

Effects of Mannitol Hypertonicity on Water and Ion Contents in the Alga *Hydrodictyon reticulatum*

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Abstract. Long-term effects of mannitol hypertonicity on the water and ion contents in the fresh-water alga *Hydrodictyon reticulatum* were studied. The cells did not behave as osmometers and a closer examination suggested that wall elasticity was related to the medium tonicity and that the rate of sodium extrusion from the cells depended on the wall (turgor) pressure.

Key words: Alga *Hydrodictyon reticulatum* — Cell volume regulation — Mannitol hypertonicity — Wall (turgor) pressure — Sodium transport

Introduction

A study of effects of medium tonicity on water and osmolyte contents of cells is likely to bring out new aspects of cell membrane transport even when such phenomena are non-physiological and hardly occurring in nature. Changes brought about in the fresh-water green alga *Hydrodictyon reticulatum*, whose habitat are clear ponds, by prolonged incubation in hypertonic media containing mannitol are of this kind. Plant cells can react to medium hypertonicity in three ways (some combination of all the three being probable): (i) part of the cell water content is lost, (ii) intracellular osmolarity is changed by transport of ions or molecules across the plasmalemma, by synthesis of osmotically active substances or by disintegration of macromolecules, (iii) the wall pressure (turgor) is changed. The primary aim of the present study was to investigate the relative importance and mutual dependence of the above mechanisms in the overall reaction of *Hydrodictyon reticulatum* to mannitol hypertonicity.

Materials and Methods

Small nets of *Hydrodictyon reticulatum* (L.) Lagerh. (a few days after they had been released from maternal cells) were cultivated in medium containing (in mmol/l): NaCl

(0.5), KNO_3 (0.55), KH_2PO_4 (0.367), K_2HPO_4 (0.287), CaCl_2 (0.2), MgSO_4 (0.05) and Fe-citrate (0.05). Soil decoction and trace elements were added as described earlier (Rybová et al. 1988). After 10 days the algae were transferred into media without mannitol (controls), media containing 50 mmol/l mannitol, and media containing 100 mmol/l mannitol. In these media the algae grew mostly for about another 10 days (precise data are given in Results).

In samples of about 50 mg fresh weight the content of water was determined from loss of weight after drying at 95 °C overnight. Extractions of dry solids in 0.01 mol/l H_2SO_4 were used for assays of sodium, potassium and chloride ions. Sodium and potassium contents were estimated using the EEL flame photometer, and potentiometric titration was used to determine the content of chloride anions.

Table 1. Effect of 50 mmol/l and 100 mmol/l mannitol in medium on water contents of the alga *Hydrodictyon reticulatum*. Averages of five experiments carried out for 9 to 11 days.

	Controls (APW)	APW + 50mmol/l mannitol	APW + 100mmol/l mannitol
H_2O (kg/kg dry solids)	14.94 ± 0.15 [n = 64]	11.65 ± 0.21 [n = 64]	11.03 ± 0.23 [n = 64]

Results

The effect of 50 mmol/l and 100 mmol/l mannitol on the water content of *Hydrodictyon reticulatum* was determined in five experiments carried out for 9 to 11 days (Table 1). The elevation of the mannitol concentration in the medium from 50 mmol/l to 100 mmol/l caused only a little further shrinkage of the cells (significant only at the 5% level). This, however, is the average, even if the most frequent, behaviour. (It is also supported by an experiment, not included in the average, in which the algae were incubated only in the medium containing 50 mmol/l mannitol and in the medium containing 100 mmol/l mannitol just for three days: the results were 13.30 ± 0.36 kg H_2O /kg dry solids [$n = 10$] and 13.22 ± 0.27 [$n = 10$], respectively.)

When the individual experiments, however, were evaluated separately, the picture was somewhat different. In two of five experiments the algae reacted in a different way. Obviously, they differed in their properties, albeit they were grown under analogous conditions and in one case (denoted as III in Fig. 1) even at the same time and just next to the batch displaying the „average” behaviour. The water contents in the two experiments deviating from the average behaviour are

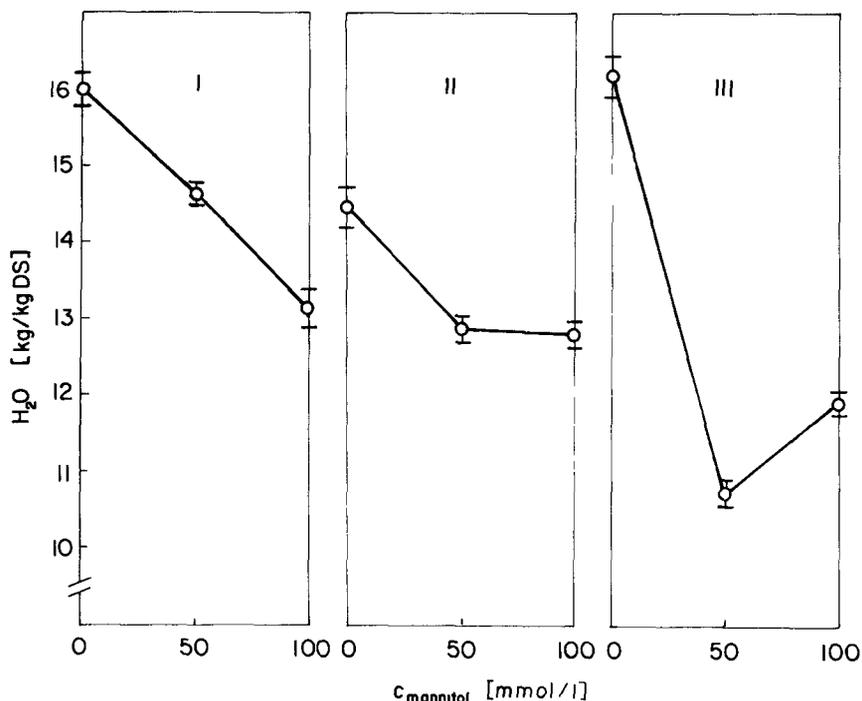


Figure 1. Three individual experiments displaying the dependence of water contents in the alga *Hydrodictyon reticulatum* on the mannitol tonicity in the medium. I: incubation time 10 days, $n = 10$; II: 9 days, $n = 12$; III: 11 days, $n = 11$.

shown (as I and III) in Fig. 1, which summarizes three experiments purposely chosen to be analysed in detail; only the one in the middle (II) has shown the average response. The difference in the mannitol osmolarity between 50 mmol/l and 100 mmol/l may occasionally bring about either a highly significant decrease or a highly significant increase in the water content, i.e., in the volume of the algal cells.

The closer analysis of the three so different results consisted in the determination of contents of major ions in the cells. The changes in the chloride anion content brought about by the mannitol hypertonicity followed exactly those in the water content (Fig. 2, compare with Fig. 1). Also the changes in the potassium content resembled them rather closely (Fig. 3), whereas the sodium ion pattern was somewhat different (Fig. 4). The ion contents divided by corresponding water contents give apparent concentrations of ions; in view of the large central vacuole of the alga *Hydrodictyon reticulatum*, in which the ions are not likely to be bound, they may well reflect the real concentrations (with the possible exception of the

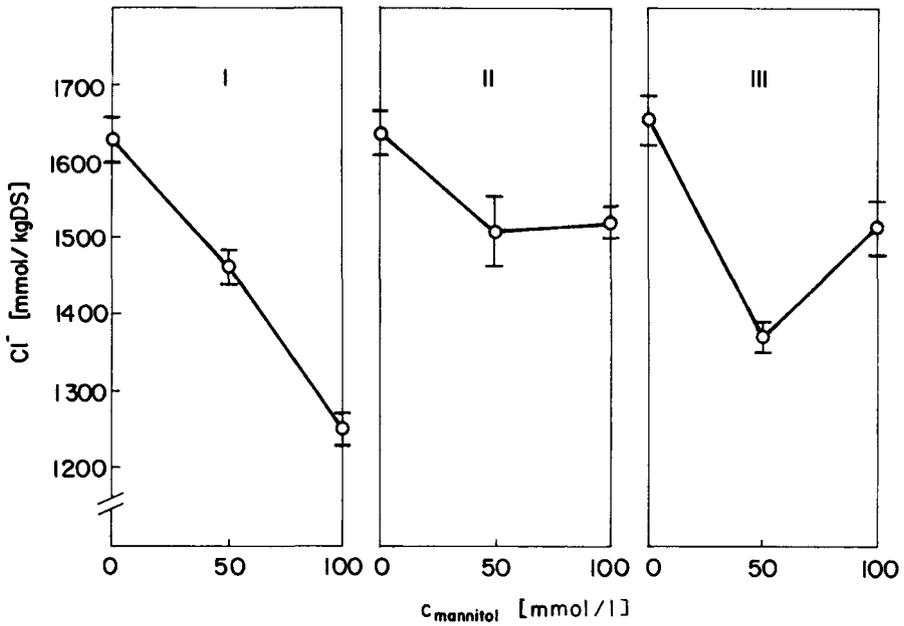


Figure 2. Dependence of chloride ion contents of the alga on mannitol tonicity in the medium. The same experiments as in Fig. 1.

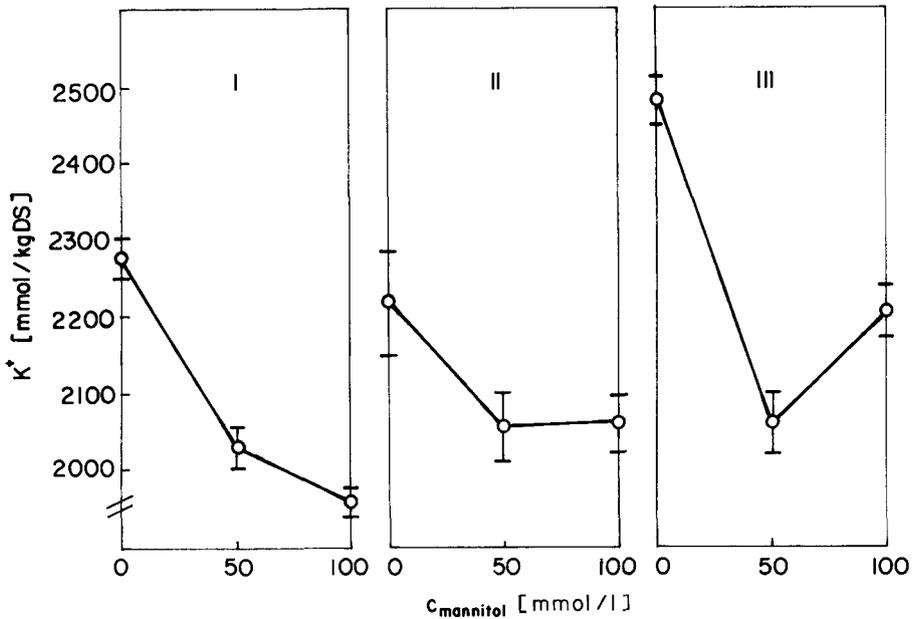


Figure 3. Dependence of potassium ion contents of the alga on mannitol tonicity in the medium. The same experiments as in Fig. 1.

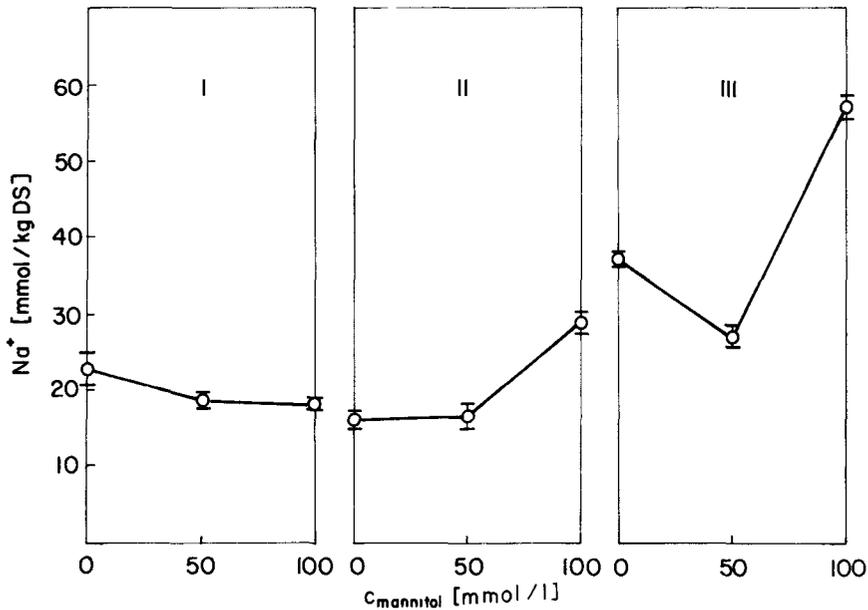


Figure 4. Dependence of sodium ion contents of the alga on mannitol tonicity in the medium. The same experiments as in Fig 1

Table 2. Effects of mannitol osmolarity on intracellular potassium chloride and sodium concentrations (mmol/l) in the alga *Hydrodictyon reticulatum*

Experiment	Controls	50 mmol/l mannitol	100 mmol/l mannitol	
[K ⁺]	I	142.4 ± 1.7	138.7 ± 2.1	149.3 ± 2.7
	[n = 10]		0.20 < p < 0.25	0.005 < p < 0.01
	II	153.2 ± 3.5	157.2 ± 3.5	163.0 ± 2.6
[n = 12]		0.40 < p < 0.50	0.10 < p < 0.20	
[Cl ⁻]	III	153.5 ± 2.4	193.3 ± 4.7	185.4 ± 3.2
	[n = 11]		p << 0.001	0.10 < p < 0.20
	I	101.8 ± 2.1	99.9 ± 1.0	95.5 ± 1.8
[n = 10]		0.40 < p < 0.50	0.025 < p < 0.05	
[Na ⁺]	II	113.3 ± 1.1	116.7 ± 3.3	118.8 ± 1.0
	[n = 12]		0.40 < p < 0.50	0.50 < p < 0.60
	III	102.6 ± 2.2	128.3 ± 2.3	127.4 ± 2.6
[n = 11]		p << 0.001	0.70 < p < 0.80	
[Na ⁺]	I	1.28 ± 0.14	1.28 ± 0.04	1.38 ± 0.06
	[n = 10]		p > 0.9	0.10 < p < 0.20
	II	1.11 ± 0.08	1.31 ± 0.13	2.00 ± 0.09
[n = 12]		0.20 < p < 0.25	p < 0.001	
[Na ⁺]	III	2.30 ± 0.05	1.93 ± 0.04	4.79 ± 0.13
	[n = 11]		p << 0.001	p << 0.001

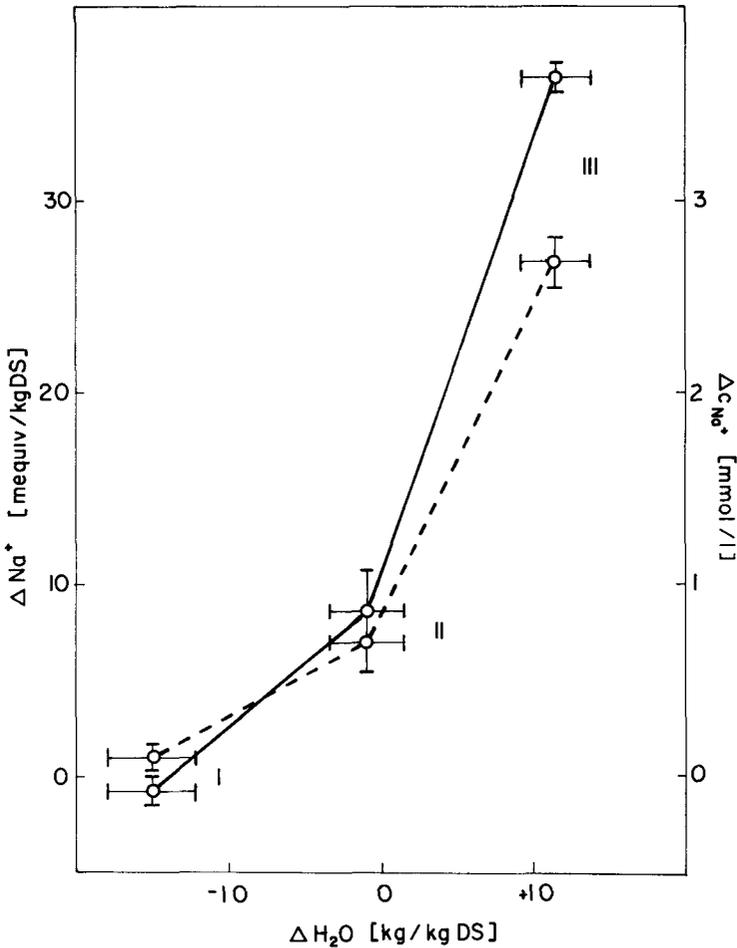


Figure 5. Dependence of difference in sodium ion contents and sodium ion concentration between algae incubated with 100 mmol/l and 50 mmol/l mannitol on the corresponding water content differences. Full line—contents; dashed lines—concentrations.

sodium ions, part of which appears to be bound in the cytoplasm (Rybova et al 1972). The concentrations obtained in this way are summarized in Table 2. The shifts in ion concentrations are either absent or unable to compensate quantitatively for the large changes in the external osmotic pressure. It will be argued in the Discussion that the same is likely to be true for other osmolytes too. An impressive correlation was found between the sodium ion contents or concentration and the corresponding water content differences, i.e. the differences between algae

incubated with 50 mmol/l and 100 mmol/l mannitol, respectively (Fig. 5). Still, the sodium concentrations involved are too small to serve as a basis for osmoregulation.

Discussion

We believe that there are two new and possibly interesting findings in the present study: (i) the algal cell behaves only exceptionally as an osmometer when the medium tonicity increases over a certain level, (ii) the differences in cell sodium content (as well as the sodium ion concentration) between algae incubated with 50 mmol/l and 100 mmol/l mannitol display a very high positive correlation with the respective differences in the water contents.

Let us first discuss the lack of osmotic behaviour in the hypertonic media. We can imagine for the sake of argument that mannitol penetrates into the cells. May its intracellular osmotic effect explain the observed behaviour? The following consideration seems to exclude such a possibility. If mannitol were a permeating substance it would have had ample opportunity to equilibrate during the experiments which lasted 9 to 11 days. The contents of dry solids of the cells would then increase. Using the data of Table 1, dry solids of the cells in 100 mmol/l mannitol would be 1.106 kg instead of 1 kg. This would result by itself in an apparent difference in the water content, even if there were no transfers of water at all. To the value of 11.65 kg H₂O/kg dry solids in the 50 mmol/l mannitol medium would then correspond 10.53 kg H₂O/kg dry solids in the 100 mmol/l mannitol medium. However, the value found in the experiments was higher (11.03), and in the experiment denoted as III in Fig. 1 even a highly significant increase in the water content was demonstrated. Hence the algal plasma membrane appears to display the common impermeability to mannitol in our experiments. The above reasoning applies also to the idea that new osmolytes might be synthesized inside the cells.

The next possibility is that hypertonicity initiates a disintegration of some macromolecules; such process would not affect the total mass of dry solids. However, there may be not enough macromolecules in the cells for such purpose as calculated earlier (Nešpůrková 1983) from electronmicrophotographs of *Hydrodictyon* mature cells (cf. Marchant and Pickett-Heaps 1971), the ratio of vacuolar to cytoplasmic volume is about ten to one and it is not likely that there are many macromolecules in the vacuole. One can imagine a disintegration of macromolecules in the cytoplasm followed by transfer of osmolytes into the vacuole, but a significant amount of an organic osmolyte would then be certainly found in boiling water extracts from the algae kept formerly in 100 mmol/l mannitol by the HPLC technique, and this was not the case.

The shifts in concentrations of principal ions between the 50 mmol/l and 100 mmol/l mannitol algae (Table 2) cannot compensate quantitatively the large changes in the external osmotic pressure. Thus, to explain the lack of even a poor

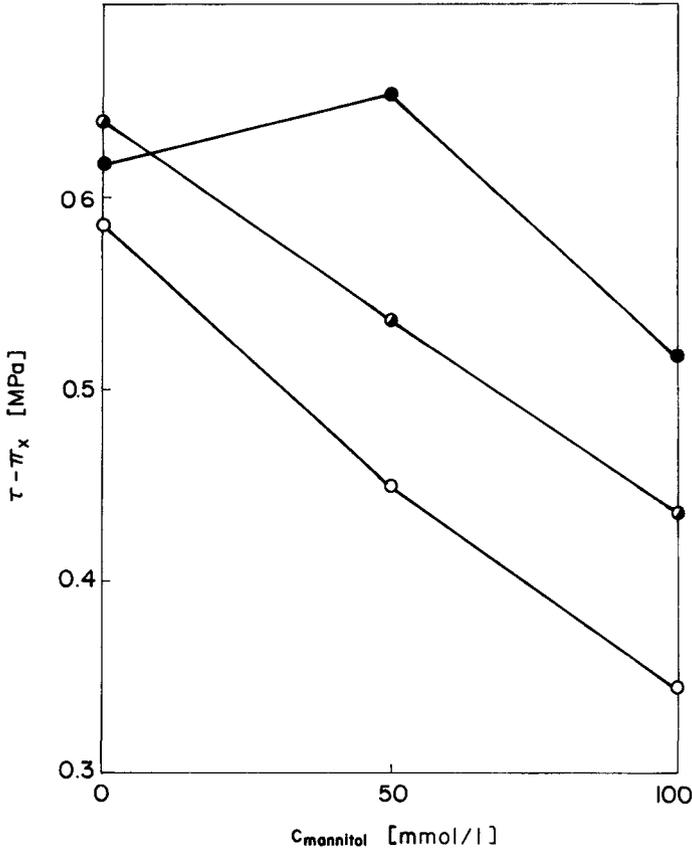


Figure 6. Dependence of wall pressure estimate on mannitol concentration in the medium in the three experiments described in the preceding Figures.

osmometric behaviour in the hypertonic region, we are left only with changes in the wall (turgor) pressure.

How can we estimate the wall pressure when the elegant pressure-probe method (Zimmermann and Steudle 1974) is not accessible in our laboratory? We can start with the water equilibrium condition

$$\pi_{\text{K}^++\text{Na}^++\text{Cl}^-} + \pi_x = \pi_{\text{medium}} + \tau$$

i.e.

$$\tau - \pi_x = \pi_{\text{K}^++\text{Na}^++\text{Cl}^-} - \pi_{\text{medium}}$$

where $\pi_{\text{K}^++\text{Na}^++\text{Cl}^-}$ is the osmotic pressure calculated from the measured intracellular concentrations of the three ions, π_{medium} is the osmotic pressure of the

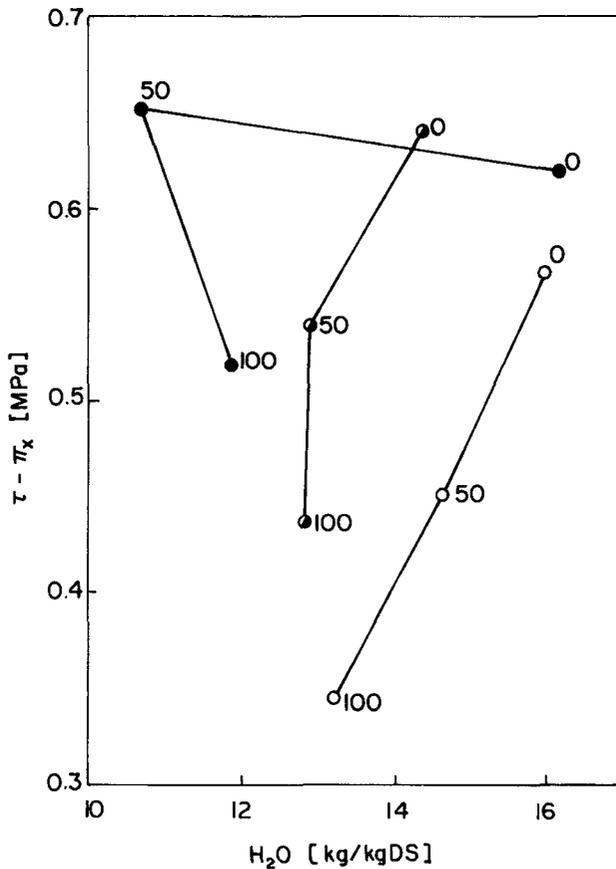


Figure 7. Dependence of the wall pressure estimate on cell water contents. Empty circles – experiment I, half-full circles – experiment II, full circles – experiment III.

medium with or without mannitol calculated from its composition (van't Hoff's formula $\pi = RTc$ was used), τ is the wall pressure. Under π_x we understand the osmotic pressure of unidentified components, perhaps mostly anions. We believe this term to be small and for practical purposes constant. Thus the value $\tau - \pi_x$ appearing in Fig. 6 should be understood as $\pi_{K^++Na^++Cl^-} - \pi_{\text{medium}}$ and this is our approximation to the wall pressure.

There was an overall drop in the wall pressure in hyperosmotic media with the exception of 50 mmol/l mannitol in experiment III (Fig. 6). This seems understandable, the pressure of the elastic wall is decreased with the cell volume shrinkage. However, the relation between our estimate of the wall pressure and the cell volume (represented by the cell water content) is rather bizarre (Fig. 7). The

Table 3. Dependence of sodium concentration on the estimated turgor pressure in the alga *Hydrodictyon reticulatum* growing in medium containing 100 mmol/l mannitol

Experiment	$\tau - \pi_x$ [MPa]	c_{Na^+} [mmol/l]
I	0.343	1.38 ± 0.06 [n = 10]
II	0.435	2.00 ± 0.09 [n = 12]
III	0.517	4.79 ± 0.13 [n = 11]

response of the cell volume to the external hypertonicity seems to depend on the way in which the cell wall itself reacts to hypertonicity. The water content of the wall equilibrates according to its matrix potential and the tonicity of the external medium, and one can imagine that the elasticity and plasticity properties of the wall are dependent on the result. These differences in properties may also reflect the fact that the cell walls during growth were stretched by different pressures. Be this as it may, the response of algae batches is not always the same. This recalls to us the deterministic chaos according to which small differences at the beginning (inoculation in our experiments) bring about considerable differences in the final behaviour of the mature cells.

Now we come to the result that the sodium content, osmotically insignificant, depends very markedly on the character of the water content difference between the 50 mmol/l and 100 mmol/l mannitol media. Table 3 shows the high positive correlation between the estimated turgor pressure and the sodium concentration in the alga. We can easily imagine that the activity of the sodium pump, described by us years ago as ouabain insensitive (Janáček and Rybová 1966), is influenced by the turgor pressure. Let us cite a general hypothesis by Bisson and Gutknecht (1980) explaining such phenomena: "The closer proximity of the membrane to the cell wall microfibrils may affect a chemical interaction, by which transport sites may be blocked or activated. Physical changes taking place in the membrane due to appression against the cell wall or stretch between the fibrils could alter the thickness of the membrane and hence the activity of proteins within it." It may well be the case. In our experiments the "closer proximity" (the higher turgor) would seem to reduce the activity of the pump extruding sodium ions from the cells.

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