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Osmotically Induced Proton Extrusion from Carrot Cells in Suspension Culture[1]

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ABSTRACT

Addition of 200 mM of a polyol to anthocyanin containing carrot (*Daucus carota* L.) cells in suspension culture decreased turgor pressure to zero and induced hyperpolarization of the membrane potential and acidification of the medium due to H^+ extrusion. These changes were shown to be slightly affected by vanadate. In parallel, a decrease in intracellular ATP and total adenylate concentrations were observed. However, when the osmoticum was NaCl acidification of the medium occurred in the absence of considerable changes in intracellular ATP concentration. These results are interpreted as indicating that a drop of turgor, by addition of a polyol, triggers a proton extrusion activity which is only slightly inhibited by vanadate but apparently ATP utilizing. The observed decrease in ATP level occurs without a change in respiration rate and is accompanied by a drop in total adenylate pool. However when NaCl is the osmoticum it is assumed that $\Delta\mu_{H+}$ is enhanced through a Na^+/H^+ antiporter. The difference between the two types of osmotica as related to their ability to penetrate through the cellular membrane is discussed.

It has been suggested that either the plasmalemma itself (2, 17, 34) or the plasmalemma-sited proton pump (which extrudes protons) might play a central role in the ability of plant cells to sense a change in turgor (22). The pump might be considered to be either the detector or the effector (or both) in turgor maintenance (2, 22). Indeed, changes in external osmoticum have been shown to induce modulations in membrane potential (11, 16, 17, 23, 26).

Transfer of anthocyanin-containing carrot cells grown in suspension culture to a saline medium is accompanied by a change in the absorption peak of the anthocyanin present in the vacuole from 537 nm to 545 nm. This change in absorption indicates an increase in vacuolar pH which is accompanied by acidification of the external medium (23). Since these cells, after being transferred to saline conditions, first plasmolyze and then eventually deplasmolyze and resume growth (25). The possible involvement of the plasmalemma-sited proton pump in the adaptation processes was investigated. Therefore, changes in external pH and membrane potential as affected by various osmotica were studied.

MATERIALS AND METHODS

Growth and Preparation of Cell Suspension. The carrot cell suspension was a gift from Dr. D. Aviv from the Weizmann Institute, Rehovot. This cell line was originally developed from a culture prepared from carrot roots by Dr. J. Reinert, Free University of Berlin, and has since been used in many laboratories (9). Cells were grown in Gamborg *et al.* (6) B_5 salt medium with the following additions: sucrose 20 g/L; casamino acids 600 mg/L; nicotinic acid 1 mg/L; thiamine HCl 10 mg/L; pyridoxine HCl 1 mg/L; *myo*-inositol 100 mg/L; 2,4-D 0.2 mg/L. The cells were grown in 100 ml medium in 250 ml Erlenmeyer flasks with continuous shaking (120 rotations/min) in continuous dim light at 26°C. Cells were harvested 6 to 8 d after inoculation (beginning of stationary phase) by collecting them on a 100 μm nylon screen, washed with 30 ml 0.2 mM $CaSO_4$, and suspended in 500 ml of 0.2 mM $CaSO_4$. Cells were collected again on a 100 μM screen and resuspended in a small volume at the concentration as indicated in the legend for each figure so that cell density would be about 1 gfw[3] per 5 ml of suspension (about 6×10^5 cells per 15 ml of the incubation medium).

Fresh weight was determined as the weight of the cells left on a Watman GF/A filter after filtering the cells under vacuum. One gfw is about 3 ml packed cell volume or 2×10^5 cells.

Membrane Potential Determination. Indication of the changes in membrane potential were deduced from the accumulation of $[^3H]TPP^+$ from the medium by the cells (24, 27). Cells were suspended in various media containing sorbitol and $CaSO_4$. In all experiments 16 μM of nonlabeled TPP^+ was added to 6×10^6 dpm of $[^3H]TPP^+$ to prevent adsorption (24). Since the radioactivity in the medium practically did not change during the assay, uptake of $[^3H]TPP^+$ above the control value was considered to indicate hyperpolarization. Aliquots of cell suspension were taken from all treatments at intervals and filtered on a GF/A filter under vacuum. The cells on the filter were washed with 10 ml of nonradioactive suspension medium, the filter plus the cells were put in a minivial without water and counted in Triton X-100:toluene (1:2) scintillation fluid. Data are expressed as the difference in radioactivity between t_{1min} and t_{120min}. The method was checked against $[TPP]_{in}$:$[TPP]_{out}$ ratio measurements (24) and was found satisfactory. The amount of radioactivity in the external medium was high enough so that the amount taken up by the cells did not affect the external concentrations and the

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[3] Abbreviations: gfw, gram fresh weight; TPP^+, tetraphenylphosphonium; FCCP, carbonyl cyanide-*p*-trifluormethoxyphenylhydrazone; PD, membrane potential difference; FC, fusicoccin; AEC, adenylate energy charge; MeG, 3-*O*-D-methylglucose; TEA, tetraethylammonium.

specific activity of [TPP^+]. Since diffusion of TPP^+ from the cells is slow there was no leakage of TPP^+ during the rinse.

Determination of Changes in External pH. Changes in pH were measured using a combined electrode (Radiometer GK2401B) either in cell free medium or directly in the cell suspension. Cell free media were prepared either by centrifugation (2000*g*) or by filtration through a GF/A filter. Cell suspensions were shaken during pH measurement or CO_2 free air was bubbled through the suspension. This last treatment eliminated the acidification due to CO_2 arising from respiration.

Malic acid was determined enzymatically (8).

Enzyme Inactivation and Nucleotide Extraction and Determination. Cells were frozen in liquid N_2 and thawed in 0.6 M cold TCA, vortexed, and passed through a GF/A filter. The acid was removed from the filtrate by extracting three times with cold diethyl ether. Traces of the ether were eliminated from the filtrate by bubbling air and the extracts adjusted to about pH 6 with KOH.

ATP was assayed by a bioluminescence method with a pico ATP biophotometer (Jobin et Yvon, Longjumeau, France). ADP and AMP were converted into ATP by the method of Pradet (19), as modified by Saglio *et al.* (28).

Chemicals. TPP^+ bromide was obtained from Merck, West Germany. [^{3}H]TPP^+ bromide 2510 mCi/mmol (1 mCi/ml) was from the Nuclear Research Center, Israel. All other chemicals were analytical grade.

RESULTS

Hyperpolarization of carrot cells and acidification of the external medium occurred following exposure of the cells to decreasing external osmotic potentials, induced by sorbitol (Fig. 1, A and B). Acidification of the medium was measured 60 min after exposure of cells to osmoticum, however at this time $[TPP^+]_{in}$ had not yet reached equilibrium and was therefore measured 120 min after addition of osmoticum. Maximal acidification and hyperpolarization occurred at 200 mM sorbitol (Fig. 1, A and B). At higher sorbitol concentrations decrease in the extent of acidification and depolarization of the cells was observed.

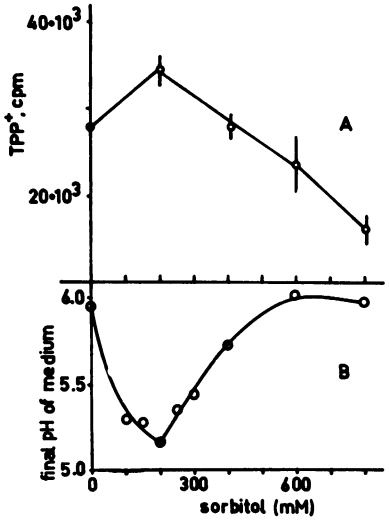

FIG. 1. Membrane potential of carrot cells and acidification of external medium as a function of increasing sorbitol concentrations. (A) An indication of membrane hyperpolarization is obtained by the increase in TPP^+ accumulated in the cells during a 120 min incubation period. Data are expressed as the difference in radioactivity between $t_{1\ min}$ and $t_{120\ min}$. Each point is an average of triplicates (±SD). (B) Acidification of medium by carrot cell suspension as a function of external sorbitol concentration in the presence of 0.2 mM $CaSO_4$. Cells were shaken in open flasks on a rotary shaker. After 60 min incubation pH was determined on cell free medium as described in "Materials and Methods." The initial pH was about 6.3. Each point is an average of duplicate.

Microscopic observations showed that 50% plasmolysis was obtained at 230 mM sorbitol (Fig. 2). Turgor pressure of the cells can be calculated as the difference between the osmotic potential yielding 50% plasmolysis, *i.e.* −5.1 bars, and the osmotic potential of the growth medium at the time of the experiment (6–8 d after inoculation) which was −0.7 bars. The calculated turgor pressure was approximately +4.5 bars, therefore addition of 200 mM polyol or 100 mM NaCl (−4.1 to −4.48 bars) should have brought turgor pressure close to zero.

In order to check whether the acidification caused by addition of 200 mM sorbitol was a specific effect of polyols or a general effect of osmotica, cells were incubated in the presence of 100 mM NaCl, 100 mM KCl, and 100 mM mannitol which all decreased osmotic potential by −4.0 to −4.5 bars. Tables I and II show that all these compounds induced enhanced acidification but the salts were more effective than the polyols. This acidifi-

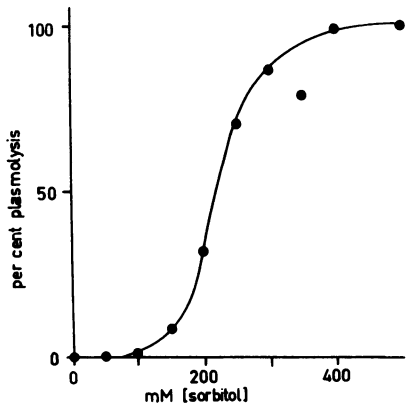

FIG. 2. Percent plasmolysis of carrot cells versus sorbitol concentration. Carrot cells 7 d after inoculation were collected on nylon mesh, rinsed with 0.2 mM $CaSO_4$, and resuspended in 0.2 mM $CaSO_4$ as described in "Materials and Methods." Various concentrations of sorbitol were added. The number of plasmolyzed cells was determined 5 to 10 min after addition of various sorbitol concentrations; at each sorbitol concentration 100 cells were counted.

Table I. *Acidification of External Medium Due to Addition of Various Osmotica*

A. Carrot cells from 8 d old cultures were filtered and resuspended in 2 mM Na-MES buffer, pH 5.9, containing 0.4 mM $CaSO_4$, 2 mM KCl ± mannitol or NaCl. After 2 h suspensions were centrifuged and pH values were measured before and after insufflating with N_2 for 15 min. For more details see text.

Media	pH after 2 h	pH after N_2 bubbling
Buffer 2 mM Na MES	6.19	6.22
Mannitol 200 mM (+ buffer)	5.79	5.84
NaCl 100 mM (+ buffer)	5.33	5.40

B. Cells were collected as described in methods. Measurements were made in duplicates after 3 h of incubation in osmotica at −4.5 bars. All media contained 0.2 mM $CaSO_4$.

Osmoticum	Initial pH	pH after 3 h
Sorbitol 200 mM	6.39	5.09
NaCl 100 mM	6.30	5.00
KCl 100 mM	6.36	4.96
Water[a]	6.26	5.51

[a] Water means cells incubated in 0.2 mM $CaSO_4$ only.

Table II. *Effect of Various Inhibitors on Proton Extrusion from Carrot Cells in Suspension Culture*

Cells were incubated with the various inhibitors in the presence of either 100 mM NaCl + 1 mM $CaSO_4$, 200 mM sorbitol + 1 mM $CaSO_4$ or 1 mM $CaSO_4$. Protons were measured by back titration. Data are expressed as percent of control at the same osmotic potential in the absence of inhibitor.

Inhibitors	Treatments		
	1 mM $CaSO_4$	100 mM NaCl + 1 mM $CaSO_4$	200 mM sorbitol + 1 mM $CaSO_4$
None[a]	100[b]	100[c]	100[d]
Vanadate 500 μM	0	68	86
Oligomycin 10 μM	17	58	45
Amiloride 10 μM	112	25	112
TEA 10 μM	88	96	76

[a] The absolute rates of proton extrusion in nmol/gfw·h are: [b] 37; [c] 382; and [d] 106.

cation of the external medium was not due to the respiratory CO_2; increased proton extrustion after addition of a polyol or salt occurred even when CO_2 was removed from the medium (CO_2 free air bubbling during the experiments summarized in Table II or N_2 bubbling through the cell free medium at the end of the experiment summarized in Table IA). Cells treated with either sorbitol or NaCl did not leak more malic acid than control cells.

Proton extrusion induced by NaCl was almost completely inhibited by amiloride (a Na^+/H^+ exchanger and Na^+ chanel inhibitor) and inhibited approximately 40% by vanadate (a plasmalemma ATPase inhibitor) or oligomycin (a mitochondrial ATPase inhibitor) (Table II). On the other hand sorbitol induced proton extrusion was neither inhibited by amiloride nor by vanadate, but was inhibited about 50% by oligomycin. Proton extrusion from control cells was inhibited by vanadate and oligomycin but not by amiloride (Table II). TEA, a potassium exchange inhibitor, did not inhibit proton extrusion induced by NaCl but slightly inhibited proton extrusion from control or sorbitol treated cells.

Since after 60 min incubation in sorbitol no change in respiration was observed (data not shown), enhanced activity of the proton pump might be accompanied by a decrease in ATP level. Figure 3A shows that treatment with 200 mM (−4.5 bars) sorbitol caused a decrease in ATP level of 60%, a drop of AEC from 0.95 to 0.8 (Fig. 3B) and a decrease in the adenylate pool (Fig. 3C). Treatment with about −4.5 bars of NaCl only lowered ATP level, during this time, by about 10% at the most and had no effect on AEC or total adenylate pool (Table IIIA). Mannitol had the same effect as sorbitol and KCl had the same effect as NaCl on both ATP level, AEC and total adenylate pool (data not shown). Table IIIA and Figure 3B show that the decrease in ATP 60 min after addition of sorbitol was not accompanied by a corresponding increase in ADP and AMP but rather that the total amount of the adenylate pool was lowered (Fig. 3C); however, with time the ATP level and total adenylate pool seem to be restored (Fig. 3). Table IIIB also shows that FC induced a drop in ATP levels as was reported previously (8, 10, 20). It also seems that NaCl prevented the FC-induced decrease in ATP level. Vanadate, a known inhibitor of the plasmalemma proton pump, caused an increase in ATP levels (Table IIIB) apparently due to the inhibition of the endogenous pump as shown in Table II. Inhibition by vanadate of sorbitol or NaCl induced proton extrusion, taking into account its buffering capacity, was limited (Table II).

FC is known to enhance proton extrusion in plant cells (13, 14). It is thought that this effect is due to enhancement of the plasmalemma proton pump. Figure 4 shows the effect of 10 μM FC on medium acidification by carrot cells. The control, curve 1, shows alkalization during the first 30 min followed by slow acidification. This is a usual behavior of cells. FC treated cells, curve 2, showed a greater acidification during the first 2 h than the control. Also in this case there was alkalization during the first 10 to 20 min (Fig. 4B) indicating a lag in the response of these cell to FC. Curve 3 in Figure 4A shows that addition of 200 mM sorbitol induced acidification which resembled the acidification induced by FC. Curve 4, in Figure 4, shows that the effects of FC and sorbitol were practically additive when present together in the reaction mixture. There was no increase in malic acid leakage from the cell in these treatments and no increased buffering capacity of the medium in sorbitol-treated cells.

Since Ca^{2+} is known to play a role in membrane tightness and to reverse membrane leakiness (32), its effect on proton extrusion was investigated. It can be seen from Figure 5 that sorbitol enhanced proton extrusion to the same extent at any Ca^{2+} concentrations from 0.1 mM to 10 mM. Other divalent cations had no effect (Table IV). Moreover Ca^{2+} ions not only enhanced acidification but their presence was essential for its demonstration; Ca^{2+} ions seem to be necessary for plasmalemma integrity. When cells were rinsed in medium which did not contain Ca^{2+} the acidification induced by 200 mM sorbitol was very low, probably due to plasma membrane damage (Table IV, 'water').

DISCUSSION

Hyperpolarization of membrane potential and acidification of the medium by cells upon exposure to decreasing osmotic potential have been reported in the literature. Marrè *et al.* (13) and Cleland (4) showed an increase of the medium acidification by mannitol up to 0.2 M with pea stem segments and *Avena* coleoptiles, respectively. Okazaki *et al.* (17) have shown that hypertonic treatment caused a slow hyperpolarization but did not significantly affect membrane resistance. Reinhold *et al.* (22) have shown that there is an osmotically induced net proton efflux from segments of *Senecio* leaf strips and the optimal osmotic potential for the effect was at −6.7 bars of polyol. This increase in osmoticum also enhanced MeG uptake which was not due to diminished MeG efflux. Okamoto *et al.* (16) have shown that 200 mM sorbitol caused a slow hyperpolarization of membrane potential in pea hypocotyl segments under aerobic conditions. From the results in the literature and from our results summarized in Figure 1 and Table I it is not possible to differentiate between enhanced proton pump activity and reduced proton influx. However the results concerning the effects of inhibitors (Table II) of FC (Fig. 4), of Ca^{2+} (Fig. 5 and Table IV) and the changes in ATP level (Fig. 3 and Table III) do enable us to suggest a possible mechanism for the effect of the different osmotica on the behaviour of the plasmalemma.

From Figure 4 it is apparent that the enhanced acidification induced by osmoticum and FC are practically additive. If 10 μM FC does cause maximal rate of proton extrusion via the proton pump (14) the osmoticum should be affecting a parameter different from that affected by FC. This conclusion is in agreement with the fact that although vanadate seems to inhibit the endogenous proton pump (29, 30) it only slightly inhibits osmotically induced proton extrusion (Table II). However, this result must be considered with caution as vanadate is an incomplete inhibitor *in vivo* (5, 32). The effect of osmoticum at −4.5 bars was also additive to the effect of Ca^{2+} on proton extrusion (Fig. 5). Since Ca^{2+} ions are known to reduce membrane leakage (31) by membrane tightening it would not be expected that the effect of sorbitol would be additive to that of the Ca^{2+} if sorbitol also affects membrane tightness. If sorbitol would act through the same mechanism as Ca^{2+} the two curves would probably not be so parallel (Fig. 5). It seems therefore that FC, Ca^{2+} and polyol at −4.5 bars affect different parameters of the plasmalemma proton extrusion apparatus.

Table III. *Effect of Sorbitol, NaCl, FC, and Vanadate on Adenylates*

A. Carrot cell suspensions were incubated for 60 min as in Figure 3. Adenylates were extracted according to "Materials and Methods". The data are from six measurements ($n = 6$).

Treatment	ATP	ADP	AMP	Σ Adenylates	AEC
			nmol/gfw ± SD		
Control	57.1 ± 1.3	10.3 ± 1.3	0.00	68.0 ± 3.5	0.92
Sorbitol 200 mM, 1 h	22.3 ± 1.0	9.7 ± 0.7	2.0 ± 0.5	34.0 ± 1.9	0.80
NaCl 100 mM, 1 h	52.0 ± 3.7	9.3 ± 0.7	0.6 ± 0.6	62.0 ± 4.0	0.91

B. Carrot cells from 8 d old cultures were washed in 0.2 mM $CaSO_4$. Cells were incubated for 1 h in solutions containing 0.2 mM $CaSO_4$ with the additions as listed below. ATP was determined according to "Materials and Methods" ($n = 3$).

Treatment	ATP Content
	nmol/gfw ± SD
Control	57 ± 2
Sorbitol 100 mM	37 ± 2
Sorbitol 100 mM	29 ± 2
NaCl 100 mM	67 ± 7
NaCl 100 mM + FC 10 mM	63 ± 3
FC 10 μM	37 ± 2
Vanadate 1 mM	85 ± 2

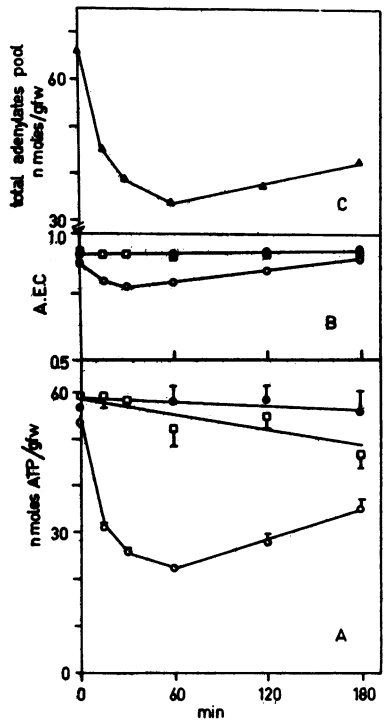

FIG. 3. ATP levels and calculated adenylate energy charge (AEC) of carrot cells as a function of time after addition of sorbitol or NaCl. Cells were incubated for indicated times in 200 mM sorbitol +1 mM $CaSO_4$ (○), 100 mM NaCl + 1 mM $CaSO_4$ (□), and in 1 mM $CaSO_4$ only (●). Total adenylate charge induced by sorbitol (▲). Each point represents the mean of three independent determinations done in duplicates ± SD.

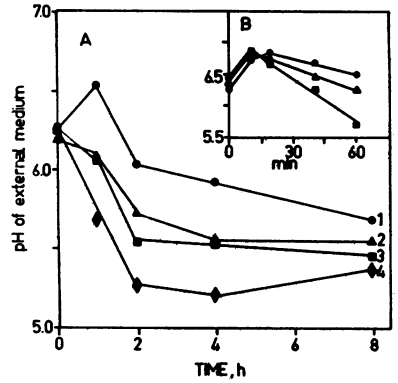

FIG. 4. Acidification of medium by carrot cell suspensions as a function of incubation time after addition of FC or sorbitol. *1*, control cells (●); *2*, cells + 10 μM FC (▲); 3, cells + 200 mOsmol sorbitol (■); *4*, cells + 10 μM FC + 200 mOsmol sorbitol (◆). All media contained 0.2 mM $CaSO_4$. (A) Kinetics during 8 h. (B) Detail of kinetics during the first 60 min.

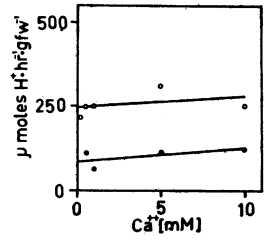

FIG. 5. Effect of Ca^{2+} concentration on sorbitol induced proton extrusion. Cells were washed in the indicated $CaSO_4$ concentrations and resuspended in that Ca^{2+} concentration with (○) or without (●) sorbitol. Cell free medium prepared from cell suspension incubated for 2 h was titrated back by KOH.

Tables II and III show that polyol-induced acidification requires metabolic energy (ATP). From Figure 3 and Table III it is apparent that there is a difference in ATP concentration in carrot cells treated for 1 h with sorbitol or NaCl. Addition of sorbitol triggers a mechanism that leads to a large decrease in ATP and total adenylate pool. The decrease in the ATP level is not due to phosphorylation of sorbitol as demonstrated by TLC analysis (data not shown). Decreases in ATP level in plant tissues due to FC enhanced acidification were reported by Rasi-Caldogno *et al.* (20), Guern *et al.* (8) and Hourmant and Cleland (10) thus indicating the possibility that the decrease in ATP levels observed by us is of the same nature, *i.e.* through the proton pump. If this explanation is applicable to the sorbitol induced acidification one would expect a much stronger inhibition by vanadate than was observed (Table II). Another possibility is that the loss of turgor induces a conformational change in membrane structure (18, 33) thus affecting the properties of the proton pump and making it vanadate insensitive (3).

Table IV. *Effect of Various Divalent Cations on Sorbitol (−4.5 bars) Induced Acidification*

Cell preparation and measurement were as in Figure 1B. Measurements were made in duplicates after 3 h incubation. Cells were washed before incubation in 2 mM solutions of the sulfates of the cations used that did not contain 0.2 mM $CaSO_4$. And then incubated in fresh solutions for measurements. ΔpH is expressed as the difference between t_0 and t_{3h}, Ca^{2+} was present in the incubation medium when Ca^{2+} is the cation used. In the water treatment the cells were washed only with distilled water. Data are means of duplicates.

Cation	ΔpH
Ca^{2+}	1.52
Mg^{2+}	1.05
Mn^{2+}	1.15
None[a]	1.24
Water[b]	0.3

[a] None means a medium containing 0.2 mM $CaSO_4$ and cells were washed in 0.2 mM $CaSO_4$. [b] Water means a medium that did not contain any Ca^{2+} and cells were washed in distilled water only.

On the other hand, NaCl caused higher rates of acidification than polyols (Tables I and II) while the decrease in amount of ATP in the cells was much smaller or none (Fig. 3, Table III). It can be presumed that in this case acidification by NaCl is mainly due to a mechanism not utilizing ATP such as a Na^+/H^+ exchanger (3, 23). This hypothesis is further strengthened by the fact that NaCl induced proton extrusion was inhibited by amiloride a Na^+/H^+ exchanger inhibitor (3) (Table II).

It should be emphasized that the effect of 100 mM NaCl is a combination of increased proton extrusion due to activations of a Na^+/H^+ exchanger and the effect of the increased ionic strength by displacement of Ca^{2+} from the plasmalemma (12). This might be a real possibility since, in the presence of 0.2 mM $CaSO_4$, 100 mM NaCl inhibited FC-induced ATP decrease (Table III), however proton extrusion was not inhibited when Ca^{2+} concentration was increased from 0.2 to 1 mM (from pH 6.5 to 5 in 0.2 mM $CaSO_4$ and from pH 6.3 to 4.7 in 1 mM $CaSO_4$).

It would be expected that a decrease in ATP level should be accompanied by a parallel increase in ADP and AMP (1). Table IIIA shows that this is not the case. Sorbitol caused a decline in ATP and a subsequent decline in the total adenylate pool without causing an increase in ADP and AMP within the first hour of treatment (Fig. 3 and Table IIIA).

During prolonged exposure to sorbitol there was a slow restoration of both the ATP level and the total adenylate pool (Fig. 3). It might be that the decrease in ATP level was due to a mechanism that not only utilizes ATP but somehow also prevents the increase in ADP and AMP levels without affecting respiration as mentioned above.

When living cells are exposed to treatments, such as a transfer to anoxia, which decrease the rate of ATP regeneration relative to the rate of ATP utilization, the ATP level and the adenylate energy charge, and in some cases the total adenylate pool are decreased (1, 15, 21). The kinetics of the drop in ATP, AEC, and total adenylates observed after sorbitol addition in this study are similar to those observed in other studies after an energy metabolism reduction (15, 21) and suggest that sorbitol transiently induces an imbalance between ATP regeneration and utilization. The factors which control the level of the adenylate pool are still poorly understood: a drop in total adenylates has been observed under stress conditions such as water stress, tissue injury, transfer to anoxia, etc. (21).

From Figures 1 and 2 it is apparent that maximal enhancement of $\Delta\mu_{H^+}$ occurs at the osmotic potential which abolishes turgor pressure. It would seem that the drop in hydrostatic pressure caused by loss of turgor might change the conformation of the plasmalemma (2, 18, 34); thus the plasmalemma proton pump will be in a different environment which might modify its characteristics (3). The change in plasmalemma conformation induced by the drop in turgor does not seem to cause a change in the passive permeability of the membrane (Fig. 5) (17, 22).

The results reported here suggest that sorbitol and NaCl affect carrot cell differently. From growth experiments (25) it is apparent that NaCl is a relatively fast permeating osmoticum, while sorbitol is a slow permeating one. Greenway *et al.* (7) already reported that slow and fast permeating solutes have different effects on metabolism. Our data may lead to a better understanding of the difference between the effects of the two types of osmotica. Our experimental data show short time effects on what seems to be a membrane component that might be involved in turgor maintenance. It has already been suggested by some authors (10, 11, 22, 34) that this component is the proton pump. Although our results show that the phenomenon seems relatively vanadate insensitive, the explanation may still be valid if we assume that the plasmalemma proton pump might have changed characteristics under conditions of low turgor and become vanadate insensitive (18).

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