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A SIMPLE THEORETICAL MODEL OF STEADY-STATE ELECTROGENIC TRANSFER OF REACTIVE SPECIES ACROSS MEMBRANES

THE CASE OF *CHARA CORALLINA*

A. JENARD, H.D. HURWITZ and F. HOMBLE

Laboratoire de Thermodynamique Electrochimique, Faculté des Sciences, Université Libre de Bruxelles, C.P. 160, Av. F.D. Roosevelt, 50, B-1050 Bruxelles, Belgium

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Transfer across membranes is treated for simultaneous active pumping and passive flow of the main ionic constituents of plant cells. The chemical equilibrium conditions for H_2CO_3 and H_2O dissociation prevailing in the external and internal solutions are taken into account. Our model assumes steady-state conditions, zero electrical current, electroneutrality inside the membrane and, in both solutions, finite space charge densities in two diffuse regions in contact with both sides of the membrane. A computer program has been developed for the integration of a set of flux equations. The results are displayed in terms of ionic flux parameters, each proportional to the ionic pump flow and to the reciprocal of the passive ionic mobility. The discussion emphasizes the role of anion inward pumping and of non-diffusible anions. The cationic pumps serve to establish different ionic balances in the cell. Transmembrane (bulk to bulk) proton pumping affects essentially the pH regulation in the cell.

1. Introduction

For many years, the value of the resting membrane potential in plant cells was derived from ionic passive diffusion. For the purpose of quantitative estimation, the validity of the Goldman equation [1] was accepted, though mainly in the case of simplified systems with a small number of ionic species. In the Goldman treatment, the assumption of separate zero electric current conditions for both active and passive ion transfers dissociates, in fact, on one side, the creation of ionic composition differences between the external and internal media of the cell from, on the other side, the creation of a potential difference. As a consequence, the generally used Goldman equation takes the form of the expression giving the passive junction potential with the constant field hypothesis.

Much experimental evidence has proved the inadequacy of this method [2,3].

At the present time, we acknowledge the fact that differences between experimental results and theoretical predictions based on the Goldman equation bear evidence as to the existence of the electrogenic processes across the membrane, and therefore to the existence of nonvanishing total active current and total passive current, in which case, however, the problem remains of determining the ionic species which are actively transferred. Some investigators [4–8], in considering the strong external and/or internal pH dependence of both membrane potential and conductance, have tried to assess the role of a proton

pump. Such a model is particularly attractive, since a perfect selective proton transfer mechanism is easily conceivable [9]. The sign of the resting membrane potential, and other experimental results [3,10], seem perfectly consistent with the occurrence of an active proton efflux displaying analogous properties with the sodium-potassium exchange mechanism proposed by Rapoport [11]. However, the impossibility of performing direct proton-permeability measurements by the use of tritium in radiotracer methods might notably weaken one's confidence in this model.

The present approach has for its origin the following consideration, especially obvious in the case of giant cells of aquatic plants, such as Characeae.

A permanent qualitative and/or quantitative difference in the ionic composition (and pH) between a plant cell and the surrounding medium must be constantly maintained to counteract the passive diffusion of all the constituents, the only exception being those species causing Donnan equilibrium. We avoid therefore in this treatment, a priori, separating diffusion and ion pumping, both appearing at once as antagonistic and complementary processes.

We keep this theoretical overall investigation of the stationary state in the living cell system at the formal level; thus, no provision is made for specific molecular models responsible for the membrane ionic permeability properties, either passive or active.

2. Definition of the stationary state

The system considered in the present treatment consists of a single compartment of finite volume in contact, across a living membrane with an external medium of fixed composition and unlimited extension. We will eventually treat here as a membrane a much more complex system than a lipidic bilayer with protein inclusions; the whole layer which lies between the vacuole of an aquatic plant cell and the aqueous solution in which it lives will also be treated globally as a single living membrane.

The following consequences may be drawn from the above considerations:

(a) No passive transfer can occur into or out of a finite-volume compartment unless an active pump acts in the reverse direction, creating and maintaining permanently the electrochemical potential difference which drives the passive transfer.

(b) An external medium of infinite extension can play the role of a source or a sink for any chemical species. This allows us to treat the production of any substance or its presence inside the cell as an inward active transfer from an eventually vanishing external concentration and, reciprocally, the consumption of any substance as an outward active flux.

3. Model specification

The following assumptions are supposed to be valid:

(a) Continuity of the electrical potential from the bulk of the surrounding medium to the center of the cell. This means that there is no Donnan equilibrium across sharp boundaries between the interior of the membrane and both inner and outer media of the cell, and that consequently there is no concentration discontinuity for any ionic species.

(b) Continuity of the dielectric displacement: thus there is no true surface charge at the interface (dissociated functional group or strongly adsorbed ions).

(c) Electroneutrality inside the membrane: this amounts to the constant field hypothesis for the membrane interior.

(d) Finite space charge densities occur in two diffuse regions in contact with both sides of the membrane. Their presence results from the action of the specific pumpings of ionic species which are not totally neutralized by the retrodiffusion of the pumped species. The thickness of these disturbed (non-homogeneous) regions is supposed to be small in comparison with the cell dimensions, and they are assumed to be at equilibrium with their respective bulk media.

(e) The Boltzmann distributions of the ions in the disturbed regions may be linearized around the bulk potentials.

(f) Transversally, the membrane is homogeneous. This accords with a one-dimensional x coordinate system perpendicular to the membrane.

In principle, an increased sophistication of the model, resulting from the assumption of, e.g., sets of ion conductances of separate specific channels, spatial distinction between tonoplast and plasmalemmal membranes, etc., should not affect the overall validity of the phenomenological relations here derived. The enhanced complexity of the model might resolve the transmembrane phenomenological mobilities into several factors. These factors remain implicit parameters of the phenomenological mobilities on the present quasi-thermodynamic level of approach.

The existence of a nonvanishing effective electric surface charge on the membrane may affect more seriously the validity of the treatment. We are compelled therefore to assume that the potential, at the outer Helmholtz plane, which is induced by the effective surface charges, is screened to a major extent by counterion-ionic site pairing in the Nernst layer.

We endeavour to consider more explicitly these effects in a following publication.

4. Principle of the treatment

We have to consider three classes of transferable chemical species:

(1) Species which are neither actively transferred by themselves, nor involved in chemical reactions (at equilibrium) with others which would be themselves actively transferred. The condition of stationarity and the finiteness of the cell volume impose the equilibrium distribution for these species.

(2) Species which are actively transferred and not involved in chemical equilibrium. The balance of fluxes is expressed by:

$$J_i^{\mathrm{A}} + J_i^{\mathrm{P}} = 0$$

where J_i is the flux of species i, and superscripts A and P denote active and passive, respectively.

The two classes so far defined will be called unreactive species. Our calculations involve up to seven ionic species, three monovalent anions (Cl^-, X^- ...), two monovalent cations (K^+, Na^+) and two divalent cations (Mg^{2+} and Ca^{2+}).

(3) Species involved in at least one chemical equilibrium (reactive species). We assume that the transferred species do not interact inside the membrane (independent channels). However, their flux balance must take into account the chemical reactions in both lateral phases. We handle here only two types of equilibria: the water dissociation and the first dissociation of the carbonic acid.

The three flux balances of the five chemical species which are interconnected by the conditions for the equilibria are as follows:

$$J^{\mathrm{A}}_{\mathrm{H}^+} + J^{\mathrm{P}}_{\mathrm{H}^+} + J^{\mathrm{A}}_{\mathrm{H_2O}} + J^{\mathrm{P}}_{\mathrm{H_2O}} + J^{\mathrm{A}}_{\mathrm{H_2CO_3}} + J^{\mathrm{P}}_{\mathrm{H_2CO_3}} = 0 \tag{1a}$$

$$J^{\mathrm{A}}_{\mathrm{OH}^-} + J^{\mathrm{P}}_{\mathrm{OH}^-} + J^{\mathrm{A}}_{\mathrm{H_2O}} + J^{\mathrm{P}}_{\mathrm{H_2O}} = 0 \tag{1b}$$

$$J^{\mathrm{A}}_{\mathrm{H_2CO_3}} + J^{\mathrm{P}}_{\mathrm{H_2CO_3}} + J^{\mathrm{A}}_{\mathrm{HCO_3^-}} + J^{\mathrm{P}}_{\mathrm{HCO_3^-}} = 0 \tag{1c}$$

As a matter of fact, water molecules are never supposed to be actively transferred ($J^{\mathrm{A}}_{\mathrm{H_2O}} = 0$).

Integration of the Nernst-Planck equation, including all the model specifications, leads to

$$C_i^{\mathrm{II}} = \left(C_i^{\mathrm{I}} - \frac{J_i^{\mathrm{P}} l}{z_i u_i F\left(\psi_{\mathrm{s}}^{\mathrm{II}} - \psi_{\mathrm{s}}^{\mathrm{I}}\right)} \left\{ \exp\left(\frac{z_i \psi_{\mathrm{s}}^{\mathrm{II}} F}{RT} \right) - \exp\left(\frac{z_i \psi_{\mathrm{s}}^{\mathrm{I}} F}{RT} \right) \right\} \right) \exp\left(- \frac{z_i V_{\mathrm{m}} F}{RT} \right) \tag{2}$$

which reduces to the equilibrium condition when $J^{\mathrm{P}} = 0$.

In eq. 2, u_i, C_i and z_i are respectively the mobility, concentration and number of charge (with sign) of the ionic species, ψ_{s} the potential at the membrane surface, l the membrane thickness and V_{m} the transmembrane potential difference (from bulk to bulk). The potential is taken as zero in the bulk of the external medium. Superscripts I and II refer to the outer and inner compartment, respectively.

We observe that the factor $J_i l/u_i$ represents in this form a parametric quantity. The introduction of this parameter emphasizes the fact that the efficiency of a particular active ion flux in generating composition and potential differences is

accounted for not only by its magnitude but also by the ability of the species to flow back passively through the membrane. As illustrated by the fact that $J_i l/u_i RTC_i$ is dimensionless, this ability is proportional to the mobility in the membrane and to the reciprocal of the membrane thickness. In the following computation, $FL_i = (J_i l/u_i F)$ is indicated as the flux parameter (of dimension J mol l^{-1} C^{-1}).

It can be observed that the combination of eqs. 1 and 2 requires introducing into our computation a hypothesis as to the values of the ratios between the passive mobilities of H^+, OH^-, CO_3H^-, H_2CO_3 and H_2O.

The results reported in this paper were obtained with the assumption of a high proton mobility ($u_{H^+} = 10u_{OH^-} = 100u_{HCO_3^-}$).

Finally, the system of eqs. 1 and 2 will be completed with the expressions of the chemical equilibria in both solutions and with expressions resulting from model specification (a) which relates the surface potentials ψ_s^I and ψ_s^{II} to V_m, to the ionic strength of both inside and outside ionic solutions and to the dielectric constants of the solutions and inside the membrane.

We wish to stress the point that a charged species whose analytical concentration vanishes in the unlimited outer medium but is finite in the inner solution will add a Donnan contribution to the transmembrane steady-state potential. The presence of this ion in the cell can be formally treated as arising from both a very small virtual active inward flux and a very small passive outward flux, thus characterized by a very low mobility in the membrane. These two factors lead to a finite value of the flux parameter FL_i of the ion, although the particle is, in reality, not actively transferred across the membrane. It is not a paradoxical result because the virtual active flux has been taken for the chemical flux which produces the particle in the cell.

5. Strategy of computation

A first program requiring a set of C_i^I and of FL_i solves the system of equations by means of three nested iterative procedures; it yields a set of C_i^{II} and the corresponding value of V_m.

This first program is introduced into a second one, where it serves as the kernel of an extension of the Newton-Raphson method. In this way, starting from a first tentative set of FL_i, the calculation uses these parameters as adjustable variables which are compelled to satisfy selected sets of C_i^I and of C_i^{II}, as well as the selected value of V_m.

All computations were made on a series 1000 Hewlett-Packard computer, with an E processor and a 96 kbyte central memory.

6. Presentation of results

Our data have been calculated with a membrane thickness of 10^{-8} m, and a dielectric constant of 80 for both the internal and external media and of 6 inside the membrane. Test calculations were performed using other reasonable values of ϵ inside the membrane. They indicate that a change of ϵ by a factor of two has a negligible influence (< 2–3%) on the flux parameters or the internal concentrations. Besides the principal results presented here, many others were obtained which have allowed us to investigate the extent of their dependence on the conditions selected for the computation. References to these results will be made incidentally in the text.

The results displayed in figs. 1 and 2 were obtained for 'standard external conditions' which are close to real living conditions of fresh water plants. These are as follows:

Total ionic concentration (normality) = YTE = 0.002 mol l^{-1}

Total carbonate concentration: $[HCO_3^-] + [H_2CO_3] = SCE = 0.001$ mol l^{-1}

Equivalent fraction of monovalent cations: $[M^+]/([M^+] + 2[M^{2+}]) = FAE = 0.5$

pH = PHE = 7

Sodium ion fraction = $[Na^+]/([Na^+] + [K^+]) = FNAE = 0.9$

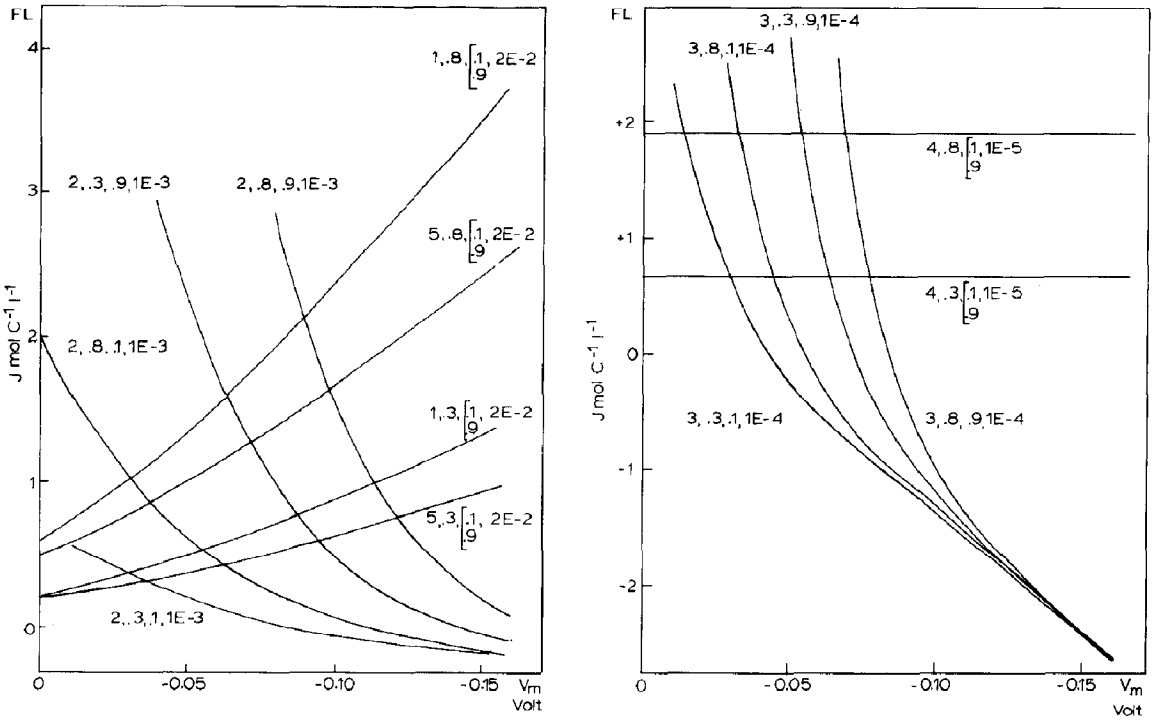

Fig. 1. Flux parameters needed to obtain a variable value of V_m at standard external conditions and fixed values of PHI = 7 and FXI = 0.6. Data on each curve are successively: the index of the flux parameter, YTI, FAI and the scaling factor for the ordinates.

Calcium ion fraction = $[Ca^{2+}]/([Ca^{2+}] + [Mg^{2+}])$ = FCAE = 0.8.

In the presentation of results, the same notations ending with letter I instead of E concern the internal medium.

The following nomenclature ending either with E or I is also used:

$$FX = \sum_i [X_i] / \left(\sum_i [X_i] + [HCO_3^-] \right)$$

Basically, the calculations involve a set of five

Table 1

Flux parameters vs. the total external concentration YTE, and ratios of separate flux parameters of the cations of the same charge

Ratio of Na^+ to K^+: external = 9, internal = 0.11. Ratio of Ca^{2+} to Mg^{2+}: external = 4, internal = 0.01. $i = 1$, unreactive anions; $i = 2$, monovalent cations; $i = 3$, divalent cations; $i = 4$, H^+; $i = 5$, HCO_3^-; YTE, variable; FAE = 0.5; PHE = 7; SCE = YTE/2; $V_m = -0.140$ V; YTI = 0.3 mol l^{-1}; FAI = 0.9; PHI = 7; FXI = 0.6.

YTE (mol l^{-1})	FL_1 (10^{-1})	FL_2 (10^{-3})	FL_3 (10^{-3})	FL_4 (10^{-5})	FL_5 (10^{-1})	FL (Na)/ FL(K)	FL(Ca)/ FL(Mg)
10^{-4}	0.2145	0.3288	−0.0365	0.7137	0.1502	0.068	4.23
2×10^{-4}	0.2254	0.2600	−0.0481	0.7140	0.1575	0.025	4.11
5×10^{-4}	0.2363	0.1853	−0.0796	0.7137	0.1647	−0.107	4.04
10^{-3}	0.2421	0.1214	−0.1270	0.7126	0.1686	−0.335	4.02
2×10^{-3}	0.2465	0.0275	−0.2149	0.7104	0.1715	−0.829	4.01
5×10^{-3}	0.2505	−0.2126	−0.4605	0.7032	0.1740	−2.74	4.01

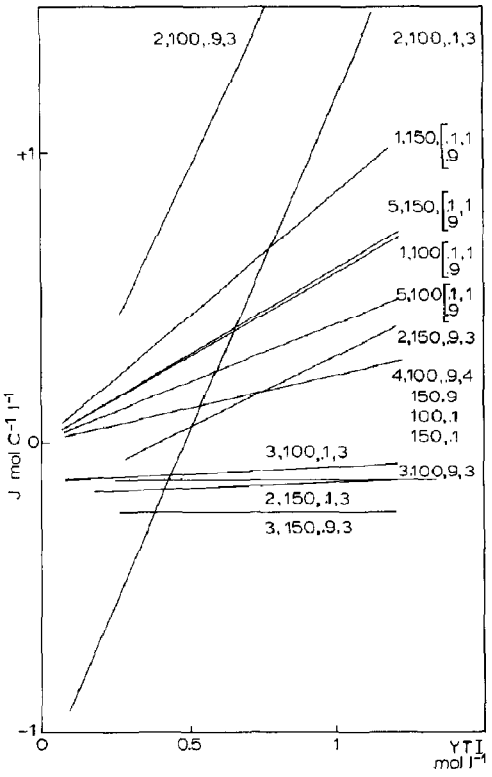

Fig. 2. Flux parameters needed to obtain a variable value of YTI at standard external conditions and fixed PHI = 7 and FXI = 0.6. Data on each curve are successively: the index of the flux parameter, V_m, FAI and $-\log_{10}$ (scaling factor for the ordinates).

flux parameters corresponding to the species indicated in table 1. No discrimination is made at the start between unreactive species bearing the same charge.

As for the reactive species, the couple of particles H^+ and HCO_3^- represents one of the possible choices among H^+, OH^-, HCO_3^- and H_2CO_3 whose concentrations are related through chemical equilibria. An abundant number of studies concerning proton pumping has led us to choose the variable $[H^+]$. Moreover, our first computational attempts emphasize clearly the necessity of an inward anion pumping. We have determined (figs. 1 and 2) the flux parameter values that are required to fulfill simultaneously the standard external conditions and five internal conditions. The latter had been changed within a rather large range in order to point out how the internal cell conditions are dependent upon the flux parameters. Hence, the choice of internal variable range is as follows:

$$PHI = 7,\ 0 \leqslant FAI \leqslant 1,\ -160\ mV \leqslant V_m \leqslant 0,$$

$$1 \leqslant YTI \leqslant 1.2,\ 0 \leqslant FXI \leqslant 1$$

It was not possible to obtain results for all combinations of variables, in particular, the computa-

Table 3

Flux parameter vs. external pH

YTE = 0.001, SCE = YTE/2, FAE = 0.5, $V_m = -0.140$ V, YTI = 0.3, FXI = 0.6, FAI = 0.9, PHI = 7.

PHE	FL_i (J mol C^{-1} l^{-1})				
	FL_1 (10^{-1})	FL_2 (10^{-3})	FL_3 (10^{-3})	FL_4 (10^{-5})	FL_5 (10^{-1})
4	0.2420	0.1310	−0.1150	−1.1367	0.1684
5	0.2421	0.1224	−0.1258	0.5209	0.1685
6	0.2421	0.1215	−0.1269	0.6896	0.1685
7	0.2421	0.1215	−0.1270	0.7127	0.1685
8	0.2421	0.1214	−0.1270	0.7164	0.1686
9	0.2421	0.1214	−0.1270	0.7169	0.1685
10	0.2421	0.1214	−0.1270	0.7175	0.1686

Table 2

Internal variable vs. total external concentration at constant flux parameter

$FL_i = 0.2145\times10^{-1}$, 0.3288×10^{-3}, -0.3654×10^{-4}, 0.7137×10^{-5}, 0.1502×10^{-1}.

YTE (mol l^{-1})	V_m (V)	YTI (mol l^{-1})	FAI	FXI	PHI
0.0001	−0.140	0.300	0.9	0.60	7.00
0.00025	−0.121	0.324	0.65	0.60	7.03
0.0005	−0.105	0.360	0.43	0.60	7.08
0.001	−0.091	0.399	0.31	0.60	7.12
0.002	−0.080	0.439	0.24	0.60	7.16

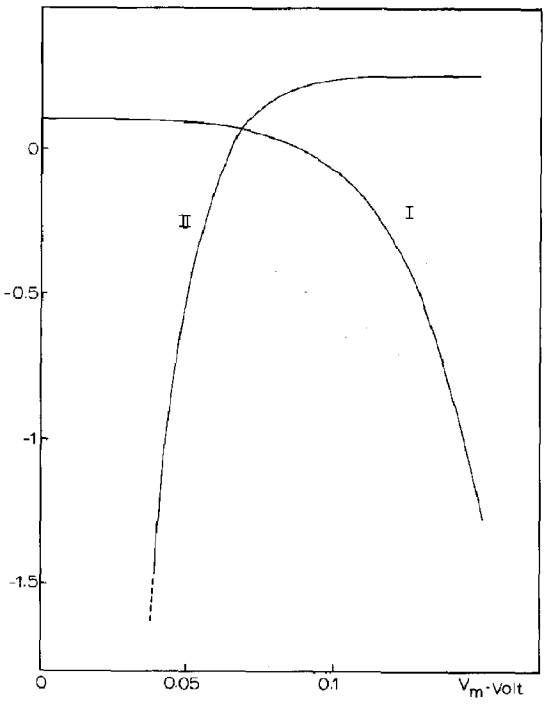

Fig. 3. Ratio of Na^+ to K^+ flux parameters (curve I) and of Mg^{2+} to Ca^{2+} flux parameters (curve II) as a function of the membrane potential V_m. Standard external conditions, YTI = 0.3 mol l^{-1}, FXI = 0.6, PHI = 7, FAI = 0.9, FNAI = 0.1, FCAI = 0.01.

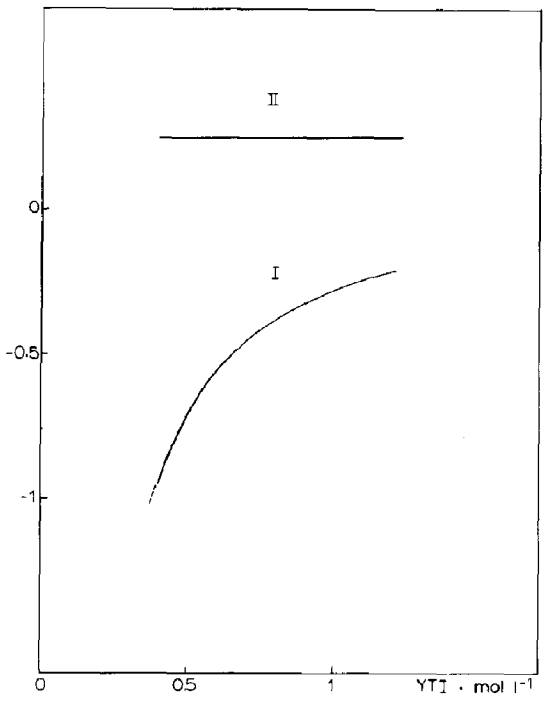

Fig. 4. Ratio of Na^+ to K^+ flux parameters (curve I) and of Mg^{2+} to Ca^{2+} flux parameters (curve II) as a function of the total internal ionic concentration YTI. Standard external conditions, $V_m = -0.150$ V, FXI = 0.6, pHI = 7, FAI = 0.9, FNAI = 0.1, FCAI = 0.01.

tion procedure fails to produce at the same time high absolute values of V_m (> 140 mV) and low values of YTI ($\leqslant$ 0.3 N). Therefore, realistic internal conditions are found at the border of the explored domain.

Some explorations were made in the domain of variable external conditions. They concern mainly external pH variations and external ionic strength changes. They are summarized in tables 1–3.

Finally, figs. 3 and 4 report results obtained when, in the situation of standard external conditions, a splitting of FL_2 and FL_3 is performed in order to reproduce the K^+/Na^+ and Mg^{2+}/Ca^{2+} internal balances.

7. Discussion

On the basis of the very few published studies about the cytoplasmic and vacuolar ionic content of freshwater algae [12–14] and of some results obtained in our laboratory [15], we infer that the following calculated cytoplasmic conditions correspond best to the real state of the cell; thus:

$V_m = -0.140$ to -0.150 V; YTI = 0.3 mol l^{-1}

FAI = 0.9 (net monovalent cation predominance)

PHI = 7; FNAI = 0.1; FCAI = 0.01 or even less

FXI remains in the range 0.5–1.0 when SCI is maintained below 0.15 mol l^{-1}. Furthermore, it is worth stressing the fact that a comparison of flux parameter values relevant to different chemical species is not tantamount to comparing pump rates, unless the ratios between the permeability coefficients are known (those coefficients are reduced here to their mobility factor inside the

membrane). * Consistent membrane potentials and internal conditions are generated if the flux parameters relevant to the anions (FL_1 and FL_5) are positive (inward flow) and are of one order of magnitude larger, at least, than all other parameters, as reflected by figs. 1 and 2. Such a result expresses the fact of a high inward anion pumping rate and/or of a low membrane permeability with regard to anions. Diffusible anion concentrations inside the cell, notably that of Cl^-, are of the same order of magnitude as that of diffusible monovalent cations [13]. The existence of actively transferred anions appears thus of prime importance for the generation of both the membrane potential and the overall internal ionic concentration.

Regarding the effects of the other pumps, their role, essentially, appears to fulfill the requirements of the internal ionic, particularly cationic, specific balance, meaning the respective proportion of Na^+ to K^+, of Mg^{2+} to Ca^{2+} and of monovalent to divalent cations. This acceptation of the ionic transfer process has been deduced in the following way.

Computed results not reported here [15] indicate that the combination of solely cationic pumps is unable to generate V_m values of more than a few millivolts, or of the correct sign, or with the internal medium more concentrated than the external one. Indeed, the cationic fluxes are prevailingly outward and will thus empty the cell. In contrast, inward pumping of anions only readily produces V_m values of about 100 mV with the correct sign and correct values of YTI. This time, however, the cation distribution in the cell happens to be unbalanced (the pH and the concentration of monovalent cations are both too low).

The addition of cation pumps able to restore the correct ion balance increases the absolute value of V_m, while YTI remains in the range of acceptable values as reported in the present paper.

Consequently, large relative variations of V_m and YTI are correlated with large relative variations of FL_1 and FL_5 (fig. 1a) and with larger variations of FL_2, to the point of changing their sign (figs. 1b and 2).

However, the variations of FL_1 and FL_5 appear as causal whereas the changes of FL_2 and FL_3 are generated by the necessity of preserving the cationic balance. Moreover, the absolute values of FL_2 and FL_3 remain in a range of two orders of magnitude lower than FL_1 and FL_5.

Both the monovalent and divalent cation active flux parameters present the common feature of tending toward negative values at so-called 'real' values of V_m and YTI, thus nearly corresponding to the real living situation (cf. fig. 1a, curves V–VIII and fig. 1b, curves I–IV). Moreover, in the same range of V_m, the active efflux of divalent cations is no longer correlated to the values of YTI nor FAI (fig. 2, curves VII, VIII, IX). Our tests have shown that this behaviour is quite general and independent of the composition of the external medium (YTE, PHE). The cationic distribution FAI appears, in these states, to be subordinated to its sole dependent on the Na^+, K^+ pumps.

The division of the cation flux parameters between the cationic species for real values of V_m and YTI leads simultaneously to a ratio of Na^+ to K^+ flux parameter lower than -1 (figs. 3 and 4, curves I) and to a fixed ratio of divalent cations actively expelled (figs. 3 and 4, curves II).

This property combines with the fact that the total monovalent cation active flux parameter FL_2 is negative and thus confirms, if $|J_{K^+}| \simeq |J_{Na^+}|$, that the cell membrane, in its resting state, displays a much larger passive permeability for K^+ than for Na^+ [13].

As for the ratio of divalent cation effluxes, it is worth noting that it becomes identical to the concentration ratio of Mg^{2+} and Ca^{2+} in the external medium. Experimental proofs are lacking to confirm this quite surprising result. As a matter of fact, we remain quite ignorant as to the influence of the composition of the external medium on the cytoplasmic free Ca^{2+} and free Mg^{2+} concentration [16,17]. Among cytoplasmic properties that are well known are to be found the values of the K^+/Na^+ concentration ratio, which is much larger than unity, and of the Mg^{2+}/Ca^{2+} concentration

* Regarding this problem, let us mention that concentration factors should be included in flux parameters that are related to models assuming, for instance, concentration discontinuities at the membrane/solution interphase, hence being more sophisticated than our model.

ratio, which is even larger. In addition, we know that green plants need Mg^{2+} in order to synthesize chlorophyll. Some properties of the Ca^{2+}, Mg^{2+}, Na^+ and K^+ pumps, once they are interrelated in the real system, can be inferred from the following results: (a) considerable variations of both the monovalent and divalent cation global flux parameters are observed as a function of changes of YTE (table 1); (b) at a constant ratio of their external concentrations, the Ca^{2+}/Mg^{2+} flux parameter ratio is practically constant; (c) the ratio of the Na^+ and K^+ flux parameters changes considerably under the same conditions. These results suggest that the Ca^{2+}/Mg^{2+} expelling pumps work at a rate which is dependent on the external conditions, and that the pumping of Na^+ and K^+ adjusts to the external medium in a delicate and complex way, in order to maintain the internal Na^+/K^+ balance. This kind of regulation involves two types of effects: the pumping rate, of course, but also the passive permeability ratio of the membrane to both monovalent ions. We wish finally to focus attention on the problem of the proton flux parameter. Calculations which are not reported in more detail here indicate that variations of FL_4 alone around any fixed condition reported in tables 1–3 and figs. 1–4 rapidly drive the internal pH to unreasonable values without appreciable change of the other internal variables [15].

If now, all ion pumping being at work, we keep constant all internal and external variables, again under any condition reported in figs. 1–4 and tables 1–3, but change considerably the ratios of the reactive particle mobilities, we observe that this change does not affect appreciably the flux parameter values, except for the proton whose flux but not the flux parameter is kept quasi-constant. This seems to reflect the fact of lower H^+ concentration (and of course OH^-) than the concentration of other species in both internal and external medium.

Considering this result and upon examination of fig. 1b (curves V and VI) it also appears clearly that, at constant pH, there are no marked effects of the internal variable on the proton flux requirement, except in the case of the total internal ionic strength variable (fig. 2, curve X). Furthermore, table 1 displays the values of the flux parameters which are needed to keep the internal variable constant while the external composition is changed quantitatively. Here too, it is shown that the proton flux is only weakly affected. With variable external pH (table 3), the proton flux changes appreciably only in external acid solutions.

It is reasonable to conclude from all these facts that the role of the proton transfer in the course of the ionic transfer process between the external and internal phases is restricted to the cytoplasmic and vacuolar pH regulation. The proton transfer exerts a minor effect on the other internal parameters. Its action is controlled by the absolute (active) protonic flux J_{H^+} irrespective of its passive mobility u_{H^+} across the membrane.

In *Chara,* the existence of H^+/Cl^- cotransport is well established [11,19–26]. However, it has not yet been possible to prove whether the proton transport produces only the energy for the active Cl^- transport or both the energy for the transport and the resting membrane potential. Our approach helps to elucidate this problem. From the data presented above, it seems that the membrane potential results essentially from the active anionic transport (Cl^- and HCO_3^- at alkaline pH) which itself might be driven by a proton-motive force. The weak selectivity displayed by the anionic pumps might account for the small differences of electrophysiological effects between the anionic species.

8. Conclusions

At the present state of our investigations, we believe that we can draw the following conclusions:

(a) Under physiological conditions all ions seem to be more or less pumped. Two different hypotheses might be suggested to explain this: either every ion has its own specific pump mechanism, or alternatively there is a small number of pumps, but they are not limited to a single ion specificity.

(b) Anions are essential: anion inward pumping and/or Donnan effects fix the sign and the order of magnitude of the cell potential and also

the higher vacuolar ionic concentration.

(c) Global proton pumping, from bulk to bulk, acts primarily as an internal pH regulator. Experimental evidence of the pH influence on (1) vacuolar composition, (2) resting potential and (3) isotopic flux, must not be confused with evidence of proton pumping, but rather should be considered as indicating that ion pump activities are pH dependent, which is the general rule for enzyme activities. Nevertheless, the quasi-independence of the internal pH to the external one evidently proves the occurrence of proton pumping. A tempting hypothesis consists in assuming that the active pumping of protons, combined with a very high passive permeability, constitutes the primary energetic driver of other ion pumpings, which derive their energy from the local passive retrodiffusion of protons. What is known, for instance, about the first stages of photosynthesis in the thylakoid membrane contributes some arguments for this assumption [3]. In this case, the proton circulation would not be apparent at the level of the bulk media. Only its indirect influence on other ion pumpings would be apparent in our results, because this proton circulation is at the level of the internal mechanism of the pump.

(d) The cation balances (M^+/M^{2+}, Na^+/K^+, Ca^{2+}/Mg^{2+}) probably depend on various cation pumps.

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