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Cation Effects on Volume and Water Permeability in the Halophilic Algae *Dunaliella parva*

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Summary. Rates of change of cell volume were measured in suspensions of the halophilic green algae *Dunaliella parva* subjected to changes in cation composition and concentration of the outside medium. Measurements were made with a particle size analyzer and results were checked by direct microphotography. For any one salt solution, changes in cell volume with concentration were consistent with the Boyle-Van't Hoff model of an osmometer. Nonosmotic volume comprised 60–80 % of total cell volume and was sensitive to the nature of the cation, increasing in the order Cs < K < Na < Ca < Mg. Kinetics of volume change in response to changes in outside salt concentration are best described by two kinetic coefficients differing by one order of magnitude and dependent on the nature of the outside cation (decreasing in order Cs > K > Na > Mg) as well as on direction of water flow.

Volume is an important extensive parameter in the analysis of a thermodynamic system because it is the conjugated capacity parameter of hydrostatic pressure in the Gibbs equation

$$dU = TdS - PdV + \Sigma \mu dn_i \tag{1}$$

where U = internal energy, T = absolute temperature, S = entropy, P = hydrostatic pressure, V = volume, μ = chemical potential and n_i = numbers of moles. Thus, in the analysis of the osmotic relations of the cell, it is important to measure cell volume. Since most of the living cell is occupied by water, the cell volume is nearly equal to the volume of cell water, and consequently, any change in the amount of cell water should be reflected as a change in volume of the cell. The amount of cell water is primarily dependent on its interaction with solutes; any transport of solute into or out of the cell should be reflected in a change of cell volume, although in most plant cells such changes are limited by the rigid cell wall. Cell volume may also be determined by interactions between water and macromole-

cules. In a typical plant cell, however, where most of the cell water is in the central vacuole, there can be little direct interaction between the cell water and its macromolecules and this type of interaction should have little effect on the volume of the water.

Dunaliella parva, a moderately halophilic unicellular algae isolated from the Dead Sea, differs from the typical plant cell in many important respects. It is not surrounded by a rigid cell wall and has no central vacuole, either large or small. Furthermore, it withstands variations of 0.4 to 4 M in the salt concentration of the surrounding medium, and we could expect to observe large changes in cell volume in response to such variations. Ben-Amotz (1973) has shown that volume changes in *Dunaliella parva* following changes in the outside salt concentration are transient only; after about 90 min the algal cell returns to its original volume. Ben-Amotz and Avron (1973) attributed the volume changes to changes in the concentration of glycerol in the cell. They confirmed former findings that glycerol is the main soluble component of the *Dunaliella* cell. They found that the glycerol changes in concentration in response to the changes in salt concentration that occur in the outer medium. This form of volume-regulation is unique to some unicellular wall-less algae (Ben-Amotz, 1973; Kauss, 1973) and most unlike the more usual type of osmotic regulation which depends on the pumping of ions between the outside medium and the cell.

One may ask whether the changes in internal glycerol concentration can fully explain the volume regulation of these cells. To test the hypothesis it is necessary to know whether the *Dunaliella* cell behaves as an osmometer, ideal or otherwise. If so, the volume should behave according to the equation which describes the behavior of an osmometer (Dick, 1972). This is the well-known Boyle-van't Hoff equation:

$$\pi_o(V-b)=\phi R T n_i \tag{2}$$

where π_o = osmotic pressure of the outside solution, V = cell volume, b = nonosmotic volume of cell, ϕ = average osmotic coefficient of the intracellular solute, R, T = gas constant and absolute temperature, respectively, and, n_i = number of moles of solute in the cell.

On differentiating Eq. (2) with respect to π we obtain:

$$\frac{\pi}{V}\frac{dV}{d\pi}=-\left[1-\frac{b}{V}\right]\left[1-\frac{\pi}{\phi}\frac{d\phi}{d\pi}\right]. \tag{3}$$

Eq. (3) relates the ratio of the fractional cell volume changes to changes in the fractional osmotic pressure.

If the membrane of the cell is semi-permeable, only two factors have to be taken into account. These are b, the nonosmotic cell volume, which in many cells is 20–30 % of the total volume, and $d\phi/d\pi$, the change in the average osmotic coefficient. The latter may be of importance for concentrated protein solutions. In *Dunaliella parva*, since glycerol is the main solute, it is to be expected that $d\phi/d\pi=0$ (Scatchard, Hamer & Wood, 1938), and therefore the osmotic behavior will be governed solely by the first factor of Eq. (3), provided that interactions of macromolecules and water be unimportant. In nonvacuolated cells such interactions may be important and therefore osmotic behavior may be affected by all the factors in the equation.

In this work the transients of adjustment of cell volume to π_o were followed and from them were calculated the kinetic parameters of volume changes, as related to water permeability. Cell volumes were measured by a particle size analyzer (PSA). Most previous measurements of cell volume were made by direct microscopic observation so that changes in the linear dimension only were followed. Such a method is highly susceptible to error since changes in linear dimension are related by the third power to the volume; thus, considerable changes in volume are reflected by only minor changes in length.

The measurement of particle volume by PSA is not straight-forward, and the signal obtained is a function of cell shape, orientation in the electrical field and relative electrical resistance between the medium and the particle (Grover *et al.* 1969*a*, *b*, 1972). The first part of this paper is devoted to the establishment of these factors. The osmotic behavior of the cells is then described, together with the kinetics of volume changes which were shown to be strongly dependent on the nature of the cation in the outer solution.

Materials and Methods

Measurements of Volume by Particle Size Analyzer (PSA)

Measurements of cell volume were made by means of a PSA, improved and modified by Grover *et al.* (1969*a*, *b*, 1972). The orifice used in the present study was cylindrical with a diameter of 50×10^{-4} cm and a length of 100×10^{-4} cm. The linear velocity of the cells through the PSA was about 400 cm/sec. A "delay" knob ensured that registration of the signal started only when the cells had penetrated the orifice by distance of at least one radius from the plane of the entrance, thus ensuring residence of the cells in a homogeneous electrical field.

Coincidence of cells in the orifice was minimized by diluting the suspension until no further change in distribution was obtained with further dilution.

Error in the estimation of the cell volume may arise from effects of the electrical field intensity on the cells (Naaman, 1970). Naaman (1970) found a critical field intensity E_c above which cell volume appeared to decrease with increase in E.

$$E=\frac{I\rho}{\pi R^2} \tag{4}$$

where E = field intensity, ρ = resistivity of the medium, R = radius of the orifice and I = current. Eq. (4) shows that for constant values of R and I, the value of E is determined by the resistance of the medium. In the present work the concentrations of the solutions used were an order of magnitude higher than those of the more usual physiological solutions. Consequently, the resistances were much lower, and it seemed likely that the values of E used were far removed from E_c. This was checked experimentally by changing I.

The PSA yields an electrical size measurement, which under optimal conditions is a product of the cell volume V, and of the shape factor γ, only:

$$v=\gamma V \tag{5}$$

(γ = shape factor, v = electrical size).

Fig. 2 in Grover *et al.* (1969*a*) gives the shape factor as function of the ratio of the axes for ellipsoids of revolution and is therefore applicable to our work, since *D. parva* cells are spheroids throughout the concentration range employed. The ratio of the axes for *D. parva* was obtained by measurement of microphotographs of cells in solutions of different tonicities.

Microphotographs were also used for the direct measurement of cell volume and the results were compared with those obtained by PSA. As the definition of the photomicrographs was good, the size of cells could be measured by projecting photographs onto sheets of paper and drawing round the cell boundaries. The scale being known, the mean volume of the cell population was then calculated. The main sources of error in this estimate were due, firstly to the orientation of those cells which did not lie parallel with the camera lens, and secondly to uncertainties in the estimation of the length of the cells. The former one is a systematic error and tends to reduce the estimate of the cell volume, whereas the second one is presumably random and was minimized by measuring no fewer than 250 cells per culture. Table 1 shows that the two methods agree reasonably well for the cultures measured. The

Table 1. Comparison of cell volumes of *Dunaliella parva* measured by photomicroscopy or particle size analyzer (PSA) (for details of the method see text).

Culture	Method	
	Photomicrograph (Volume μ^3)	PSA
A	160	162
B	138	145
C	64	69

PSA measurements quoted are those of highest frequency (modal values), rather than mean values, for reasons discussed below. Measurements obtained by direct observation were always slightly lower than those from the PSA, indicating that the electrical conductivity of the particles was much lower than that of the medium (Grover *et al.*, 1969*a*).

Yet another difference between the two methods must be discussed. Figs. 1 and 2 show histograms of the distribution of volume and "electrical size", respectively, the "electrical

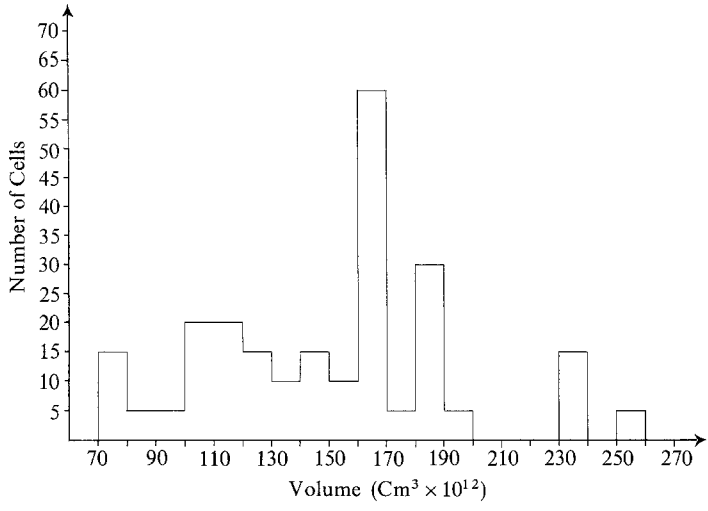

Fig. 1. Size-distribution histogram of *Dunaliella parva* cells, as obtained by microphotography (for details *see text*). Refers to same population as shown in Fig. 2

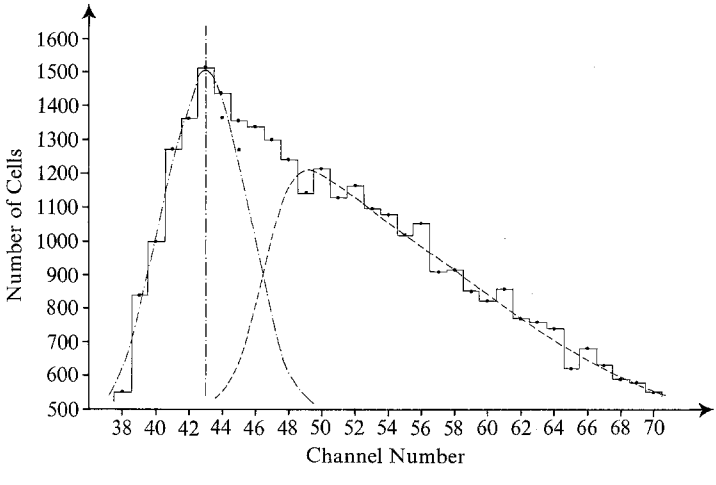

Fig. 2. Size-distribution histogram obtained by PSA of *Dunaliella parva* cells. The solid line is that of the experimental data. - · - · - · represents calculated normal distribution for population A *(see text)*. - - - represents population B obtained by subtraction

size" histogram being rather more skewed. It is suggested that the skewness is due to the superposition of two populations, as seen in Fig. 2. A symmetrical population around the mode represents cells in which the long axis of the cell is oriented parallel with the long axis of the orifice. The second population would arise from the presence of cells oriented at an angle to the long axis of the orifice. The mean of the first population agrees well with measurements obtained from the photomicrographs (Table 1).

The physical basis of the second population was thoroughly discussed by Grover *et al.* (1969*a*, 1972). Fig. 2 of their paper (Grover *et al.*, 1969*a*) shows that the value of the shape

factor γ changes considerably when the orientation of the particle in the orifice is altered from the parallel to the perpendicular direction.

Fluid flowing through the orifice either passes through the "core" at the center of the orifice or in the boundary layer which lies between the "core" and the orifice wall. In the "core" the direction of flow is parallel to the long axis of the orifice whereas in the boundary layer there are gradients of velocity of fluid flow in the radial direction away from the boundary. When cells are suspended in the fluid, 65–80 % of them must be in the boundary layer at some stage during their passage through the orifice (Rabinowitch, 1974) and must then be oriented at an angle to the longitudinal axis and therefore to the electrical field. This difference in cell orientation results in an "apparent shape factor", γ'':

$$\gamma'' = \gamma \cos^2 \psi + \gamma' \sin^2 \psi \tag{6}$$

where γ = shape factor of cell oriented parallel to the electric field, γ' = shape factor of cell oriented perpendicular to the electric field and ψ = angle of orientation of the long cell axis to the longitudinal axis of the orifice.

Using Eq. (6), it is possible to calculate the "apparent shape factor" γ'' for any given ψ. Since the ratio of the axes for the appropriate spheroid was obtained by measurement of the microphotographs, the shape factors for *D. parva* cells could be calculated with the aid of Fig. 2 in Grover *et al.* (1969*a*) and gave values of 1.21 for γ and 1.70 for γ'. Using these values, the parameters of population B in Fig. 2 can be caculated:

	γ''	ψ
median	1.4	52°
maximum	1.32	39°.

These values appear to be consistent with the hydrodynamic conditions prevailing in the orifice (Rabinowitch, 1974).

It is concluded that the skewness of the electrical size distribution arises from the different orientations of the cells within the orifice. There is no evidence for the existence of two populations of cells with different cell volumes. The conclusion is reinforced by two additional observations. Firstly, two main pulse shapes are seen on the oscilloscope screen; these resemble the shapes seen for spheroid particles by Grover *et al.* (1972) who account for them in terms of orientation of particles in the orifice. Secondly, the skewness is considerably reduced for *D. parva* cells in 0.6 M NaCl, at which concentration the cells are almost spherical.

Thus, the true size of *D. parva* cells is given by the distribution of the A population in Fig. 2. Volumes presented in the present work are therefore calculated from the mode rather than from the mean channel.

Cell Suspensions

Dunaliella parva was isolated from the Dead Sea. Cultures were grown at 28 °C in a growth medium adapted from McLachlan by addition of 1.5 M NaCl, 5 mM $NaNO_3$ and 20 mM Tris-HCl buffer at pH 7.4, as described by Ben-Amotz and Ginzburg (1969).

Cells were harvested usually at mid- or late logarithmic phase by centrifugation at 1500–2000 × *g* for 5 min. They were resuspended in 1.5 M NaCl with 50 mM Tris-HCl buffer at pH 7.4 and were used for experiments during the first 2 hr after preparation.

Cells suspended in the solution of a salt other than NaCl were rinsed once with an isotonic solution of the required salt and then resuspended in the same solution. Thus, the original NaCl was diluted at least 10^4 times. Usually cells were kept only for a short time in solutions of salts other than NaCl.

For determination of cell volume at equilibrium, 2 ml of cell suspension in solutions isotonic with 1.5 M NaCl were added to 50 ml of the desired solution and the first measure-

ments were taken 3–4 min later. A few more measurements were made to ensure that the volume had reached a constant value. 25,000–100,000 cells were used for each measurement, each being repreated 3–4 times. From comparisons of replicates the standard error of the mean was found to be less than 1 %.

For measurements of rates of volume change, cells with a known initial volume were used. A solution, differing in nature or concentration from that in which the cells were suspended, was pumped through the orifice; the current was turned on and 2 ml cell suspension was added by means of a syringe by one operator. This operation lasted less than 0.5 sec and at the moment it occurred a stop-watch was started by a second operator. Four consecutive measurements were then performed and registered on a multichannel analyzer which was subdivided into four 100-channel units. The times of the measurements were noted by the operator with the stop-watch. Each measurement took less than 0.5 sec and about 5000 cells were measured each time. The first measurement was taken 5 sec after the mixing operation; there is, however, an uncertainty of 10 % in the estimation of this time which is particularly unfortunate since the cell volume was still in the process of rapid change. The uncertainty in the estimation of time was smaller for the later measurements; the rate of change of cell volume was also slower for these measurements. The agreement within a group of four replicates was to within 2–3 %.

Table 4 shows that the variability of the material was quite large. The source of this variability is not known since growth conditions were uniform. It was observed, however, only between cells from different cultures; measurements made on cells from the same culture and on the same day varied by less than 1 % of the mean.

All experiments were done in a room with temperature controlled at 22 ± 1 °C.

Salts used were of analar grade. The osmotic pressures of solutions were calculated from tables in Robinson and Stokes (1959).

Results

Behavior of Cell as Osmometer

By plotting the cell volume at equilibrium as a function of $1/\pi_o$ it is possible to test whether the cells behave according to Eq. (2). A straight-line relationship is an indication of consistency with Eq. (2) and with the classical Boyle-Van't Hoff osmometer model. Figs. 3 and 4 show that a straight-line relationship is obtained. The intercept on the ordinate gives b, the nonosmotic volume.

Table 2 presents results obtained from measurements on eight cell suspensions in NaCl or KCl. Despite the spread in cell size, it is clear that the cells were consistently smaller in KCl than in NaCl.

For another set of measurements, the results of which are presented in Table 3, cells were suspended in solutions of four different chloride salts isotonic with 1.5 M NaCl. These cells were smaller than those described in Table 2 and the "nonosmotic volume", as proportion of the total volume, was larger (60 % in Table 2 as against 80 % in Table 3). Table 3 shows that the cell volume differs according to the nature of the cation

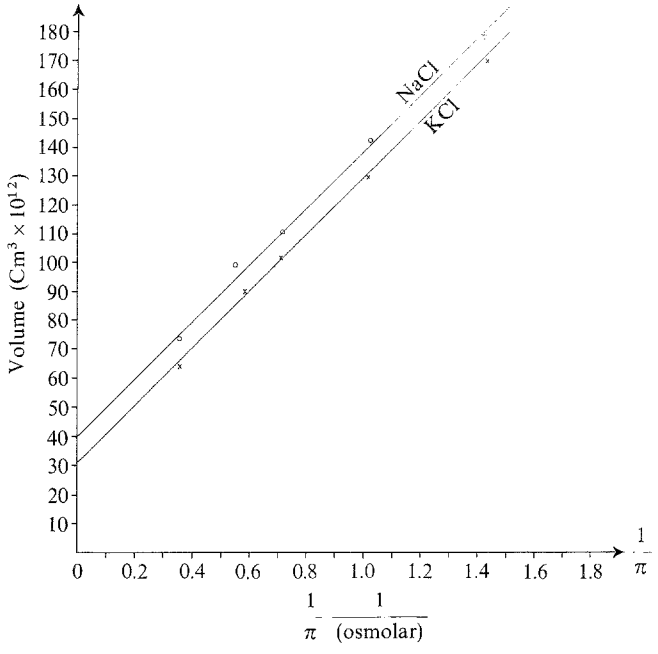

Fig. 3. Cell volume of *Dunaliella parva* as a function of the reciprocal of osmotic pressure of the suspending medium. The equilibrium volume at each concentration was reached 3–5 min after resuspension in the appropriate medium. The salt solutions included 50 mM Tris-HCl at pH 7.4

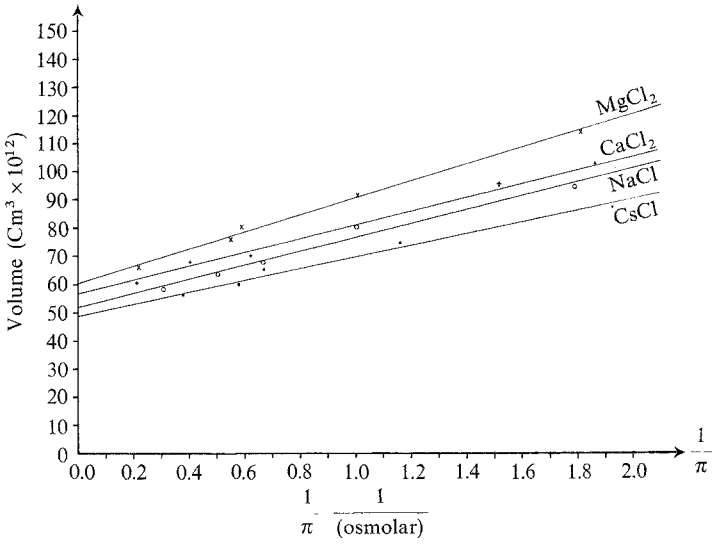

Fig. 4. Cell volume of *Dunaliella parva* as a function of the osmotic pressure of the suspending medium. The equilibrium volume at each concentration was reached 3–5 min after resuspension in the appropriate medium. The salt solutions included 50 mM Tris-HCl at pH 7.4

Table 2. The parameters of the "Boyle-Van't Hoff" osmometer model for *Dunaliella parva* cells suspended either in NaCl or KCl

Culture	NaCl			KCl		
	V_o	b	$\frac{b}{V_o} \times 100$	V_o	b	$\frac{b}{V_o} \times 100$
1	98	61	62	92	59	64
2	82	52	63	78	45	58
3	95	64	67	90	54	60
4	72	43	60	67	44	66
5	125	81	65	116	73	62
6	134	89	66	128	80	63
7	165	107	65	157	102	65
8	102	62	61	95	59	62
Mean	109.1	69.9	63.6	102.9	64.5	62.5

V_o = volume of cell at "isotonic solution" (equivalent to 1.5 M NaCl).
b = the "nonosmotic volume" – i.e. the intercept of the line of volume *vs.* $1/\pi_o$.
All measurements are in μ^3.

Table 3. The parameters of the "Boyle-Van't Hoff" osmometer model for *Dunaliella parva* cells suspended in different salt solutions

Solution	V_o (μ^3)	b (μ^3)	$\frac{b}{V_o} \times 100$
CsCl	64	53	83
NaCl	69.5	57	82
$CaCl_2$	74	62	84
$MgCl_2$	82	67	82

in the ambient medium; there is an increase in the following order:

$$Cs < Na < Ca < Mg.$$

Cells in CsCl differ by about 25 % from cells in $MgCl_2$. The Table also shows that the difference can be attributed chiefly to differences in b, the nonosmotic volume.

Water Permeability

Fig. 5 shows a typical time-curve of the changes in cell volume which followed a change in concentration of the outside salt concentration. The volume changes rapidly for the first few seconds of the experiment;

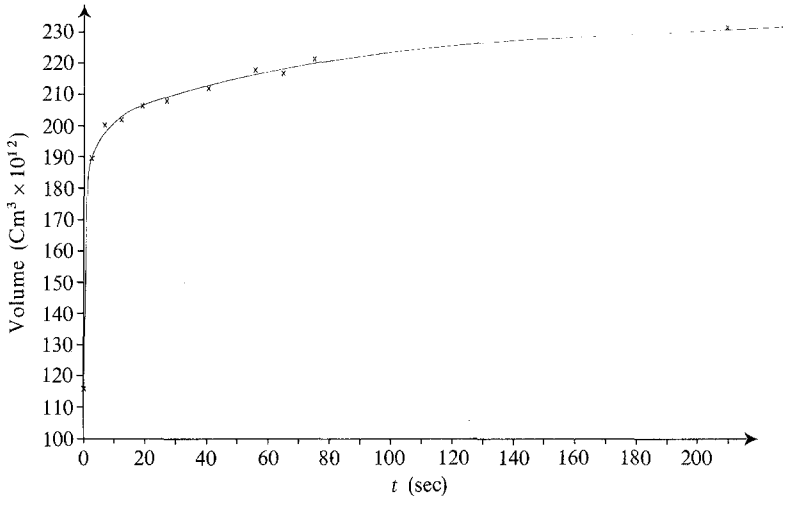

Fig. 5. Change in volume of *Dunaliella parva* cells as a function of time after dilution of suspending medium from 1.5 to 0.7 M. Medium included 50 mM Tris-HCl at pH 7.4

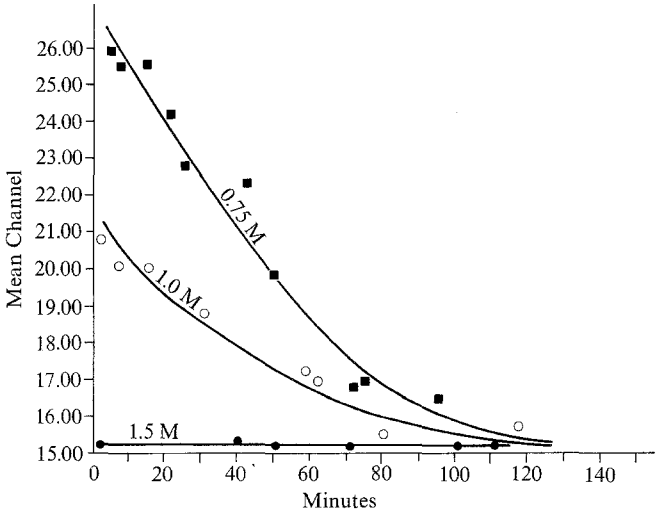

Fig. 6. Change in volume of *Dunaliella parva* cells as a function of time after dilution of suspending medium from 1.5 to 1.0 and 0.75 M, respectively. Medium included 50 mM Tris-HCl at pH 7.4

for the next 200 sec or so the change was much slower until finally a constant volume was reached.

Fig. 6 shows a typical time-curve of changes in cell volume which followed a decrease in concentration of the outside salt concentration;

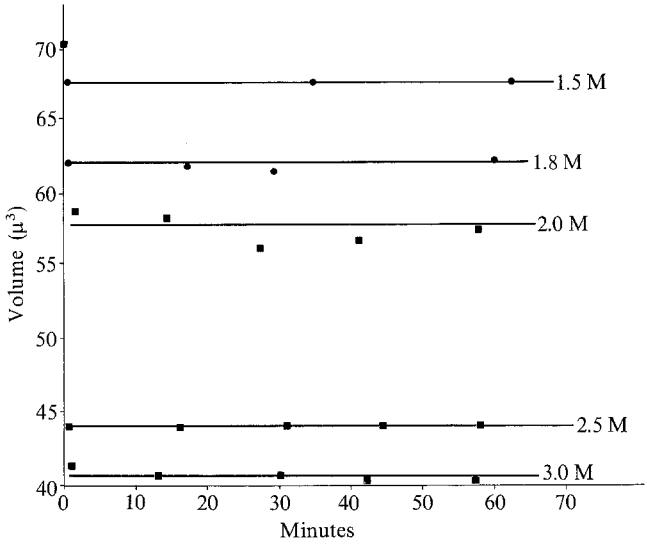

Fig. 7. Change in volume of *Dunaliella parva* cells as a function of time after increase of the concentration of suspending medium. Medium included 50 mM Tris-HCl at pH 7.4

in contrast to Fig. 5, the time scale is in minutes rather than in seconds. It is seen that the cells first swell; after about 10 min there is a spontaneous reduction in volume, which proceeds until the volume has returned to the original volume. The kinetics of this volume change are almost identical with production of glycerol that occurs under similar conditions (Ben-Amotz & Avron, 1973). Fig. 7 shows a time-curve of the changes in cell volume which followed an increase in concentration of the outside salt solution. Here we do observe a decrease in cell volume. Once the cells have arrived at a given volume, their volume stays constant for at least 90 min. It can be assumed that they reached osmotic equilibrium.

As the cells do not have a rigid cell wall, it can be assumed that there is no hydrostatic pressure difference across the membrane. The volume flow J_v can be described by a simple phenomenological equation: (Kedem & Katchalsky, 1958)

$$J_v = \frac{1}{A}\frac{dV}{dt} = -\sigma L_p \Delta\pi \tag{7}$$

where A = area of outer surface, V = cell volume, σ = reflection, or selectivity coefficient, L_p = hydraulic coefficient and $\Delta\pi$ = osmotic pressure difference. Eq. (7) can be cast in a more explicit form:

$$\frac{1}{A}\frac{dV}{dt} = -\sigma L_p RT\left(\frac{\Sigma n_i}{V} - C_o\right) \tag{8}$$

where C_o = concentration of outside solution. Integrating Eq. (8) yields

$$\int \frac{1}{A} dV = -\sigma L_p RT\left(\Sigma n_i \int \frac{dt}{V} - C_o \int dt\right). \tag{9}$$

It is assumed that n_i does not change for the duration of the experiment. As the cell suspension is very dilute, C_o can also be considered constant.

The numerical integration of Eq. (9) for short intervals of time yields the value of σL_p for each time interval. Note was taken of the fact that a change in volume was accompanied by a proportionate change in area.

A typical example of such an analysis is shown in Fig. 8. It can be seen that during the first time interval the value of (σL_p) is 5.2×10^{-12} cm^3/dyne sec. For all the other time intervals the values of (σL_p) are similar to each other and are about 25 times smaller than the first (σL_p). No assumption was made concerning the value of σ.

The same pattern was obtained with all the measurements reported here. It is concluded that the osmotic volume changes of the *Dunaliella* cells can best be described by two consecutive kinetic parameters, $(\sigma L_p)_1$ and $(\sigma L_p)_2$, which differ from each other by about one order of magnitude.

Table 4 presents a collection of kinetic coefficients, (σL_p), for a number of cultures. There are big differences between values obtained on different days. A similar spread was found in measurements of cell volume in isotonic solutions; there was no correlation between the initial volume (in

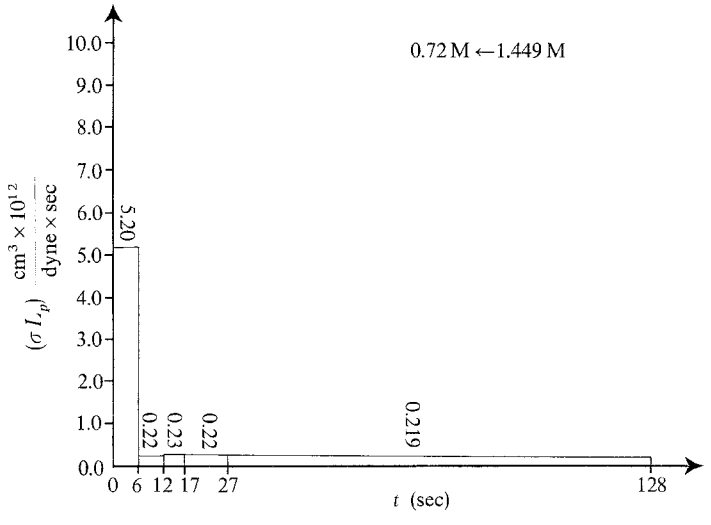

Fig. 8. Kinetic coefficient (σL_p) of volume change of *Dunaliella parva* when the suspending volume was diluted from 1.5 to 0.7 M. Medium included 50 mM of Tris-HCl at pH 7.4. (σL_p) was obtained by numerical integration of Eq. (9)

Table 4. The kinetic coefficients, $(\sigma L_p)_1$ and $(\sigma L_p)_2$, of the volume change of *Dunaliella parva* cells, when changed from solutions of NaCl or KCl isotonic with 1.5 M NaCl to a solution of 0.6 M NaCl or KCl

Culture	NaCl $\left(\frac{cm^3}{sec \times dyne} \times 10^{12}\right)$		KCl $\left(\frac{cm^3}{sec \times dyne} \times 10^{12}\right)$	
	$(\sigma L_p)_1$	$(\sigma L_p)_2$	$(\sigma L_p)_1$	$(\sigma L_p)_2$
1	5.07	0.35	5.26	0.58
2	3.7	0.60	–	–
3	7.6	0.47	12.6	0.50
4	0.46	0.14	0.78	0.22
5	4.3	0.40	7.04	0.50
6	3.7	0.65	–	–
7	5.2	0.23	6.3	0.30
8	2.54	0.25	3.2	0.34
9	3.75	0.12	3.8	0.25
10	4.35	0.17	4.5	0.20
11	1.48	0.40	1.6	0.43
12	1.75	0.50	1.9	0.57
Mean	3.7	0.35	4.7	0.39

(σL_p) has been calculated according to Eq. (7). The data are for cultures grown on different days. Measurements made at 22 ± 1 °C.

isotonic solution) and the kinetic parameters. As discussed before, the variability was not due to experimental error: measurements made under the same conditions on the same day had a standard error of no more than 2–3 %. It thus seemed that comparisons must be made between cells taken from the same culture subjected to different treatments on the same day.

Two major observations can be made from these data. Firstly, the kinetic data are described by two kinetic coefficients, $(\sigma L_p)_1$ and $(\sigma L_p)_2$. Secondly, there was a consistent difference between the σL_p of cells in NaCl and that of cells in KCl: the values are consistently higher for cells in KCl. Even more pronounced differences are seen in Table 5 which shows results for three species of cations. σL_p for cells in CsCl is almost twice as high as for cells in $MgCl_2$; these differences exist clearly for $(\sigma L_p)_1$ and are smaller, or nonexistent, for $(\sigma L_p)_2$.

Table 6 shows that the kinetic coefficients are dependent upon the direction of the volume flow; there is a noticeable asymmetry in the system. σL_p was twice as large for water-*in*flow as for water-*out*flow in the 0.7–1.5 M concentration range and was 6 times as large (in/outflow) in the 1.5–2.0 M

Table 5. The kinetic coefficients, $(\sigma L_p)_1$ and $(\sigma L_p)_2$, of the volume change of *Dunaliella parva* cells, suspended in different salt solutions, when changed from solution isotonic to 1.5 M NaCl to 0.7 M of the given salt solution

Culture	$\frac{cm^3}{sec \times dyne} \times 10^{12}$	
	$(\sigma L_p)_1$	$(\sigma L_p)_2$
Culture 1		
CsCl	1.64	0.25
NaCl	1.20	0.25
$MgCl_2$	0.90	0.22
Culture 2		
CsCl	1.37	0.30
NaCl	0.98	0.20
$MgCl_2$	0.80	0.15
Culture 3		
CsCl	2.25	0.176
NaCl	2.05	0.119
$MgCl_2$	1.45	0.134
Mean		
CsCl	1.75	0.21
NaCl	1.41	0.22
$MgCl_2$	1.05	0.17

Table 6. The kinetic coefficients, $(\sigma L_p)_1$ and $(\sigma L_p)_2$, of volume change of *Dunaliella parva* cells when subjected to an increase or decrease in concentration of the outer solution

NaCl $\left(\frac{cm^3}{sec \times dyne} \times 10^{12}\right)$			KCl $\left(\frac{cm^3}{sec \times dyne} \times 10^{12}\right)$		
Conditions of experiment	$(\sigma L_p)_1$	$(\sigma L_p)_2$	Conditions of experiment	$(\sigma L_p)_1$	$(\sigma L_p)_2$
1.5 → 0.72 M	5.2	0.22	1.5 → 0.74 M	6.3	0.32
0.72 → 1.5 M	2.9	0.16	0.74 → 1.5 M	3.3	0.13
1.5 → 2.83 M	8.8	0.40	1.5 → 3.05 M	9.0	0.50
2.83 → 1.5 M	1.30	0.138	3.05 → 1.5 M	1.56	0.106

range. This asymmetry is consistent with a system of two membranes in series, where the membranes have dissimilar permeability parameters (Ginzburg, 1962; Kedem & Katchalsky, 1963). When $\sigma_1 \neq \sigma_2$, the asymmetry is consistent with the description of a system with two kinetic coefficients.

Discussion

This paper has presented measurements of the cell volume of *Dunaliella parva* and the changes occurring in the parameter after transferring cells to a solution at a different concentration or of a different salt. These measurements have led to a description of the osmotic behavior of the cell, and have made it possible to calculate the kinetic parameters relating to changes in cell volume. Surprisingly enough, both the osmotic behavior and change in cell volume were found to be strongly dependent on the nature of the cation component of the salt solution.

Figs. 5, 6 and 7 demonstrate three kinetic parameters, two referring to increase in cell volume (apparent half times: 1–2 sec and 30 sec) and the third to the decrease in volume which is thought to be due to glycerol production (apparent half time: 40–50 min). It is clear that the influence of the third parameter on the first two is negligible and does not affect the maximum volume measured due to the decrease in salt concentration.

For any one salt solution, the cell volume followed the Boyle-Van't Hoff model. Were the cells to behave as an ideal osmometer, however, its changes in volume should be independent of the nature of the cation in the ambient salt solution. In fact, the maximum volume was found in cells in solutions of $MgCl_2$, and the smallest volume in solutions of CsCl, the whole sequence being $MgCl_2 > CaCl_2 > NaCl > KCl > CsCl$. When cell volume was plotted against $1/\pi_o$, as in Figs. 3 and 4, a series of straight lines was obtained, each line referring to a different salt solution. It can be concluded that the effect of the cation was obtained on the intercept and thus on the nonosmotic volume, rather than on the slope, or osmotic volume of the cell.

Even though the nonosmotic volume (parameter *b*) has been much discussed, its physical meaning is not well understood (Dick, 1966; Gary-Bobo & Solomon, 1968; Dick, 1971). The nonosmotic volume usually represents 20–30 % of total cell volume and 60 % of the volume of organelles such as mitochondria and chloroplasts (Dick, 1966). In *D. parva* cells in 1.5 M NaCl the nonosmotic volume represents 60–80 % of the total cell volume. Under the same conditions the dry weight is 40 % of the total fresh weight. Were the density to be 1, the dry matter would comprise 40 % of the cell volume so that the nonosmotic volume would consist of the dry matter plus a considerable portion of the cell water; in other words, not less than half the amount of cell water does not participate in osmotic volume changes, as interpreted by Eq. (2), an unacceptable conclusion. Such a conclusion is made even less acceptable by the fact that the main cell solute appears to be glycerol (Ben-Amotz & Avron, 1973).

Scatchard *et al.* (1938) showed that the osmotic coefficient for glycerol is 1 and that $d\phi/d\pi \simeq 0$ for concentrations up to 10 M. Thus, unless either glycerol and/or water have very strong interactions with the macromolecules or gel structures within the cell, they should both participate in osmotic volume changes.

The single huge chloroplast, which occupies over half of the cell volume, may contribute towards the high value of b, though it cannot solely account for it. As already mentioned, isolated chloroplasts have high b values.

It is difficult to see how b could be sensitive to the nature of the cation component of the outside solution unless the compartment comprising the nonosmotic volume were isolated from the osmotic volume and in direct communication with the outside solution. In summary, the nature of b is hard to understand.

The other parameters measured were the kinetic coefficients of volume change. These were calculated from Eq. (7) which applies to systems behaving like osmometers, and which therefore can be applied to *D. parva* cells for any one salt solution. The kinetic coefficient is the product of the hydraulic conductivity, L_p, and the reflection or selectivity coefficient, σ. It was intriguing to observe that two distinct kinetic coefficients, $(\sigma L_p)_1$ and $(\sigma L_p)_2$, were necessary to describe the kinetics of the volume changes. These could refer to two distinct cell populations each with its own characteristic water permeability (e.g. young and old cells), or they could refer to a single cell population in which the change in cell volume consisted of two distinct phases. The latter alternative is suggested as the more probable on the following grounds. Fig. 9 shows a time series of the changes

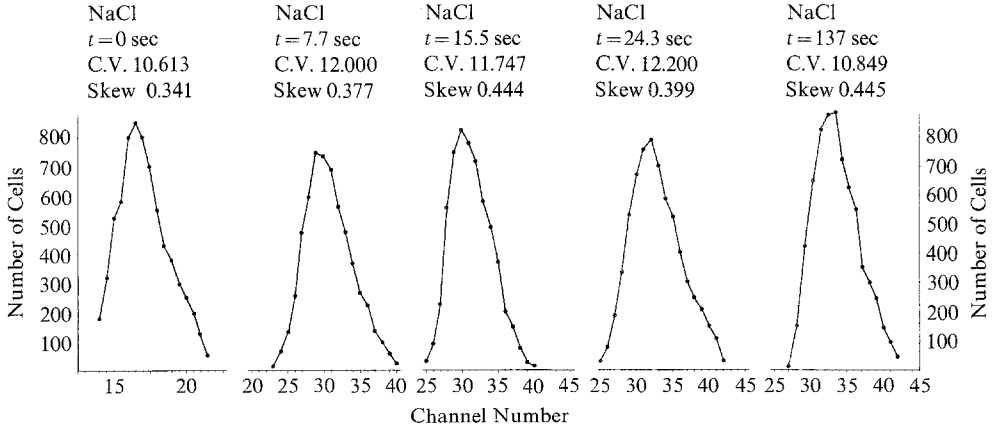

Fig. 9. Time sequence of size-distribution histograms of *Dunaliella parva* cells, when the suspending medium was changed from 1.5 to 0.7 M NaCl. C.V. = Coefficient of Variation

in cell volume caused by a change in concentration of the outside solution. If there were to be two cell populations, one with a fast-changing cell volume and the other with a more slowly-changing cell volume, one would expect to see a change in the asymmetry with time, especially at intermediate times; the skewness should grow to a maximum and then fall. In fact, there was no development of skewness with time.

Furthemore, calculations were made to predict the behavior of two distinct cell populations, N_1 and N_2 with kinetic coefficients $(\sigma L_p)_1$ and $(\sigma L_p)_2$ differing from each other by one order of magnitude. At time zero the maximum channel is the same for both populations; let this be channel 30 with coefficient of variation of 10 %=3. At time=7 sec there should be two distinct cell populations since N_1 has almost doubled in volume whereas N_2 has swelled by only 10 %:

	Mode	Standard deviation
N_1	60	6
N_2	33	3.3

Each population should be symmetrical around its mode. A Gaussian curve for N_1 is described by

$$0.7 \exp\left[-\tfrac{1}{2}\left(\frac{x-60}{6}\right)^2\right], \tag{10}$$

x=a given channel and 60=channel of maximum frequency. The corresponding equation for N_2 is

$$0.3 \exp\left[-\tfrac{1}{2}\left(\frac{x-33}{3.3}\right)^2\right]. \tag{11}$$

The solutions for the two populations are given below:

N_1		N_2	
channel no.	(exp)	channel no.	(exp)
50	0.175	23	0.003
55	0.492	28	0.096
60	0.70	33	0.3
65	0.492	38	0.096
70	0.175	43	0.003

It is clear that two separate populations emerge, with distinct maxima and almost no overlapping.

This calculation does not correspond at all to the data shown in Fig. 9. On the contrary, it can be concluded that the two kinetic coefficients $(\sigma L_p)_1$ and $(\sigma L_p)_2$ must refer to one and the same cell population. The *D. parva* cell contains two membranes to which the parameters might apply, the cell membrane to which $(\sigma L_p)_1$ may be assigned, and the chloroplast membrane (either the outer or the thylakoid) for $(\sigma L_p)_2$.

The outer surface area of the cell was used as the numerical value of the area A for the calculation of both $(\sigma L_p)_1$ and of $(\sigma L_p)_2$ in Eq. (9). Since $(\sigma L_p)_2$ is presumed to refer to the chloroplast membrane, the calculated numerical value of $(\sigma L_p)_2$ must be cut by a factor depending on the ratio of the areas of the chloroplast outer membranes. It seems probable that the surface area of the chloroplast is about twice that of the cell membrane. Thus, the quoted value of $(\sigma L_p)_2$ is probably twice its real value, should it indeed refer to the chloroplast. Should $(\sigma L_p)_2$ refer to the thylakoid membrane, which is even larger, then the value of $(\sigma L_p)_2$ must be even smaller.

Fig. 5 shows that $(\sigma L_p)_1$ describes about 60–70 % of the total volume change while $(\sigma L_p)_2$ is responsible for the remaining 30–40 %. It is important to try to assign these proportions to different regions in the cell. It is unfortunate that electron-microscopic sections have not yet been made of cells in the process of changing volume. There are, however, sections of cells at equilibrium in 1.5 M NaCl; in these sections about half of the total cell area is occupied by the chloroplast. This latter is packed with starch grains which undoubtedly do not change in volume with changes in the tonicity of the outside solution. Thus, the fraction of the chloroplast which is capable of participating in changes of volume must represent less than half of the total cell volume. There is therefore some correspondence between the relative volumes to which $(\sigma L_p)_1$ and $(\sigma L_p)_2$ are assigned and the relative volumes of the cell and the chloroplast.

A model of two membranes in series can be made to account for the asymmetry of $(\sigma L_p)_1$ and $(\sigma L_p)_2$ with respect to direction of water flow and the dependence of the two parameters on the concentration of C_o (Ginzburg, 1962; Kedem & Katchalsky, 1963). A necessary condition for asymmetry is that $\sigma_1 \neq \sigma_2$. The size of the asymmetry indicates a large difference between σ_1 and σ_2. However, the degree of asymmetry also depends upon the other membrane parameters and on the rate of water flow.

$(\sigma L_p)_1$ must now be discussed for cells moved from equilibrium conditions at 1.5 M NaCl to 0.7 M NaCl. The value of $(\sigma L_p)_1$ depends on the nature of the cation present in the outer solution, being highest in CsCl

and lowest in $MgCl_2$; the following sequence was observed: Cs > K > Na > Mg.

One way to explain the phenomena, but not necessarily the only one is as follows: At high salt concentration the values of such parameters of solution as viscosity (Stokes & Mills, 1965) and self-diffusion of water (Wang, 1954) depend pronouncedly on the nature of the cation component; viscosity decreases in the order Cs > K > Na > Mg. This same order was found for the values of $(\sigma L_p)_1$. The similarity of the sequences suggests that the viscosity of the solution is a determining factor in the entry of water into the cell. This could be the case if solutions at high concentration pass through pores in the cell membrane which would therefore have a low selectivity for ions relative to water ($\sigma = 0.1 - 0.01$). If σ were to be so low, then $(L_p)_1$ would have a value of $2 - 5 \times 10^{-11}$ cm^3/dyne sec, a very high value indeed.

As shown before, only 20–40 % of the total volume of the cell takes part in volume changes. The compartment related to $(\sigma L_p)_1$ must then represent 60–70 % of this osmotic volume. Thus, some 10–20 % of the total cell volume must equilibrate quickly with the surrounding solution while the remainder of the cell is virtually impermeable to ions. One would then expect the average concentration of NaCl within cells at equilibrium with 1.5 M NaCl to be 0.3 M. In fact, Ben-Amotz (1973) measured the cell concentration of NaCl and found it to be 0.2 M, in good agreement with the amount expected here.

The postulated low selectivity of the outer membrane of ions relative to water explains the sensitivity of the nonosmotic volume to the nature of the cation in the bathing medium.

The osmotic volume related to $(L_p)_1$ roughly fits the cytoplasm volume of the cell, excluding the nucleus. One would like to conclude that the NaCl concentration in the cytoplasm is the same as in the bathing medium. This conclusion does not, however, fit the observation of Ben-Amotz and Avron (1972) that several enzymes of *D. parva* are inhibited by high salt concentrations. Furthermore, it is hard to see how soluble vital substances escape from being leached from the cytoplasm.

Full explanation of the phenomena described here, requires further types of measurements for adequate proof or disproval. Measurements of ion content might help to resolve the puzzling questions which arose in the course of the investigation.

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