J. Membrane Biol. 124, 255–263 (1991)

The Journal of
Membrane Biology

Ion Fluxes in *Acetabularia:* Vesicular Shuttle

H. Mummert* and D. Gradmann**
Institut für Biologie I der Universität, Auf der Morgenstelle 1, D-7400 Tübingen, Germany

Summary. Ion flux relations in the unicellular marine alga *Acetabularia* have been investigated by uptake and washout kinetics of radioactive tracers ($^{22}Na^+$, $^{42}K^+$, $^{36}Cl^-$ and $^{86}Rb^+$) in normal cells and in cell segments with altered compartmentation (depleted of vacuole or of cytoplasm). Some flux experiments were supplemented by simultaneous electrophysiological recordings. The main results and conclusions about the steady-state relations are: the plasmalemma is the dominating barrier for translocation of K^+ with influx and efflux of about 100 nmol · m^{-2} · sec^{-1}. K^+ passes three- to sevenfold more easily than Rb^+ does. Under normal conditions, Cl^- (the substrate of the electrogenic pump, which dominates the electrical properties of the plasmalemma in the resting state) shows two efflux components of about 17 and 2 μmol · m^{-2} · sec^{-1}, and a cytoplasmic as well as vacuolar $[Cl^-]$ of about 420 mM ($[Cl^-]_o$ = 529 mM). At 4°C, when the pump is inhibited, both influx and efflux, as well as the cellular $[Cl^-]$, are significantly reduced. Na^+ ($[Na^+]_i$: about 70 mM, $[Na^+]_o$: 461 mM), which is of minor electrophysiological relevance compared to K^+, exhibits rapid and virtually temperature-insensitive (electroneutral) exchange (two components with about 2 and 0.2 μmol · m^{-2} · sec^{-1} for influx and efflux). Some results with Na^+ and Cl^- are inconsistent with conventional (noncyclic) compartmentation models: (i) equilibration of the vacuole (with the external medium) can be faster than equilibration of the cytoplasm, (ii) absurd concentration values result when calculated by conventional compartmental analysis, and (iii) large amounts of ions can be released from the cell without changes in the electrical potential of the cytoplasm. These observations can be explained by the particular compartmentation of normal *Acetabularia* cells (as known by electron micrographs) with about 1 part cytoplasm, 5 parts central vacuole, and 5 parts vacuolar vesicles. These vesicles communicate directly with the central vacuole, with the cytoplasm and with the external medium.

Key Words *Acetabularia* · compartmentation · flux analysis · ionic relations · vesicular transport

Introduction

The ion transport properties of the giant cells of the unicellular marine alga *Acetabularia* have provided several surprises, such as a powerful Cl^- ATPase (for review *see* Gradmann, 1989), which seems to be inhibited by green light (Gradmann, 1978), or very slow action potentials (some 100 sec, Mummert & Gradmann, 1991). Energy metabolism is apparently involved in these action potentials (Gradmann, 1976), which are accompanied by losses of large amounts of Cl^- (Gradmann, Wagner & Gläsel, 1973). Action potentials in *Acetabularia* seem to have a regulatory function for the osmotic relations (Mummert & Gradmann, 1976; Wendler, Zimmerman & Bentrup, 1983). Controversial opinions have been stated about a vesicular shunt between the vacuole and outside (Gradmann & Mummert, 1984; Wendler, Zimmerman & Bentrup, 1984). Part of this discussion referred to results (Mummert, 1979), which have not been published yet in regular papers. The essential data are given here within a wider context. These data comprise combinations of information which are only available from *Acetabularia* so far, i.e., nonsteady state flux measurements with simultaneous voltage recordings from individual cells. Another favorable circumstance is that measurements can be carried out not only on cells with natural compartmentation but also on cell segments with artificially simplified compartmentations. The entire ensemble of results strongly confirms the operation of a vesicular ion shuttle between outside and the vacuole in *Acetabularia*, a mechanism suggested long ago for Characean cells (MacRobbie, 1969). In addition, there is evidence for a vigorous, electroneutral Na^+/Na^+ exchange at the plasmalemma.

Materials and Methods

Cells and Media

Acetabularia acetabulum (formerly *A. mediterranea*) has been cultured as previously described (Gradmann, 1975). Young, approximately cylindrical cells of 15–35 mm in length and 0.2–0.4

* *Present address:* Edemannswisch, D-2240 Norderwöhrden, Germany.
** *Present address:* Pflanzenphysiologisches Institut der Universität, Untere Karspüle 2, D-3400 Göttingen, Germany.

mm in diameter were used; a typical cell had a volume of about 2 μl and a surface area of about 25 mm^2. At least three days before an experiment, the cells were equilibrated in artificial seawater (ASW) containing (in mM): 461 Na^+, 10 K^+, 53 Mg^{2+}, 10 Ca^{2+}, 529 Cl^-, 28 SO_4^{2-}, 2 HCO_3^-, 10 Tris/HCl, pH 8.0, under continuous white light (3.2 W · m^{-2}, Osram L 65W/30) at 21 ± 1°C. For low temperature experiments, all steps—beginning with three days of equilibration—have been performed in a cold (4°C) room. For labeling of the cells with radioisotopes, the concentration of no component of ASW was changed by >0.05%. The incubation media contained one of the tracer ion species with the following radioactivities (in 10^{12} Bq · m^{-3}): 5 $^{42}K^+$, 8–20 $^{86}Rb^+$, 0.8–2.3 $^{22}Na^+$, 0.8–2.3 $^{36}Cl^-$.

Since the electrogenic pump of *Acetabularia* is inhibited by temperatures <10°C, experiments have been carried out at normal room temperature (21 ± 1°C) and in the cold (4 ± 0.5°C) with the aim of possible assignment of specific effects to passive or active ion transport.

Compartments

The cytoplasm of *Acetabularia* cells (about 10% of the cell volume) can essentially be separated from the vacuole by centrifugation for 20 min at 600 × *g*. Thus, cytoplasmic cell segments (virtually without vacuoles) and vacuolar cell segments (with about 15% cytoplasm compared to normal cells, according to Goldfarb, Sanders & Gradmann, 1984*a*) are available after appropriate dissection. This separation has already been used successfully (Saddler, 1970; Freudling & Gradmann, 1979; Goldfarb et al., 1984*a*; Goldfarb, Sanders & Gradmann, 1984*b*). Whereas the cytoplasmic cell segments are very robust, the vacuolar ones are relatively tender. The integrity of the latter has been examined by the uptake of neutral red. Healthy vacuolar cell segments are turgescent and take up neutral red vigorously. Although uncentrifuged cells did not display significant differences in the electrical properties whether the vacuole was normal or stained with neutral red, the measurements here on vacuolar cell segments have been carried out with unstained preparations, because the proper state of these segments could also be examined reliably by the apparent turgor.

Preparations of isolated cell walls have been obtained by cutting normal cells on both ends and perfusing them with ASW. These empty cell walls have been tied off at both ends and incubated in radioactive media for three days for control purposes, just as normal cells.

Influx

Unidirectional influx of ions was recorded by the uptake kinetics of radioactive tracers into cells of known surface area. For this purpose, cells have been incubated for different times in radioactive media and washed in nonradioactive medium for about 100 sec. The washout kinetics below demonstrate that this time is long enough to elute >95% of the radioactivity from the cell wall (τ < 30 sec) and short enough not to release >2% of the radioactivity from the protoplast (τ > 400 sec).

Efflux

The experimental setup for efflux measurements consists of a slanted (about 10°) plexiglass baseplate with a groove to hold a cell. During a washout experiment, nonradioactive medium passed the cell in the groove and was collected dropwise as described previously (Mummert & Gradmann, 1976). The experimentally determined delay of about 5 sec between passage of the medium and collecting of the drops in a scintillation vial reflected the temporal resolution of the apparatus. This delay was taken into account for the kinetic analysis. The washout apparatus was supplemented for simultaneous electrophysiological recordings according to Gradmann (1975).

For determination of unidirectional effluxes, the release of radioactivity from the prelabeled (mostly equilibrated) cells was monitored by sampling of the washing medium in intervals of 15 sec or more. Scintillation fluid (Unisolve-1, Koch-Light Laboratories) was added to the samples, and β-radioactivity ($^{42}K^+$, $^{86}Rb^+$, $^{36}Cl^-$) was measured in a liquid scintillation counter. Corrections for quenching were done with internal standards (dispensible in the case of $^{86}Rb^+$ with its high decay energy of 17.8 MeV). For the determinations of γ-radioactivity (of $^{22}Na^+$), the following empirical procedure (which may be based on the Compton effect) turned out to work sufficiently: aqueous samples were collected in polyethylene scintillation vials (Packard), diluted (1 : 1) with Unisolve-1, and counted by a liquid scintillation counter (Packard 3385) at a window of 20–600 and 4% amplification, with a sufficient yield of about 60% and linearity from 0 to 10^7 Bq.

Backsummation of the radioactivity of the samples to the final radioactivity which remained in the cell after completing a washout procedure yields the washout kinetics of the cellular radioactivity. These washout kinetics, $A^*(t) = \Sigma\{A_{0i}^* \cdot \exp(-t/\tau_i)\}$, are the sum of n exponential components (current index i), with the amplitudes A_{0i}^* (radioactivity A_i^* at time zero of washout) and the corresponding time constants τ_i. The apparent exponential components have been identified here by usual (Cram, 1968) graphical means as illustrated in Fig. 5: in the semilogarithmic plot of $\ln(A^*(t))$ *versus* t the slowest component $A_{0n}^* \cdot \exp(-t/\tau_n)$ can be determined by the linear assymptote to the data at long times after beginning of the washout. Extrapolation of this line (dashed in Fig. 5) yields A_{0n}^* at time zero. The numerical differences between the (early) data and the extrapolated line are then plotted again semilogarithmically (inset *B* in Fig. 5) to yield the n–1st component by the corresponding procedure. Differences between this (second) linear approach and the (early) recorded data were treated the same way again to give the coefficients of the fastest component. With this empirical approach, one or two exponential components could be assessed for the cell interior.

Flux data are corrected for the decaying specific radioactivity S_j^* (in Bq · mol^{-1}) of the tracer j in the cell during the washout procedure and for the natural decay of the radioisotope j.

Kinetics of Vesicular Compartmentation

Analyzing the experimental data below by the standard model with three compartments (outside, cytoplasm, and vacuole) in series gave odd results. These problems are solved by assuming an additional vesicular compartment which exchanges ions with the vacuole, with the cytoplasm, and with the external medium (Fig. 1), these problems can be solved. Efflux kinetics of the resulting four-compartment model—no matter whether linear, branched, or cyclic—comprises three exponential components, which may explicitly be calculated with the model parameters. Since in our experiments only two intracellular components could

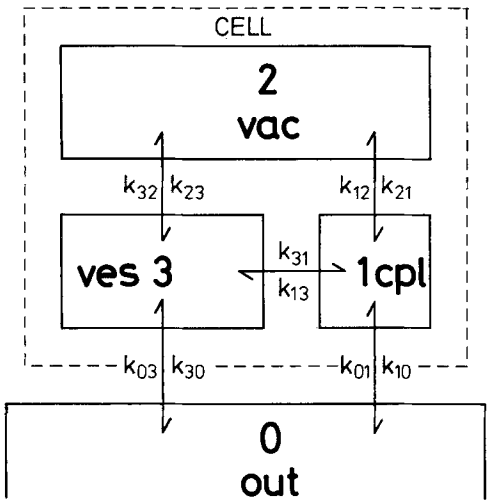

Fig. 1. Compartmentation and pathways for major ions in *Acetabularia;* model with definitions for calculations; *O, out:* outside; *1, cpl:* cytoplasm; *2, vac:* central vacuole; *3, ves:* vesicles.

be distinguished, a quantitative treatment of the explicit model is not appropriate here.

Results

Substitution of K^+ by Rb^+

In order to test to what extent Rb^+ can be used as a convenient substitute for investigations of K^+ relations, cells were incubated for up to 10 days in media in which the normal 10 mM K^+ was replaced by 10 to 100% (nonradioactive) Rb^+. This treatment did not significantly change the transplasmalemma voltage (V_m) and the response of V_m to light-on and -off (Fig. 2). Only the shoulder during the repolarization, which can be related to the Nernst equilibrium voltage of potassium, E_K, (Gradmann, 1975; Bertl & Gradmann, 1987) occurred at about 10–20 mV more positive V_m.

Cap formation was significantly inhibited (to about 30%) when the cells have been incubated for 30 days in media in which K^+ was substituted >60% by Rb^+ (Fig. 5 in Mummert, 1979). For radioactive labeling some cells have been incubated, in fact, in media with >6 mM Rb^+, but not longer than two weeks.

Influx

Uptake kinetics of $^{42}K^+$, $^{22}Na^+$ and $^{36}Cl^-$ have been determined at normal temperatures (21°C) and in the cold (4°C). The results from 21°C are illustrated by

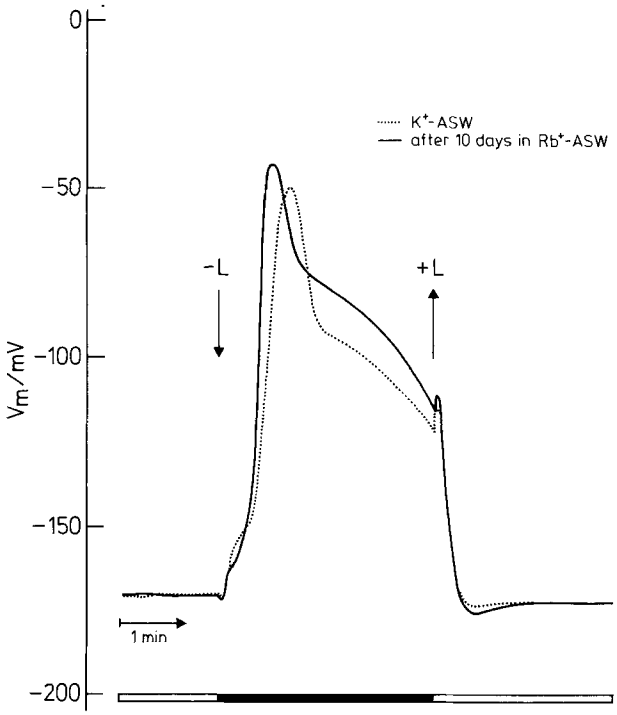

Fig. 2. Resting potential, light-off triggered action potential and light-on response during repolarization of two cells of the same culture, which were incubated for 10 days in normal artificial seawater with 10 mM K^+ (K^+ – ASW) or in Rb^+-ASW with 10 mM Rb^+ instead of K^+. Only significant difference: voltage range of slow repolarization more positive in Rb^+ – ASW.

Fig. 3 and compiled in the upper part of Table 1. Under normal conditions, the kinetics of the K^+ uptake is slow and not subdivided in several components, whereas the kinetics for the unidirectional uptake of $^{37}Cl^-$ and $^{22}Na^+$ are much faster with two clearly distinct components. For the uptake of $^{36}Cl^-$, actually, an apparent delay is indicated in the beginning by the sigmoidal kinetics. In Fig. 3, data for relative Cl^- and Na^+ uptake look similar. However, taking the size of the intracellular pools into account ($[Cl^-]_i \cong 500$ mM and $[Na^+]_i \cong 70$ mM according to Saddler (1970) and our own data in Table 2), the Cl^- influx turns out to be about seven times larger than the Na^+influx.

At 4°C (lower part of Table 1), the fast components vanished and the slow component showed a unidirectional influx which is about 50% lower than the corresponding influx at 21°C.

For the experiments in the cold, the $^{22}Na^+$ relations turned out to be more complex. There was a large scatter for the saturating $^{22}Na^+$ content after equilibration. For many cells, the data for normal conditions (about 70 mM) could be confirmed. However several cells showed considerably increased (up to 200 mM) $[Na^+]_i$. This indicates that in the

Table 1. Unidirectional uptake of $^{22}Na^+$, $^{42}K^+$, and $^{36}Cl^-$

Condition	Ion	n	Time constant (sec)		A^*_{02}/A^*_{01}	Unidirectional influx, $\Phi/\mu mol \cdot m^{-2} sec^{-1}$	
			τ_1/min	τ_2/hr		Φ_1	Φ_2
21°C							
	K^+	42	—	98.2 ± 9	—	—	0.1 ± 0.02
	Na^+	24	18.5 ± 2	3.5 ± 0.6	≅1	2.3 ± 0.3	0.2 ± 0.02
	Cl^-	43	18.8 ± 2.7	3.4 ± 0.7	≅1	17.4 ± 4.1	1.5 ± 0.3
4°C							
	Na^+	39	10.6 ± 2.1	5.2 ± 1.1	1–10	2.3 ± 0.3	0.7 ± 0.1
	Cl^-	17	—	13.8 ± 1.5	—	—	0.7 ± 0.1

Means ± SE; 21 and 4°C; white light ≅ 80 W · m^{-2}; A^*_{01} and A^*_{02}: amplitude coefficients (amounts) of apparent exponential components 1 and 2 for uptake of radioactivity.

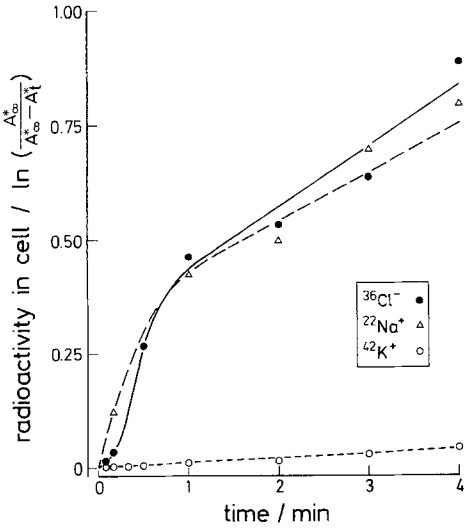

Fig. 3. Examples of uptake kinetics of $^{42}K^+$, $^{22}Na^+$ and $^{36}Cl^-$ at 21°C; normalized, logarithmic plot (for absolute data *see* Table 1) showing only one component for $^{42}K^+$ but two components for $^{22}Na^+$ and for $^{36}Cl^-$ with similar characteristics for these two ion species.

cold many cells did not sustain the electrochemical gradient for Na^+ completely but allowed some passive net uptake of Na^+.

K^+ Efflux

Example of washout kinetics of $^{86}Rb^+$ and of $^{42}K^+$ are shown in Fig. 4. There is a fast initial component which reflects the eluation of the cell wall compartment (volume: about 0.5 · 10^{-9} m^{-3} at $[K^+]_{wall} = [K^+]_o$ = 10 mM). Equivalent results have been obtained with $^{86}Rb^+$ on isolated cell walls (Fig. 14 in Mummert, 1979). Those results revealed, in addition, the time constant (≅5 sec) of the apparatus.

The $^{86}Rb^+$ kinetics in Fig. 4 show (after this initial component) a slow elution with only one exponential component ($\tau \cong 14$ days), which agrees well with previous reports (Saddler, 1970; Mummert & Gradmann, 1976). $^{42}K^+$ is washed out about seven times faster ($\tau \cong 2$ days in this example, mean of four experiments: 60.5 ± 6 hr). Due to the fast decay of $^{42}K^+$, these cells have been incubated for only about 24 hr, i.e., not to complete equilibration. In order to determine the mean efflux (140 ± 14 nmol · m^{-2} sec^{-1}) an intracellular $[K^+]$ of 450 mM has been taken from elsewhere (Saddler, 1970; Mummert & Gradmann, 1976).

Na^+ Efflux

For Na^+, the situation turned out to be more complex again. An example of washout kinetics of $^{22}Na^+$ is shown in Fig. 5. Two exponential components can clearly be identified in this example. A third (rather fast) one is not well resolved here; it can be assigned again to the cell wall compartment.

For $[Na^+]_{cyt} \cong [Na^+]_{vac}$ (about 70 mM, Saddler, 1970) and a volume ratio of the two compartments $Vol_{vac} : Vol_{cyt} \geq 10$, $^{22}Na^+$-equilibrated cells should show an amplitude ratio $A^*_{02}/A^*_{01} \geq 10$ as well. Surprisingly, only 2 of the 20 investigated cells did match this expectation by their efflux kinetics. These were just those cells which showed two peculiar properties, namely a very high Na^+ content ($[Na^+]_i \cong [Na^+]_o$) and apparently faster labeling of the large (vacuolar) compartment compared to the small (cytoplasmic) one, i.e., a higher specific radioactivity, S^*_2, in the vacuole than S^*_1 in the cytoplasm at a given incubation time. The numerical results from these cells are listed in Table 3.

From the remaining 18 cells investigated, 8 showed $[Na^+]_i < 100$ mM, which is consistent with previous reports (Saddler, 1970). The results from these cells fall again into two significantly ($P < 0.05$)

Table 2. Unidirectional efflux of Na^+ at 80 W · m^{-2} white light[a]

Condition	n	Time constant (sec)		Amplitude ratio	Efflux/μmol m^{-2} sec^{-1}		Concentration $[Na^+]_i$/mM
		τ_1/min	τ_2/hr	A_{02}^*/A_{01}^*	Y_1	Y_2	
21°C							
Norm.$_1$	3	10.4 ± 2.6	6.4 ± 1.8	0.77 ± 0.02	7.03 ± 1.34	0.10 ± 0.04	73 ± 12
Norm.$_2$	5	8.2 ± 1.4	9.0 ± 1.5	5.9 ± 0.9	2.45 ± 0.63	0.19 ± 0.02	84 ± 16
Cytopl.	2	49.5 ± 20	—	—	4.84 ± 0.20	—	165 ± 37
4°C							
Normal	8	13.5 ± 1.1	4.7 ± 0.5	1.8 ± 0.4	3.44 ± 0.73	0.24 ± 0.04	82 ± 18
Normal	6	—	13.3 ± 4.2	—	—	0.20 ± 0.02	120 ± 19

[a] Data (means ± SE) from experiments at different temperatures (21 and 4°C) and from different cell types; A_{01}^* and A_{02}^*: amplitude coefficients (amounts) of apparent exponential components 1 and 2 for release of radioactivity; at 21°C, normal: untreated cells with $[Na^+]_i < 100$ mM; norm.$_1$: normal cells with low A_{02}^*/A_{01}^*; norm.$_2$: normal cells with high A_{02}^*/A_{01}^*; cytopl.: Cytoplasmic (vacuole-depleted) cell segments; at 4°C, selected kinetics from normal cells (14 out of 40) with unambiguous behavior.

Table 3. Compartmentation of $^{22}Na^+$ of cells with $A_{02}^*/A_{01}^* \geq 10$

Cell	t_{incub}/hr	A_{02}^*/A_{01}^*	S_1^*/S_0^*	S_2^*/S_0^*	$[Na^+]_i$/mM	τ_1(influx)/hr
1	24.3	19	0.49	1.03	473	36.5
2	44.0	10	0.79	0.91	420	28.2

A_{01}^* and A_{02}^*: amplitude coefficients (amounts) of apparent exponential components 1 and 2 for uptake of radioactivity; $S_{1,2,0}^*$: specific radioactivities in component 1, component 2, and incubation medium, respectively; assumption: $[Na^+]_{vac} = [Na^+]_{cyt}$.

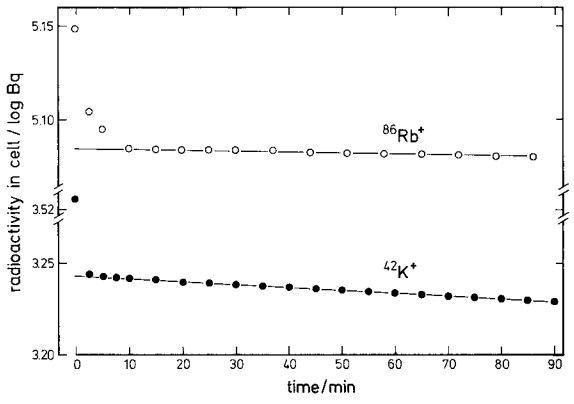

Fig. 4. Examples of washout kinetics of $^{86}Rb^+$ (upper trace) and of $^{42}K^+$ (lower trace); main features: only one (slow) exponential flux component (after initial washout of cell wall compartment) apparent in both tracings; washout of Rb^+ slower (τ = 343 hr) than of K^+ (τ = 48 hr).

distinct groups: one with an amplitude ratio $A_{02}^*/A_{01}^* \cong 6$ and another group with $A_{02}^*/A_{01}^* \cong 0.8$. The data from these cells are listed in Table 2 together with those from cytoplasmic (vacuole-depleted) cell segments. Since the bulk data on $^{22}Na^+$ presented above indicate labile Na^+ relations under these experimental conditions, the centrifuged preparations were used only if the normal membrane voltage could be recorded simultaneously. This was possible only twice with vacuole-depleted cell segments and never with cytoplasm-depleted ones.

At 4°C, the Na^+ washout kinetics also displayed a considerable scatter. From the results of 40 cells, 14 kinetics could unambiguously be analyzed, either by two exponentials (n = 8) or by one exponential (n = 6). The results are also listed in Table 2. When the cells have been incubated for only 5 min (far from equilibration), the washout kinetics at 4°C showed a transient maximum of the slope at about 20 min (data: Fig. 20 in Mummert, 1979, *not shown*).

Summarizing: at 21°C as well as at 4°C, unidirectional influx and efflux of Na^+ show both a fast component (some μmol m^{-2} sec^{-1}) and one 3 to 10 times slower; the ratio of the corresponding amplitudes (A_{02}^*/A_{01}^*) is around 1, and total $[Na^+]_i$ is <100 mM. Larger ratios (corresponding to the volumes of vacuole and cytoplasm), higher intracellular concentrations and smeared kinetics are also found which seems to be due to some injury. The fast component can be missing in the cold.

Cl^- Efflux

An example of $^{36}Cl^-$ washout kinetics has already been presented previously (Fig. 4 in Mummert, Hansen & Gradmann, 1981). These kinetics show two intracellular components after the fast initial compo-

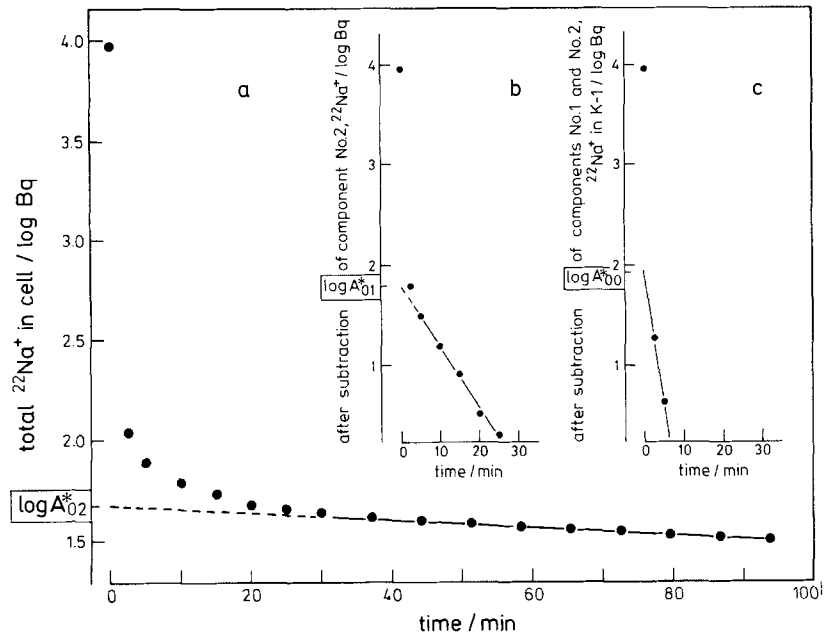

Fig. 5. Example of washout kinetics for $^{22}Na^+$ at 21°C in semilogarithmic plots. (*a*) Total radioactivity in cell; straight line marks efflux component No. 2: $A^*_{02} \cdot \exp(-t/\tau_2)$. (*Inset b*) After subtraction of component No. 2; straight line marks efflux component No. 1: $A^*_{01} \cdot \exp(-t/\tau_1)$. (*Inset c*) After subtraction of components No. 1 and No. 2; straight line tentatively marks efflux component No. 0: $A^*_{00} \cdot \exp(-t/\tau_0)$ which probably corresponds to the cell wall compartment.

Table 4. Unidirectional efflux of Cl^- at 80 W m^{-2} white light

Condition	*n*	Time constant		Amplitude ratio A^*_{02}/A^*_{01}	Efflux/μmol m^{-2} sec^{-1}		Concentration $[Cl^-]_i$/mM
		τ_1/min	τ_2/hr		Y_1	Y_2	
21°C							
Normal	10	18.7 ± 0.9	2.7 ± 0.3	1.1 ± 0.1	17.3 ± 2.1	2.3 ± 0.5	412 ± 53
Cytopl.	4	—	2.1 ± 0.3	—	—	5.5 ± 1.5	417 ± 117
Vacuol.	2	—	3.6 ± 1.5	—	—	1.6 ± 0.2	230 ± 54
4°C							
Normal	3	28.6 ± 5.6	10.9 ± 1.4	13 ± 4.5	1.89 ± 0.5	0.9 ± 0.1	284 ± 4
Normal	10	—	9.2 ± 1.2	—	—	1.3 ± 0.2	248 ± 27

Data (means ± SE) from experiments at different temperatures (21 and 4°C) and from different cell types, at 21 and at 4°C; A^*_{01} and A^*_{02}: amplitude coefficients (amounts) of apparent exponential components 1 and 2 for release of radioactivity; normal: untreated cells, cytopl.: cytoplasmic (vacuole-depleted) cell segments, vacuol.: vacuolar (cytoplasm-depleted) cell segments.

nent from the cell wall. Such experiments have been carried out with normally compartmented cells, with cytoplasmic (vacuole-depleted) cell segments and with vacuolar (cytoplasm-depleted) ones. The results from 21°C and from 4°C are compiled in Table 4.

Discussion

Methods

The numerical analysis of the flux kinetics (Tables 1 to 4) with one or two intracellular components is merely descriptive. It is assumed that the correct description would require more components, corresponding to the number of physical compartments including, e.g., plastids or the vesicular system (*see below*). When the number of possibly relevant compartments does not correspond to the number of exponential components in the apparent efflux kinetics, it would be inappropriate to analyze the simple kinetics with a complicated model, unless the required information came from other sources.

The numerical agreement of the data for influx and for efflux (Tables 1, 2 and 4) indicates the reliability of the methods and results, in particular that the steady-state condition was fulfilled, which is essential for the flux analysis of the washout experiments.

It may be recalled that in general, the amounts of unidirectional ion fluxes exceed those of corresponding net ion fluxes, which are equivalent to electrical currents. These data are only convertable for conditions far from thermodynamic equilibrium, or if the net fluxes could be reliably determined by the difference of the two opposite unidirectional fluxes.

Influx

Under physiological resting conditions, V_m is rather negative (about -170 mV) due to the activity of the electrogenic Cl^- pump (for review *see* Gradmann, 1989). This is reflected by the large Cl^- influx (Fig. 3 and Table 1), which is reduced in the cold (Table 1) when the electrogenic pump is known to be inhibited.

The uptake kinetics of Na^+, which has repeatedly been reported to play a minor role in the electrical behavior of the plasmalemma in *Acetabularia* (Saddler, 1970; Gradmann, 1970, 1975; Bertl & Gradmann, 1987), display remarkable similarities with the uptake kinetics of Cl^- (Fig. 4), especially with respect to the unexpected amplitude ratio A_{02}^*/A_{01}^* of about 1 for the two exponential components. These peculiarities may be an effect of the vesicular compartment which can exchange Na^+ and Cl^- with the external medium without affecting the electrical properties of the plasmalemma (Mummert & Gradmann, 1991).

The slow uptake of $^{42}K^+$ corresponds to the electrophysiological findings (Saddler, 1970; Gradmann, 1970, 1975; Bertl & Gradmann, 1987) that K^+ plays a minor role for the electrical membrane properties under physiological resting conditions, because the K^+ channels are inactivated at the very negative resting voltage of about -170 mV.

Efflux

One-Component Washout of $^{42}K^+$

The comparison of $^{42}K^+$ and $^{86}Rb^+$ relations (Figs. 2 and 4) results in the conclusions that the "real" K^+ influx of about 100 nmol $\cdot$ m^{-2} sec^{-1} as determined by $^{42}K^+$ is significantly higher than the "quasi" K^+ influx from $^{86}Rb^+$ experiments and that massive substitution of K^+ by Rb^+ shows some developmental changes but only after several weeks of incubation.

The fast initial component of the K^+ washout kinetics reflects the cell wall compartment as judged by the pool size. The fact that the fast initial component is followed by only one exponential component in the steady-state uptake and washout kinetics of K^+ means that the barrier for K^+ diffusion across the tonoplast is not higher than the barrier of the plasmalemma, i.e., the plasmalemma essentially controls the K^+ relations of the entire protoplast.

More-Component Kinetics

Conventional Treatment. Usual compartmental analysis of the measurements yields the data as listed in Tables 2 and 4. As expected, cell segments with only one *major* (cytoplasmic or vacuolar) compartment show only one exponential component in the washout kinetics. An electrophysiological comparison of the three different compartmental preparations has shown that the currents and conductances in cytoplasmic cell segments are 2 to 5 times larger than in normal cells and that vacuolar cell segments show correspondingly smaller currents and conductances (Freudling & Gradmann, 1979). The fluxes reported here nicely fit into this pattern: compared to normal cells, the fluxes in cytoplasmic cell segments are increased by factors in the range of 2 to 5 and correspondingly decreased in vacuolar cell segments.

$[Cl^-]$ in normal cells and in cytoplasmic cell segments turned out to be nearly as high as in the seawater outside. However, in cold-treated cells with reduced activity of the Cl^- pump, $[Cl^-]$ was significantly lower, i.e., closer to passive equilibrium, especially when it is considered that V_m is more positive (about -80 mV) in the cold than under physiological conditions (about -170 mV). Similarly, the vacuolar cell segments with their lowered transport activity—as judged by the current and conductance data (Freudling & Gradmann, 1979)—show a somehow lowered [Cl].

As for Na^+ (Tables 1 and 2), the situation seems to be unstable, but allows a consistent interpretation. Since the two-component efflux kinetics of Na^+ are remarkably independent of temperature compared to the situation with Cl^-, the electrochemical inward-directed gradient for Na^+ is probably not maintained by primary active transport but by secondary processes. It is suggested that this transport system is localized in the plasmalemma, because devacuolated, cytoplasmic cell segments (which lack the vesicular system) can show large unidirectional Na^+ fluxes (Table 2). These processes, in turn, should be electroneutral, because (compared with K^+) the unidirectional fluxes are large and the electrophysiological effects are small (Saddler, 1970; Gradmann, 1970, 1975). One could suggest an electroneutral Na^+/Na^+ exchange at the

plasmalemma. On the other hand, such a transporter should have been blocked during the experiments for Table 3, because these results were explained by a bypass of a Na^+-tight plasmalemma.

Surprises and Explanation by Additional, Vesicular Compartment. The conventional model of serial compartmentation (outside—small cytoplasm—large vacuole) is insufficient to describe the following observations.

(i) In the tracer uptake kinetics of the conventional model of linear compartmentation, the specific radioactivity in the cytoplasm is always larger than in the vacuole. However, the data in Table 3 show examples with the opposite relationship.

(ii) The amplitudes A_{01}^* and A_{02}^* of the two exponentials are similar at 21°C, as found in normal cells for Na^+ (Table 2) frequently and always for Cl^- (Table 4), and the volume ratio between cytoplasm and vacuole is about 1 : 10. With the direct assignment of components to compartments, $[Cl^-]$ would turn out to be unrealistically high (about 2.5 M) in the cytoplasm, whereas the direct determination of $[Cl^-]$ in the (sole) cytoplasmic compartment of centrifuged cell segments (Table 4) gave realistic values of about 420 mM.

(iii) There are large efflux bursts of Cl^- and of Na^+, which do not affect the electrical potential in the cytoplasm (Gradmann & Mummert, 1980; Wendler et al., 1983).

In this situation an extension of the model is suggested with an additional, vesicular compartment (Fig. 1) which corresponds, in fact, to the intracellular structure as demonstrated by electron micrographs (Bouck, 1964; Van Gansen & Boloukhère-Presbourg, 1965; Franke et al., 1977). The three observations can be explained by the model as follows:

(i) With small k_{01}, k_{10} (low permeability of the plasmalemma for Na^+, as reported by Saddler, 1970; Gradmann, 1970), large k_{03}, k_{30} (vigorous endo- and exocytotic activity of the vesicular system), small k_{13}, k_{31} (little exchange between vesicles and cytoplasm), large k_{23}, k_{32} (effective contact between vesicles and central vacuole), and "normal" k_{12}, k_{21} (contact between cytoplasm and central vacuole), the predominant part of tracer uptake into the cytoplasm will not take place directly across the plasmalemma but *via* vesicles and the central vacuole.

(ii) The large vacuolar compartment (ca. 90% of the cell volume) is subdivided into two parts of similar volume: a central vacuole and a vesicular system.

(iii) A vesicular shuttle between vacuole and outside provides a pathway for large amounts of salt (Na^+ and Cl^-) without direct effect on the electrical relationships between cytoplasm and outside. The fact that this electrically silent salt release does not take place in vacuolate-depleted cell segments strongly supports this view.

Unfortunately, the complexity of the apparent compartmentation prevents the unequivocal determination of the individual rate constants k_{jk}, not only because of the large number of parameters but also because a fundamental prerequisite for compartmental analysis (namely good stirring) is not fulfilled for the vesicular compartment which is expected to differ considerably in its ionic composition between more central and more peripheric parts.

Conclusions

In *Acetabularia* there is a large (nearly half the cell volume) vesicular system which mediates considerable exchange of Na^+ and Cl^- between the vacuole and outside bypassing the plasmalemma. There is also an electroneutral Na^+/Na^+ exchange at the plasmalemma.

This work was supported by the Deutsche Forschungsgemeinschaft. We thank Hans-Georg Klieber for valuable discussions and Anna Amtmann as well as Dr. Adam Bertl for critically reading the manuscript.

References

Bertl, A., Gradmann, D. 1987. Voltage gated potassium channels in *Acetabularia:* A patch-clamp study. *J. Membrane Biol.* **99**:41–49

Bouck, G.B. 1964. Fine structure in *Acetabularia* and its relation to protoplasmic streaming. *In:* Primitive Motile Systems in Biology. R.D. Allen, editor. pp. 7–17. Academic, New York—London

Cram, W.J. 1968. Compartmentation and exchange of chloride in carrot root tissue. *Biochim. Biophys. Acta* **163**:339–353

Franke, W.W., Spring, H., Kartenbeck, J., Falk, H. 1977. Cyst formation in some Dasycladacean green algae. I. Vesicle formation during coenocygotomy in *Acetabularia mediterranea*. *Eur. J. Cell Biol.* **14**:229–252

Freudling, C., Gradmann, D. 1979. Cable properties and compartmentation in *Acetabularia Biochim. Biophys. Acta* **552**:358–365

Goldfarb, V., Sanders, D., Gradmann, D. 1984*a*. Phosphate relations of *Acetabularia:* Phosphate pools, adenylate phosphates and ^{32}P influx kinetics. *J. Exp. Bot.* **35**:626–644

Goldfarb, V., Sanders, D., Gradmann, D. 1984*b*. Reversal of electrogenic Cl^- pump in *Acetabularia* increases level and ^{32}P labelling of ATP. *J. Exp. Bot.* **35**:645–658

Gradmann, D. 1970. Einfluß von Licht, Temperatur und Außenmedium auf das elektrische Verhalten von *Acetabularia*. *Planta* **93**:323–353

Gradmann, D. 1975. Analog circuit of the *Acetabularia* membrane. *J. Membrane Biol.* **25**:183–208

Gradmann, D. 1976. "Metabolic" action potentials in *Acetabularia*. *J. Membrane Biol.* **29:**23–45

Gradmann, D. 1978. Green light (550 nm) inhibits electrogenic Cl^- pump in *Acetabularia* membrane by permeability increase for the carrier ion. *J. Membrane Biol.* **44:**1–24

Gradmann, D. 1989. ATP-driven chloride pump in giant alga *Acetabularia*. *Methods Enzymol.* **174:**490–504

Gradmann, D., Mummert, H. 1980. Plant action potentials. *In:* Plant Membrane Transport: Current Conceptual Issues. R.M. Spanswick, W.J. Lucas, and J. Dainty editors. pp. 333–334. Elsevier Biomedical, Amsterdam

Gradmann, D., Mummert, H. 1984. Mechanism of Cl^- efflux bursts in *Acetabulaira:* Vesicle release *versus* permeability transients. *J. Membrane Biol.* **78:**81–82

Gradmann, D., Wagner, G., Gläsel, R.M. 1973. Chloride efflux during light-triggered action potentials in *Acetabularia mediterranea*. *Biochim. Biophys. Acta* **323:**151–155

MacRobbie, E.A.C. 1969. Ion fluxes to the vacuole of *Nitella translucens*. *J. Exp. Bot.* **20:**236–256

Mummert, H. 1979. Transportmechanismen für K^+, Na^+ und Cl^- in stationären und dynamischen Zuständen bei *Acetabularia*. Ph.D. Thesis. University of Tübingen, Tübingen

Mummert, H., Gradmann, D. 1976. Voltage dependent potassium fluxes and the significance of action potentials in *Acetabularia*. *Biochim. Biophys. Acta* **443:**443–450

Mummert, H., Gradmann, D. 1991. Action potentials in *Acetabularia:* Measurement and simulation of voltage-gated fluxes. *J. Membrane Biol.* **124:**265–273

Mummert, H., Hansen, U.-P., Gradmann, D. 1981. Current-voltage curve of electrogenic Cl^- pump predicts voltage-dependent Cl^- efflux in *Acetabularia*. *J. Membrane Biol.* **62:**139–148

Saddler, H.D.W. 1970. The ionic relations of *Acetabularia mediterranea*. *J. Exp. Bot.* **21:**345–359

Van Gansen, P., Boloukhère-Presbourg, M. 1965. Ultrastructur de l'algue unicellulaire *Acetabularia mediterranea*. *J. Microsc.* **4:**347–362

Wendler, S., Zimmermann, U., Bentrup, F.-W. 1983. Relationship between cell turgor pressure, electrical membrane potential, and chloride efflux in *Acetabularia mediterranea*. *J. Membrane Biol.* **72:**75–84

Wendler, S., Zimmermann, U., Bentrup, F.-W. 1984. *Reply to:* Mechanism of Cl^- efflux bursts in *Acetabularia:* Vesicle release *versus* permeability transients. *J. Membrane Biol.* **78:**82–83

Received 15 March 1991