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Generation of plasma membrane potential by the Na^+-pump coupled to proton extrusion

C. L. Bashford* and C. A. Pasternak

Department of Biochemistry, St George's Hospital Medical School, Cranmer Terrace, London SW17 ORE, England

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Abstract. Lettré cells maintain a plasma membrane potential near − 60 mV, yet are scarcely depolarized by 80 m*M* Rb^+ and are relatively impermeable to $^{86}Rb^+$. They are depolarized by ouabain without a concomitant change in intracellular cation content. Addition of K^+ to cells suspended in a K^+ free medium, or of Na^+ to cells in a Na^+ free medium, hyperpolarizes the cells. They contain electroneutral transport mechanisms for Na^+, K^+ and H^+ which can function as Na^+:K^+ and Na^+:H^+ exchanges. It is concluded that plasma membrane potential of Lettré cells, in steady-state for Na^+ and K^+, is produced by an electrogenic Na^+ pump sustained by electroneutral exchanges, and restricted by anion leakage.

Key words: Lymphocytes, Lettré cells, membrane potential, Na^+ pump, cation diffusion

Introduction

The electrical potentials which exist across the membranes of mitochondria (Mitchell 1968; Nicholls 1974), lysozomes (Ohkuma et al. 1982), chromaffin granules (Bashford et al. 1976) and intact cells are generated either by electrogenic pumping of ions such as H^+ or Na^+, or by diffusion of ions such as K^+. It has generally been assumed that, except for red cells which have rather low membrane potentials (Hoffman and Laris 1974; Sims et al. 1974), the potential across the plasma membrane of most animal cells is generated by the outward diffusion of K^+ ions down their chemical concentration gradient (Williams 1970). However all animal cells produce H^+ ions – by production either of lactic acid by glycolysis or of carbonic acid (hydrated CO_2) by respiration – and extrude these into media that can become significantly more acid than cytoplasm (e.g. Bashford et al. 1983), and all have a Na^+-pump in their plasma membrane. Hence it is theoretically possible for cells to couple their Na^+-pump to their need to export metabolic acid; certainly the Na^+-pump contributes significantly to the generation of membrane potential in a variety of cells, particularly when internal Na^+ is elevated (Thomas 1972; Pietrzyk et al. 1978; Lew et al. 1979). It appears to contribute so significantly to the membrane potential of Lettré cells, a line of malignant ascites tumour cells, that we have suggested ion pumping to be the *main* mechanism by which these, and perhaps other (Bashford and Pasternak 1985), cells generate their plasma membrane potential (Bashford and Pasternak 1984). This would imply that in Lettré cells the rate at which ions are pumped across the plasma membrane greatly exceeds the rate at which they diffuse across it; on the other hand, because membrane potential is maintained in the absence of net movement of Na^+ or K^+, it also implies the existence of electroneutral return mechanisms for the ions pumped, such as Na^+:K^+ and Na^+:H^+ exchanges (Bashford and Pasternak 1984). We show here that Lettré cells indeed possess these attributes and we come to the conclusion that the major net contributing factor to membrane potential in Lettré cells is the continuous action of the Na^+-pump; the activity of the pump is not restricted by depletion of cellular Na^+ as this is sustained by Na^+:K^+ and Na^+:H^+ exchanges, so that cells in a steady-state for Na^+ and K^+ continue to export H^+ as a consequence of Na^+-pump activity. Other workers have inferred the existence of an electrogenic H^+-pump in the membrane of Ehrlich ascites tumour cells (Geck et al. 1978; Heinz et al. 1981), but to our knowledge, this is the first demonstration that the plasma membrane potential of an animal cell is predominantly generated by the mechanism outlined above.

* To whom reprint requests should be sent

Experimental

Lettré cells and human peripheral lymphocytes were prepared as described by Bashford and Pasternak (1984). Membrane potential was assessed by the addition of oxonol-V (gift of Dr. B. Chance) to cell suspensions followed by measurement of $A_{630-590}$ in a Johnson Research Foundation Compensated Fluorimeter/Spectrophotometer as described by Bashford et al. (1983, 1985a) and by Bashford and Pasternak (1984). Cell cations, and content of $^{86}Rb^+$, was measured in cell pellets after spinning through oil (Impraim et al 1979); in some experiments, indicated in the figure legends, cells were spun directly through oil without prior dilution in choline medium (Bashford et al. 1985b). Changes in intracellular pH were measured either by preincubating cells with quene-1 ester (Rogers et al. 1983) followed by washing and measurement of fluorescence (excitation, 390 nm; emission 540 nm) or by addition of neutral red and measurement of $A_{445-540}$; in each case the Johnson Research Foundation Compensated Fluorimeter/Spectrophotometer was used. Quene-1 ester was kindly donated by Dr G. A. Smith; amiloride was a gift of Professor J. G. Widdicombe.

Results

Figure 1 (upper panel) shows that the membrane potential of Lettré cells is relatively insensitive to addition of RbCl but is then depolarized by valinomycin, an ionophore which greatly increases membrane conductance for K^+ (Harris and Pressman 1967), whereas lymphocytes are depolarized by RbCl and are then insensitive to valinomycin. Similar results have been obtained with KCl (Bashford and Pasternak 1984); the extent of depolarization of lymphocytes is consistent with potassium (rubidium) diffusion being the major determinant of membrane potential in that case. The insensitivity of Lettré cell membrane potential to Rb^+ (and K^+) suggests that the passive permeability of the membrane to these ions is very low. Certainly the rate at which $^{86}Rb^+$ leaks across the Lettré cell plasma membrane is a factor of four lower than its rate of leakage across the lymphocyte plasma membrane (Fig. 1, lower panel) which itself is known to have a relatively low density of K^+ channels (De Coursey et al. 1984; Matteson and Deutsch 1984). How much of the observed $^{86}Rb^+$ movement can be ascribed simply to diffusion cannot be established solely from tracer fluxes. However, the conditions chosen for the experiments (K^+ free medium, presence of ouabain and

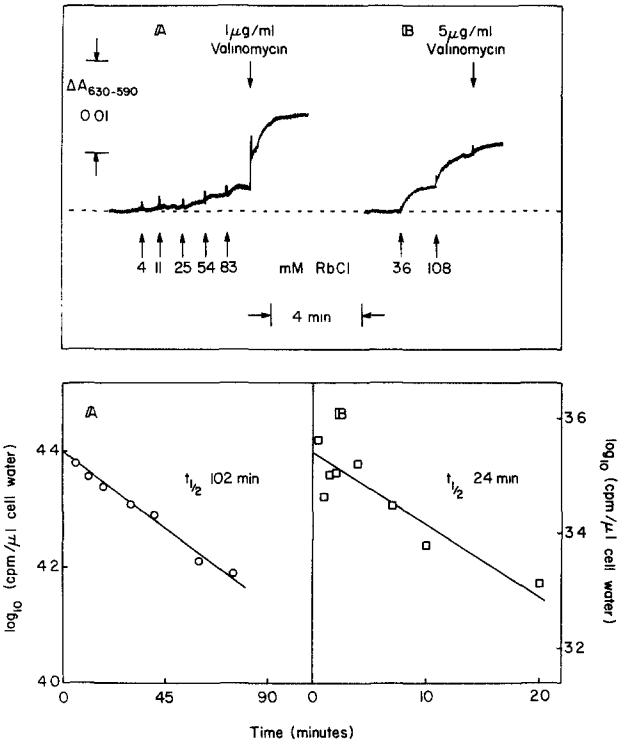


Fig. 1. Permeability of Lettré cells and lymphocytes to Rb^+. *Upper panel:* effect on membrane potential of Lettré cells (*A*) or human peripheral lymphocytes (*B*) of successive additions of RbCl (to give the final concentrations indicated), followed by valinomycin. Cells were prepared, suspended (at 4×10^6/ml) in 150 m*M* NaCl, 5 m*M* KCl, 5 m*M* Hepes, 1 m*M* $MgCl_2$, 2 μ*M* oxonol-V, pH 7.3 at 37 °C and membrane potential recorded; depolarization is indicated by an increase in $A_{630-590}$. *Lower panel:* efflux of $^{86}Rb^+$ from Lettré cells (*A*) or human peripheral lymphocytes (*B*). Cells preincubated (at $2-4 \times 10^8$/ml) with $^{86}RbCl$ were diluted (to $2-4 \times 10^6$/ml) into 150 m*M* NaCl, 5 m*M* Hepes, 1 m*M* $MgCl_2$, 1 m*M* furosemide, 1 m*M* ouabain, pH 7.4 at 37 °C and ^{86}Rb in cell pellets measured. The results are expressed as ^{86}Rb cpm per μl cell water

furosemide) were designed to minimize contributions from the Na^+-pump and electroneutral Na^+, K^+ and $2Cl^-$ cotransport (Skou and Norby 1979; Geck et al. 1980).

The anion present in the medium in these experiments was Cl^-, whose substitution by SO_4^{2-} has no effect on Lettré cell potential (Bashford and Pasternak 1984), and whose transmembrane flux in the closely related Ehrlich ascites cell is predominantly electroneutral (Hoffman et al. 1979). It seems reasonable to conclude that the Rb^+ fluxes observed represent the upper limit of Rb^+ (K^+) diffusion in the two cell types. Our results suggest that substantially fewer K^+ channels will be found in Lettré cells than in lymphocytes.

That the Na^+-pump contributes directly to the membrane potential of Lettré cells but not that of

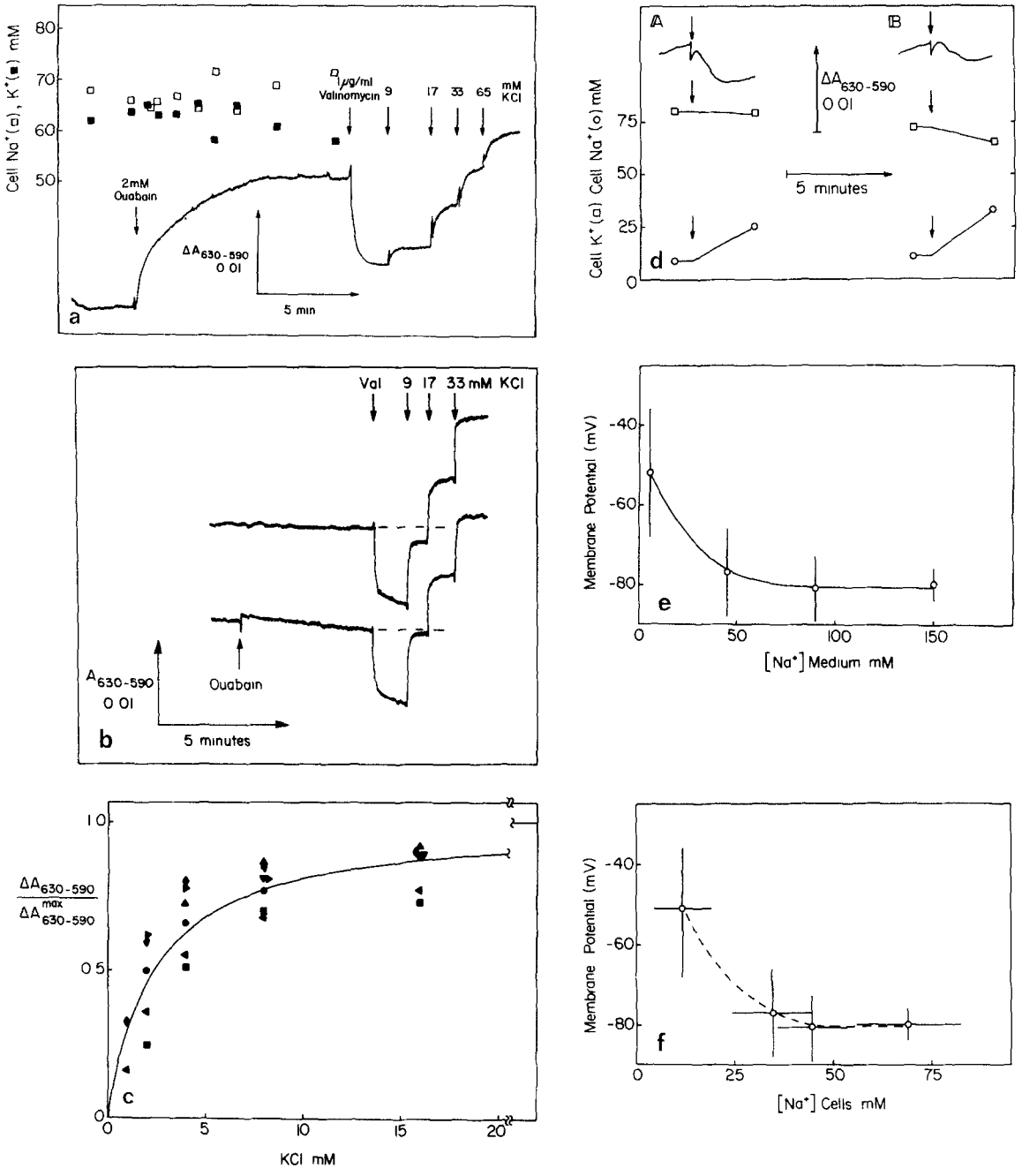
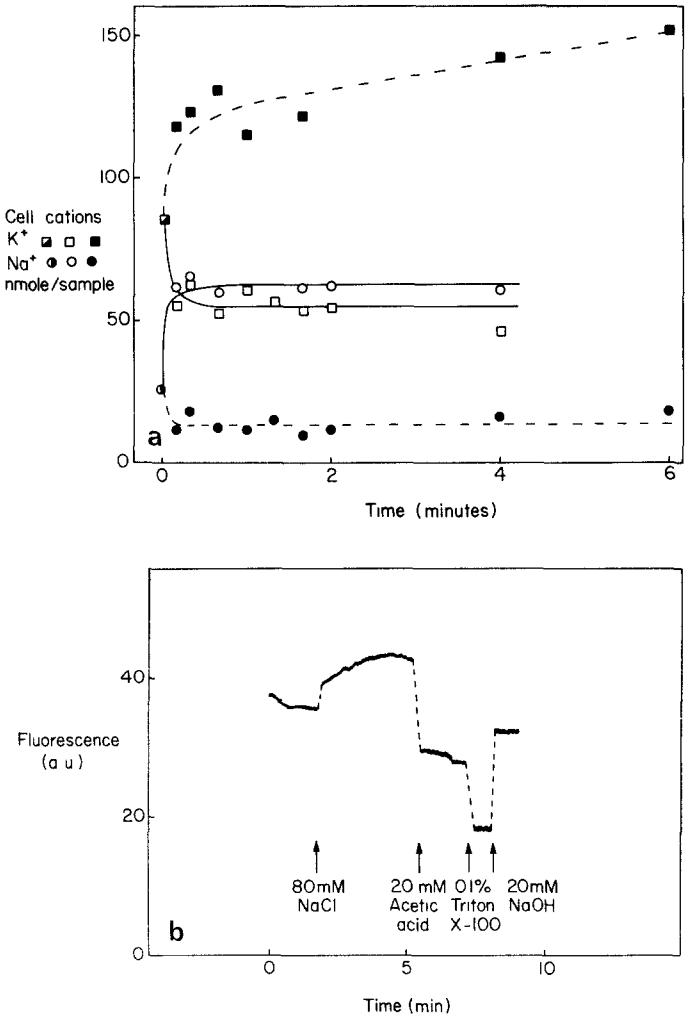


Fig. 2a–f. Contribution of Na^+ pump to membrane potential of Lettré cells and lymphocytes. **a** Effect of ouabain on membrane potential and intracellular cations of Lettré cells. 5×10^6 cells/ml in 150 m*M* KCl, 5 m*M* KCl, 1 m*M* $MgCl_2$, 1 m*M* glucose, 2 µ*M* oxonol-V, pH 7.4 at 33 °C. Valinomycin and KCl were added in order to determine the final membrane potential (−20 mV, from an initial value of −80 mV) by the null point method of Hoffman and Laris (1974; Bashford et al. 1985a). **b** Effect of ouabain on membrane potential of lymphocytes. 2.3×10^6 cells/ml in 150 m*M* NaCl, 5 m*M* KCl, 5 m*M* Hepes, 1 m*M* $MgCl_2$, 2.5 µ*M* oxonol-V, pH 7.4 (NaOH) at 37 °C. Valinomycin (2 µg/ml) and KCl (both traces) and ouabain (2 m*M*, lower trace) were added as indicated. Membrane potential was −62 mV in the absence of ouabain (*upper trace*) and −63 mV in the presence of ouabain (*lower trace*). **c** K^+-dependence of Lettré cell membrane repolarization. 4×10^6 cells/ml in 150 m*M* NaCl, 5 m*M* glucose, 5 m*M* Hepes, 1 m*M* $MgCl_2$, 2 µ*M* oxonol-V, pH 7.4 (NaOH) at 33 °C. KCl was added and the decrease in $A_{630-590}$ ($\Delta A_{630-590}$) recorded. Data from six separate preparations are presented as the extent of the change in absorbance relative to the maximum extent at high K^+ concentration. The solid curve is the best fit hyperbola of the form $\Delta A/\Delta A^{max} = [S]/(K_{0.5} + [S])$ where $K_{0.5} = 2.4$ m*M*. **d** Na^+-dependent hyperpolarization of Lettré cells. 4×10^6 cells/ml in 300 m*M* mannitol, 10 m*M* Hepes, 1 m*M* $MgSO_4$, 2.5 µ*M* oxonol-V, pH 7.4 (NaOH) at 32 °C in the absence (*A*) or presence (*B*) of 1 m*M* ouabain. 50 m*M* NaCl was added as indicated by the arrow. **e, f** Na^+-dependence of Lettré cell membrane potential. 4×10^6 cells/ml in 5 m*M* KCl, 5 m*M* Hepes, 1 m*M* $MgSO_4$ (or $MgCl_2$), 2 µ*M* oxonol-V, pH 7.4 (NaOH) and 300 mOs*M* mannitol/NaCl to give the NaCl concentration indicated. Intracellular Na^+ was measured from the pellet Na^+ and pellet H_2O (wet weight-dry weight) after spinning cells through oil omitting the dilution in choline medium (Bashford et al. 1985b). The bars indicate the standard deviation ($n = 3$) of the estimates



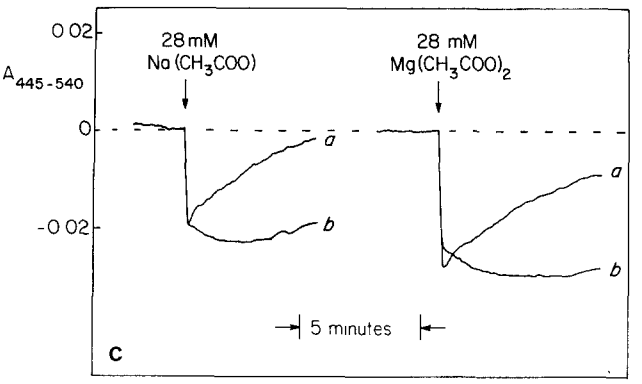


Fig. 3 a – c. Electroneutral ion movements in Lettré cells. **a** opposing movements of K^+ and Na^+. Lettré cells suspended (at 1.5×10^8/ml) in 77.5 m*M* NaCl, 77.5 m*M* KCl, 5 m*M* Hepes, 1 m*M* $MgCl_2$, pH 7.4 at 21 °C were diluted (to 6×10^5/ml) into either 155 m*M* NaCl (*open symbols*) or 155 m*M* KCl (*closed symbols*), each containing 5 m*M* Hepes, 1 m*M* $MgCl_2$, pH 7.4 at 21 °C and intracellular cations measured. The initial concentrations of K^+ and Na^+ are indicated by the partially-closed symbols. **b** Na^+-dependent movement of H^+. Lettré cells preincubated with quene-1 ester were washed, suspended (at 10^7/ml) in 200 m*M* sucrose, 50 m*M* Hepes, 1 m*M* $MgCl_2$, pH 7.4 at 37 °C and fluorescence measured. Additions gave the final concentrations as indicated. **c** Na^+-dependent, amiloride sensitive movement of H^+. 2% v/v Lettré cells in 200 m*M* sucrose, 50 m*M* Hepes, 1 m*M* $MgCl_2$, 25 μ*M* neutral red, pH adjusted to 7.4 with NaOH at 37 °C. Sodium or magnesium acetate were added to give the final concentration indicated in the absence (*a*) or presence (*b*) of 2 m*M* amiloride. Acidification is indicated by a decrease in $A_{445-540}$

lymphocytes is shown in Fig. 2. Whereas many cells slowly depolarize in the presence of ouabain as a result of changes in intracellular cation concentration and hence in concentration gradients (Thomas 1972), depolarization of Lettré cells is rapid and is unaccompanied by changes in cation content (Fig. 2a). Indeed for a depolarization of 60 mV, a 10-fold change of K^+ content would be required if the potential were due to K^+ diffusion; note that under these conditions lymphocytes are not depolarized (Fig. 2b), even though they possess a ouabain-sensitive $^{86}Rb^+$ uptake mechanism (Felber and Brand 1982), which we have observed in our preparations and which presumably reflects the activity of the Na^+-pump. The Na^+-pump is linked to the uptake of K^+ and is therefore dependent on the presence of K^+ in the extracellular medium, as well as on Na^+ in the intracellular milieu (Skou and Norby 1979). Lettré cells are depolarized in K^+-free medium and are repolarized by addition of K^+ (Bashford and Pasternak 1984); the extent of repolarization (indicated by the decrease in $A_{630-590}$) depends on the concentration of K^+ added to the medium (Fig. 2c), and follows a simple saturation function with half maximal effect at a K^+ concentration of 2.4 m*M*. Again lymphocytes behave in a different manner under these conditions. K^+ depletion hyperpolarizes slightly (relative to membrane potential in 5 m*M* K^+) and further addition of K^+ depolarizes cells (see Fig. 1). When Lettré cells are suspended in a Na^+-free medium the intracellular Na^+ concentration falls (see Figs. 2f, 3a). Under these conditions they are depolarized neither by ouabain (nor by K^+) and are hyperpolarized, in a ouabain-sensitive fashion, by the addition of Na^+ (Fig. 2d). The Na^+ concentration dependence of this hyperpolarization is shown in Fig. 2e and f: the effect is half maximal at an *intracellular* Na^+ concentration of 22 m*M*.

If the membrane potential of Lettré and other cells is indeed generated by an electrogenic Na^+-pump, (which by definition pumps out more Na^+ than it pumps in K^+), how is the intracellular concentration of Na^+ and K^+ maintained at osmotic steady-state? Certainly Lettré cells do maintain con-

stant intracellular Na^+ and K^+ over several hours, during which time their membrane potential also remains constant, at about -60 mV (Bashford and Pasternak (1984). Diffusion of Na^+ (into cells) and K^+ (out of cells) cannot account for this constancy since Lettré cells to not depolarize when K^+ or Na^+ is added. Hence some kind of electroneutral transport mechanisms must operate to maintain cellular cation levels. Figure 3b shows that Lettré cells are indeed capable of taking up Na^+ and extruding Na^+; the reverse movement, to which the K^+-pump of course contributes, is also shown. The molecular basis for these electroneutral movements is not fully understood. Furosemide, an agent which inhibits Na^+, K^+, $2Cl^-$ co-transport in Ehrlich cells (Geck et al. 1980) has little effect on the rapid movements of Na^+ and K^+ observed (within 10 s) after shifting Lettré cells to a new medium. However, furosemide does inhibit the much slower (minutes to hours) K^+ accumulation or K^+ loss (Fig. 3a) observed in these experiments. The slow, furosemide-sensitive, loss of K^+ is associated with a loss of cell chloride (assessed by equilibrium distribution of $^{36}Cl^-$). We note that the furosemide-sensitive fluxes are not accompanied by measurable changes in cellular Na^+ content; and that, unlike ouabain, furosemide has no immediate effect on Lettré cell membrane potential. It is not yet clear whether the rapid, furosemide-insensitive, movements of Na^+ and K^+ represent $Na^+:K^+$ exchange or compensating movements of $Na^+ + Cl^-$ in one direction and of $K^+ + Cl^-$ in the other direction. At first sight the slow leakage of $^{86}Rb^+$ out of cells (Fig. 1) might be thought incompatible with the rapid movement of K^+ indicated in Fig. 3b. In order to observe the latter change, however, cells were exposed to considerable changes in external milieu (cf. Geck et al. 1980) in the absence of inhibitors; under such conditions, rapid movements of $^{86}Rb^+$ do of course occur (data not shown). The point is that the slow leakage of $^{86}Rb^+$ at steady-state includes any such electroneutral Rb^+ (K^+) movements, and thus reduces the upper limit for any electrogenic Rb^+ (K^+) movement even further.

In addition to electroneutral counter-movements of Na^+ and K^+, Lettré cells, in common with other cell types (Murer et al. 1979) possess an amiloride-sensitive counter-movement of Na^+ and H^+. Thus Na^+-dependent acidification of the extracellular medium has recently been reported (Bashford and Pasternak 1984) and Fig. 3b and c illustrate situations in which intracellular H^+ levels are affected by extracellular Na^+. Figure 3b shows an experiment in which cytoplasmic pH was monitored using the intracellular, fluorescent indicator quene-1 (Rogers et al. 1983). Addition of Na^+ to cells containing quene-1 and suspended in Na^+ free medium causes an increase in fluorescence characteristic of a rise in pH (Rogers et al 1983). Under similar conditions the external medium acidifies on Na^+ addition (Bashford and Pasternak 1984) indicating that the alkalinization occurred in an intracellular compartment containing the indicator. The subsequent addition of acetic acid, which acidifies intracellular compartments because of the relatively high membrane permeability of the undissociated acid (Henderson et al. 1969), caused a decrease in fluorescence; lysis of the cells with triton X-100 caused a further fluorescence decrease indicating that the medium was more acid than the cells; subsequent addition of base confirms that an increase in dye fluorescence represents alkalinization of the dye-containing medium. Figure 3c shows the results of a complementary set of experiments in which the internal pH was deliberately altered, and the Na^+-sensitivity of the restoration of the original pH value assessed. In this case the membrane permeant indicator neutral red in the presence of highly buffered solutions to suppress changes in external pH (Junge et al. 1979; Wikstrom 1984) was used; under these conditions more than 60% of the dye is cell-associated, less than 40% of the dye remaining in the supernatant after pelleting the cells. An intracellular acid load was imposed on the cells by the addition of sodium or magnesium acetate, acidification arising from permeation of undissociated acetic acid which then dissociates within cells. The recovery of cell pH from the acid load requires the presence of Na^+ in the medium ($K_{0.5}$ approx. 40 m*M* Na^+, data not shown) and is inhibited by amiloride. While these experiments with quene-1 and neutral red clearly indicate the operation of a $Na^+:H^+$ counter-movement, the stoichiometry of the process has not yet been determined.

Discussion

The results presented above can most simply be rationalised by proposing a scheme in which the membrane potential of Lettré cells suspended in conventional ionic media is produced by a Na^+-pump whose continued activity depends on electroneutral $Na^+:K^+$ and $Na^+:H^+$ exchanges so that cells in a steady-state for Na^+ and K^+ continue to export H^+ as a consequence of Na^+ pumping; i.e. the Na^+-pump, in effect, is a H^+-pump (Fig. 4). This model envisages H^+ export as a "secondary" active process linked to the transmembrane Na^+ gradient which is analogous to the "chloride" pump found in epithelia (Shorofsky et al. 1982). Lettré cells may, in addition to the mechanism outlined above, possess a frank H^+-pump (Geck et al. 1978; Heinz et al. 1981),

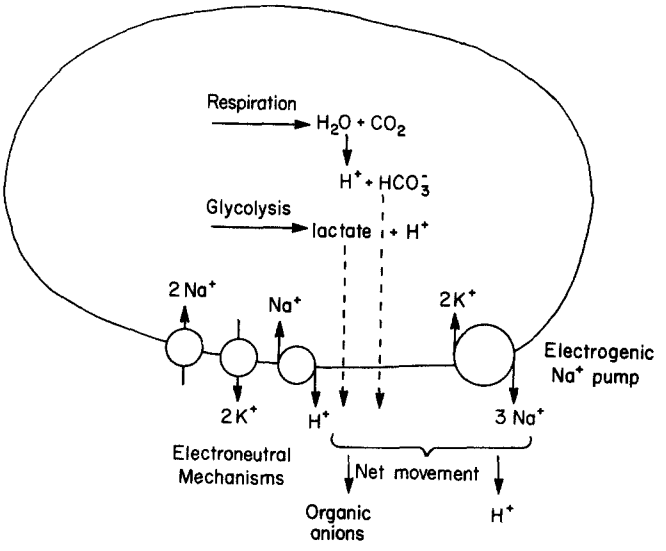


Fig. 4. Maintenance of membrane potential in Lettré cells

which may account for the potential found in cells suspended in a low ionic strength medium (see Fig. 2e). The extent to which frank H^+-pumping contributes to the potential of cells suspended in normal salines remains to be assessed. The magnitude of the pumped potential has to be restricted either by the reversal potential of the pump, or by the induction of currents equal and opposite to the pump current. In the case of mitochondrial membrane potential, for example, the outward flow of protons generated by respiration is mainly balanced by a back-diffusion of protons so that a potential of some −200 mV is set (Mitchell 1968; Nicholls 1974). Both for the inner mitochondrial membrane (Nicholls 1974) and for the membrane of photosynthetic bacteria (Clark et al. 1983), the proton permeability is non-ohmic and only becomes significant as the potential approaches its steady-state value. In Lettré cells the magnitude of the potential does not seem to depend on the diffusion of Cl^-, SO_4^{2-}, K^+, Na^+, Mg^{2+} or Ca^{2+} whereas organic anions such as lactate and bicarbonate, and to a limited extent protons, do affect membrane potential (Bashford and Pasternak 1984). We propose that it is fluxes of these latter ions at potentials around −60 mV which balance the electrical current through the Na^+-pump. In essence, the scheme depicted in Fig. 4 separates Na^+-pump-dependent acid extrusion into separate H^+ and organic anion pathways and provides cells with an effective method for excreting some of their metabolically produced acid. The relative contributions of the Na^+-pump-dependent and other pathways to acid excretion remains to be assessed. It should be stressed that the scheme presented in Fig. 4 is the simplest consistent with our observations of cation movements across the Lettré cell membrane. The cells may also possess electroneutral anion exchange systems, e.g. chloride-bicarbonate exchanges (Knauf et al. 1983; Thomas 1984). While such activities are important for maintenance both of an osmotic steady-state so far as anions are concerned and of cytoplasmic pH (Hoffman 1982), they are of only minor importance for the regulation of a membrane potential that is set by cation pumping and limited by anion leaks. Lettré cells therefore fit into a general scheme for the setting of membrane potential as indicated in Table 1; human neutrophils appear to behave similarly (Kuroki et al. 1981, 1982; Bashford and Pasternak 1985). A mechanism for generating plasma membrane potential that is in effect a H^+-pump may be of relevance to the acidification of endosomes (Galloway et al. 1983; Hopkins 1984; Yamashiro et al 1983) also. The importance of the Na^+-pump for transducing surface events into intracellular metabolism in the closely-related Ehrlich ascites tumour cell has long been stressed (Racker 1983a, b). In the light of data linking the expression of certain oncogenes to the presence of surface receptors (Downward et al. 1984) that may function as Na^+:H^+ exchangers (Moolenaar et al. 1982), it is clearly of interest to examine in what other cells membrane potential is set by Na^+-pump-dependent H^+ extrusion.

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References

Bashford CL, Pasternak CA (1984) Plasma membrane potential of Lettré cells does not depend on cation gradients but on pumps. J Membr Biol 79:275–284

Bashford CL, Pasternak CA (1985) Plasma membrane potential of neutrophils generated by the Na^+ pump. Biochim Biophys Acta, in the press

Bashford CL, Casey RP, Radda GK, Ritchie GA (1976) Energy-coupling in adrenal chromaffin granules. Neuroscience 1:399–412

Bashford CL, Alder G, Micklem KJ, Pasternak CA (1983) A novel method for measuring intracellular pH and potassium concentration. Biosci Rep 3:631–642

Bashford CL, Alder GM, Gray MA, Micklem KJ, Taylor CC, Turek PJ, Pasternak CA (1985a) Oxonol dyes as monitors of membrane potential: The effect of viruses and toxins on the plasma membrane potential of animal cells in monolayer culture and in suspension. J Cell Physiol 123, 326–336

Bashford CL, Micklem KJ, Pasternak CA (1985b) Sequential onset of permeability changes in mouse ascites cells induced by Sendai virus. Biochim Biophys Acta 814, 247–255

Clark AJ, Cotton NPJ, Jackson JB (1983) The influence of the ionic conductance on the relation between electron transport and proton-motive force in intact cells of *Rhodopseudomonas capsulata.* Eur J Biochem 130:575–580

DeCoursey TE, Chandy KG, Gupta S, Cahalan MD (1984) Voltage-gated K^+ channels in human *T* lymphocytes: A role in mitogenesis? Nature 307:465–468

Downward J, Yarden Y, Mayes E, Scrace G, Totty N, Stockwell P, Ullrich A, Schlessinger J, Waterfield MD (1984) Close similarity of epidermal growth factor receptor and *v-erb-B* oncogene protein sequences. Nature 307:521–527

Felber SM, Brand MD (1982) Factors determining the plasma membrane potential of lymphocytes. Biochem J 204:577–585

Galloway CJ, Dean GE, Marsh M, Rudnick G, Mellman I (1983) Acidification of macrophages and fibroblast endocytotic vesicles in vitro. Proc Natl Acad Sci USA 80:3334–3338

Geck P, Pietrzyk C, Heinz E, Pfeiffer B (1978) Is there an active proton pump in Ehrlich cells. In: Öbrink KJ, Flenström G (eds) Proc Symp Gastric Ion Transport, Uppsala 1977. Acta Physiol Scand Special Suppl, pp 363–372

Geck P, Pietrzyk C, Burckhardt B-C, Pfeiffer B, Heinz E (1980) Electrically silent cotransport of Na^+, K^+ and Cl^- in Ehrlich cells. Biochim Biophys Acta 600:432–447

Harris EJ, Pressman BC (1967) Obligate cation exchanges in red cells. Nature 216:918–920

Heinz A, Sachs G, Schafer JA (1981) Evidence for activation of an active electrogenic proton pump in Ehrlich ascites tumour cells curing glycolysis. J Membr Biol 61:143–153

Henderson PJF, McGivan JD, Chappel JB (1969) The action of certain antibiotics on mitochondrial, erythrocyte and artificial phospholipid membranes. The role of induced proton permeability. Biochem J 111:521–535

Hoffman EK (1982) Anion exchange and anion-cation cotransport systems in mammalian cells. Philos Trans R Soc Lond B 299:519–535

Hoffman EK, Simonsen LO, Sjoholm C (1979) Membrane potential, chloride exchange, and chloride conductance in Ehrlich mouse ascites tumour cells. J Physiol 296:61–84

Hoffman JF, Laris PC (1974) Determination of membrane potentials in human and *Amphiuma* red blood cells by means of a fluorescence probe. J Physiol 239:519–552

Hopkins CR (1984) The importance of the endosome in intracellular traffic. Nature 304:684–685

Impraim CC, Micklem KJ, Pasternak CA (1979) Calcium, cells and virus: alterations caused by paramyxoviruses. Biochem Pharmacol 28:1963–1969

Junge W, Auslander W, McGear AJ, Runge T (1979) The buffering capacity of the internal phase of the thylakoids and the magnitude of the pH changes inside under flashing light. Biochim Biophys Acta 546:121–141

Knauf PA, Law F-Y, Marchant PJ (1983) Relationship of net chloride flow across the human erythrocyte membrane to the anion exchange mechanism. J Gen Physiol 81:95–126

Kuroki M, Satoh H, Kamo N, Kobatake Y (1981) Contribution to the membrane potential of the electrogenic Na^+, K^+-pump in guinea pig polymorphonuclear leukocytes. FEBS Lett 123:177–180

Kuroki M, Kamo N, Kobatake Y, Okimasu E, Utsumi K (1982) Measurement of membrane potential in polymorphonuclear leukocytes and its changes during surface stimulation. Biochim Biophys Acta 693:326–334

Lew VL, Ferreira HG, Moura (1979) The behaviour of transporting epithelial cells. 1. Computer analysis of a basic model. Proc R Soc Lond B 206:53–83

Matteson DR, Deutsch C (1984) K channels in T lymphocytes: A patch clamp study using monoclonal antibody adhesion. Nature 307:468–471

Mitchell P (1968) Chemiosmotic coupling and energy transduction. Glynn Research, Bodmin, UK

Moolenaar WH, Yarden Y, de Laat SW, Schlessinger J (1982) Epidermal growth factor induces electrically silent Na^+ influx in human fibroblasts. J Biol Chem 257:8502–8506

Murer H, Hopfer U, Kinne R (1976) Sodium/proton antiport in brushborder membrane vesicles isolated from rat small intestine and kidney. Biochem J 154:597–604

Nicholls DG (1974) The influence of respiration and ATP hydrolysis on the proton-electrochemical gradient across the inner membrane of rat liver mitochondria as determined by ion distribution. Eur J Biochem 50:305–315

Ohkuma S, Moriyama Y, Takano T (1982) Identification and characterization of a proton pump on lysosomes by fluorescein isothiocyanate-dextran fluorescence. Proc Natl Acad Sci USA 79:2758–2762

Pietrzyk C, Geck P, Heinz E (1978) Regulation of the electrogenic ($Na^+ + K^+$)-pump of Ehrlich cells by intracellular cation levels. Biochim Biophys Acta 513:89–98

Racker E (1983a) Resolution and reconstitution of biological pathways from 1919 to 1984. Fed Proc. 42:2899–2909

Racker E (1983b) Oncogenes, transforming growth factors and protein transport: A hypothesis. Biosci Rep 3:507–516

Rogers J, Hesketh TR, Smith GA, Metclafe JC (1983) Intracellular pH of stimulated thymocytes measured with a new fluorescent indicator. J Biol Chem 258:5994–5997

Sanders D, Hansen U-F, Slayman CL (1981) Role of the plasma membrane proton pump in pH regulation in nonanimal cells. Proc Natl Acad Sci USA 78:5903–5907

Shorofsky SR, Field M, Fozzard HA (1982) The cellular mechanism of active chloride secretion in vertebrate epithelia: studies in intestine and trachea. Philos Trans R Soc Lond B 299:567–607

Sims PJ, Waggoner AS, Wang CH, Hoffman JF (1974) Studies on the mechanism by which cyanine dyes measure membrane potential in red blood cells and phosphatidylcholine vesicles. Biochemistry 13:3315–3330

Skou JC, Norby JG (eds) (1979) Na, K-ATPase Structure and Kinetics. Academic Press, New York

Thomas RC (1972) Electrogenic sodium pump in nerve and muscle cells. Physiol Rev 52:563–594

Thomas RC (1984) Experimental displacement of intracellular pH and the mechanism of its subsequent recovery. J Physiol 354:3P–22P

Wikström M (1984) Pumping protons from the mitochondrial matrix by cytochrome oxidase. Nature 308:558–560

Williams JA (1970) Origin of transmembrane potential in nonexcitable cells. J Theor Biol 28:287–296

Yamashiro DJ, Fluss SR, Maxfield FR (1983) Acidification of endocytic vesicles by an ATP-dependent pump. J Cell Biol 97:929–934