we demonstrate a relationship between uptake and swelling. This, along with the finding that the equilibrium volume is linearly related to the steady-state [Cl^-] (Fig. 7), supports the idea that the extent to which cell volume increases is determined by the uptake of Cl^- which in turn is related to the extracellular [Cl^-].

The cotransport system mediates, in addition to Cl^-, the uptake of Na^+ and K^+ as an electrically silent neutral quarternary complex. Thus, it is anticipated that with the activation of net cotransport the complex ($Na^+ + K^+ + 2Cl^-$) enters the cell driven by the sum of the chemical potentials [18]. As the gradient approaches zero, net uptake ceases and a steady state with respect to cell volume and Cl^- is established (Figs. 2, 6–9). This, however, should be contrasted with the results obtained in the absence of ouabain (Figs. 1, 5; [7]). Transport competent cells (i.e., an active Na^+/K^+ pump) also establish a steady state in which the net driving force is clearly non-zero and favors the inward directed uptake of the quaternary complex. However, in the Ehrlich as well as other cell types maintained under normal physiological conditions inhihition of the cotransport system does not result in detectable changes in either cell volume or electrolytes [22,23]. This suggests that under these conditions the cotransport system does not mediate net ion uptake even though it is energetically favorable. In view of the present results it is tempting to speculate that activity of the cotransport system and thereby RVI is somehow regulated by the intracellular [Cl^-] and not exclusively by the gradient of chemical potential ($\Delta\mu_{net}$).

Taken together the results of these as well as past studies suggest that recovery of cell volume in Ehrlich tumor cells, previously shrunken due to changes in the internal electrolytes, is due exclusively to the activity of the ($Na^+ + K^+ + 2Cl^-$) cotransport system. The extent of swelling correlates with the uptake of Cl^- and the re-establishment of its steady state. In the absence of active Na^+ and K^+ transport, the driving force for this process appears to be the chemical potential energy stored within the gradients of the electrolytes.

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