J. Membrane Biol. 43, 335–349 (1978)

Evidence for Interactions between the Energy-Dependent Transport of Sugars and the Membrane Potential in the Yeast *Rhodotorula gracilis* (*Rhodosporidium toruloides*)

Robert Hauer and Milan Höfer

Botanisches Institut, der Universität, Kirschallee 1, D-5300 Bonn 1, Germany

Received 20 March 1978; revised 5 June, 1978

Summary. A membrane potential (inside negative) across the plasma membrane of the obligatory aerobic yeast *Rhodotorula gracilis* is indicated by the intracellular accumulation of the lipid-soluble cations tetraphenylphosphonium and triphenylmethylphosphonium. The uptake of these ions is inhibited by anaerobic conditions, by uncouplers, by addition of diffusible ions, or by increase of the leakiness of the membrane caused by the polyene antibiotic nystatin. The membrane potential is strongly pH-dependent, its value increasing with decreasing extracellular proton concentration. Addition of transportable monosaccharides causes a depolarization of the electrical potential difference, indicating that the H^+-sugar cotransport is electrogenic. The effect on the membrane potential is enhanced by increasing the sugar concentration. The half-saturation constants of depolarization for D-xylose and D-galactose were comparable to those of the corresponding transport system for the two sugars. All agents that depressed the membrane potential inhibited monosaccharide transport; hence the membrane potential provides energy for active sugar transport in this strain of yeast.

Monosaccharides are actively transported into cells of *Rhodotorula gracilis* (Kotyk & Höfer, 1965; Höfer & Kotyk, 1968). Uptake is coupled to an influx of protons (Misra & Höfer, 1975), which always exhibits a stoichiometry of 1:1 (Höfer & Misra, 1978). Similar observations have been reported for bacteria (West & Mitchell, 1973; Deshusses & Reber, 1977; Lagarde & Haddock, 1977), algae (Komor & Tanner, 1976), fungi (Seaston, Inkson & Eddy, 1973; Slayman & Slayman, 1974), and higher plants (Giacquinta, 1977; Komor, Rotter & Tanner, 1977; Racusen & Galston, 1977). Hence, as West and Mitchell (1972) have suggested, the uptake of protons coupled to the transport of nonelectrolytes may be the means by which energy is usually supplied for transporting uncharged substrates. According to Mitchell, the free energy produced by

0022-2631/78/0043-0335 $03.00

the transport of protons along the gradient of their electrochemical potential drives the transport of substrates uphill. So the symport of sugars with protons would tap the energy stored (i) in the pH gradient and (ii) in the membrane potential. Such a pH gradient (inside alkaline) across the plasmalemma of *Rh. gracilis* and its utilization by monosaccharide transport has been found by Höfer & Misra (1978). The second component of the electrochemical proton gradient, the membrane potential, is shown here to exist across the plasmalemma of *Rh. gracilis* and to be used in transporting monosaccharides.

Materials and Methods

Growth

The obligatory aerobic yeast *Rhodotorula gracilis* (*glutinis*) ATCC 26194 and CBS 6681 (*Rhodosporidium toruloides*, mating type *a*) was grown as described previously (Misra & Höfer, 1975). The cells (inoculum 2×10^6 cells/ml of growth medium) were harvested in the stationary phase after 24 hr (yield approximately 10^8 cells/ml) and aerated for 6 hr before use as a 5% suspension (wet wt/vol). In all experiments involving uptake of lipid-soluble cations, the suspension was centrifuged at $5000 \times g$ after aeration for 6 hr and the pellet was stored at 4 °C overnight. After resuspension, the cells were aerated for 2 hr before use.

Uptake of Sugars

Transport of D-xylose was measured by the membrane filter technique as described by Heller & Höfer (1975). D-galactose transport was studied by monitoring the activity of ^{14}C-labeled substrate in the medium as for the lipid-soluble cations (*see* below).

Uptake of Lipid-Soluble Cations

For all experiments, the yeast was suspended in Tris/citric acid buffer (appropriate amounts of 0.3 M Tris with 0.3 M citric acid), to give 4% wet wt of yeast/vol. The experiment was started by adding ^{3}H-tetraphenylphosphonium (TPP^+) or ^{3}H-triphenylmethylphosphonium ($TPMP^+$). Samples of 0.4 ml were withdrawn and centrifuged for 12 sec at $15{,}000 \times g$ in an ECCO-Quick centrifuge (Collatz, Berlin, Germany). 0.2 ml of the supernatant was mixed with 10 ml toluene/Triton X 100/ethanol scintillation fluid (32:16:3), containing 0.6% 2,5 diphenyloxazole and 0.06% 1,4-bis-(5-phenyloxazol-2-yl)benzene and counted in a Packard 3380 liquid scintillation counter. All cpm values were corrected for quenching effects by the use of an external standard and the channel ratio method (Brewer, Pesce & Ashworth, 1974). The decrease of radioactivity in the supernatant was used as a measure of cations taken up by the cell.

Calculation of the Membrane Potential

The membrane potential was estimated by inserting the intra- and extracellular concentrations of the lipid-soluble cations into the Nernst equation. The intracellular concentration

was calculated by assuming a value of 2.0 μl intracellular water/mg dry wt (Höfer & Misra, 1978).

Experiments under Anaerobic Conditions

In order to avoid interference by traces of oxygen (*cf.* Höfer, 1971), highly purified nitrogen (99.995%) was used.

Chemicals

Nitrogen was obtained from Messer, Griesheim, Germany. ^{14}C-D-galactose was a product of Amersham-Buchler, Braunschweig, Germany. Labeled tetraphenylphosphonium-chloride (4.33 Ci/mol) was a kind gift of Dr. P. Geck, Frankfurt, and tritiated triphenylmethylphosphonium-bromide (71.5 Ci/mol) of Dr. K.H. Altendorf, Tübingen. Carbonylcyanide-*m*-chlorophenylhydrazone (CCCP) was obtained from Sigma Chemicals, Munich, Germany, carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP) from Boehringer, Mannheim, Germany, and nystatin from Serva, Heidelberg, Germany. All other compounds were of reagent grade and obtained from Merck, Darmstadt, Germany.

Results

Indirect methods are favored for measuring electrical potential differences across the membranes of small compartments because of the difficulty of using microelectrodes. For the work presented below, the lipophilic cations introduced by Liberman & Topali (1969) were used. These are ions made lipophilic by surrounding the charge-bearing moiety by a core of hydrophobic substituents. This class of ions can permeate biological membranes without the aid of carrier molecules. So the equilibrium distribution of cations between the two compartments separated by a membrane is expected to depend on the membrane potential as predicted by Nernst's equation for diffusion potentials. For this kind of distribution the net charge of the cations must be independent of pH and the cations must not be bound intracellularly. Among the lipophilic cations currently in use, the dibenzyldimethylammonium (DDA^+) has presented the greatest difficulties (Lombardi, Reeves & Kaback, 1973; Hoeberichts & Borst-Pauwels, 1975). For this reason we used the more lipophilic tetraphenylphosphonium (TPP^+) ion and the similar triphenylmethylphosphonium ($TPMP^+$) analogue.

Uptake of Lipid-Soluble Cations

As shown in Figs. 1 and 2, both cations are taken up by cells of *Rh. gracilis*. In the case of tetraphenylphosphonium (TPP^+), a sponta-

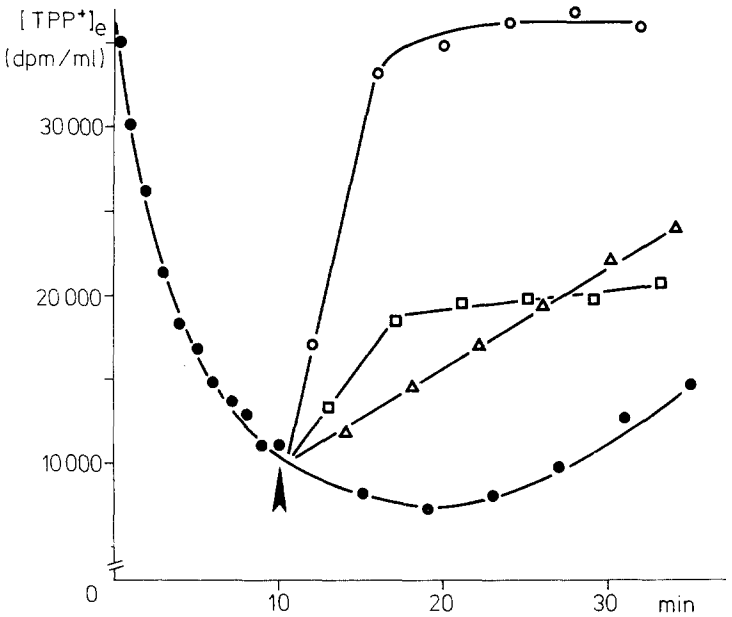

Fig. 1. Uptake of TPP^+ and its inhibition by uncouplers. 15-ml cell suspension in water (about 10 mg dry wt/ml) were incubated with 3 ml Tris/citric acid buffer, pH 7.5 (0.3 M), at 28 °C in a reciprocating shaker. The experiment was started by adding 36 µl 1-mM tetraphenylphosphoniumchloride (20 µM final concentration). 500-µl samples were withdrawn, centrifuged, and 200 µl supernatant counted in 10 ml scintillation fluid. At the time indicated by arrow, 4-ml portions were transferred to flasks containing 40 µl aqueous NaN_3 or ethanolic DNP or CCCP solutions to give the final concentrations indicated. ●: control; ○: 10 µM CCCP; △: 100 µM DNP; □: 1 mM NaN_3. (Number of experiments, $n=20$)

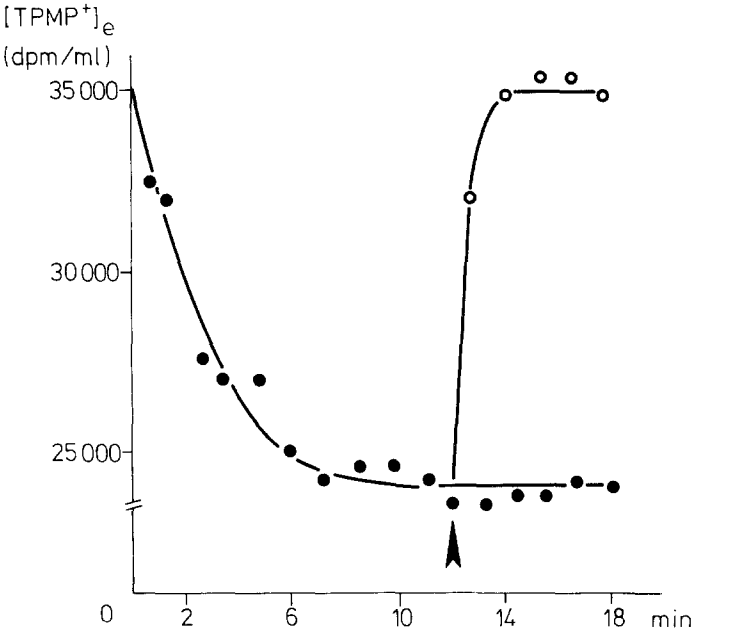

Fig. 2. Uptake of $TPMP^+$ and its inhibition by CCCP. Experimental conditions were as in Fig. 1. The final concentration of $TPMP^+$ was 1.43 µM. ● control; ○ CCCP. ($n=5$)

Table 1. Dependence of the accumulation ratio of lipophilic cations on their concentration[a]

Concentration	Accumulation ratio			
	TPP^+	ΔE	$TPMP^+$	ΔE
1 μM	n.m.	–	8.6	−55 mV
10 μM	24.0	−82 mV	9.2	−58 mV
100 μM	18.3	–	6.6	–
1 mM	3.7	–	1.4	–
10 mM	1.7	–	1.0	–

n.m. = not measured.

[a] All experiments were carried out at pH 7.5. Results from 8 experiments were pooled. The values of the accumulation ratio were inserted into the Nernst equation to calculate the apparent membrane potential (ΔE).

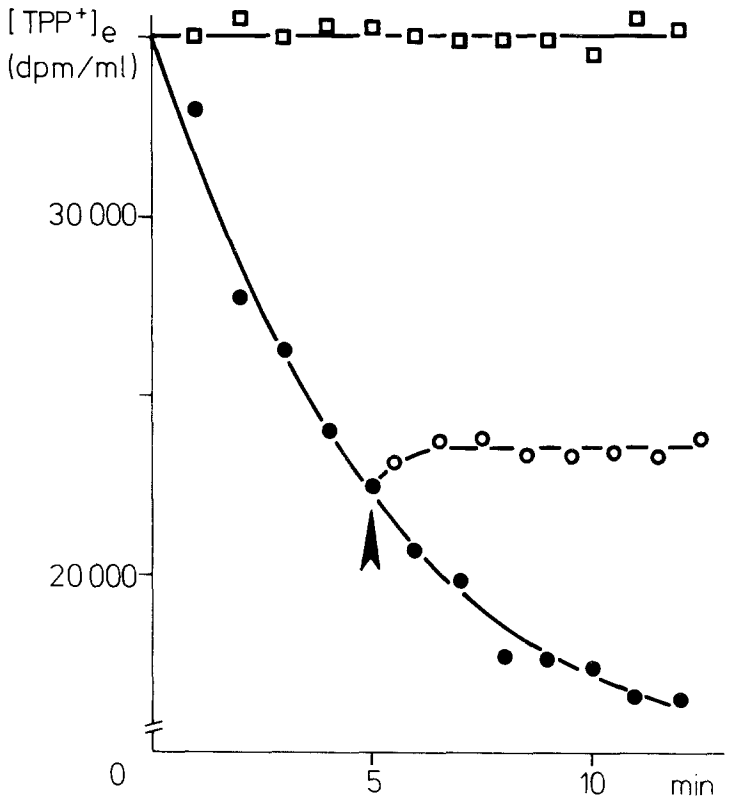

Fig. 3. Effect of anaerobiosis on the uptake of TPP^+. 8 ml yeast suspension in water were incubated with 2 ml 0.3 M Tris/citric acid buffer, pH 7.5. The experiment was started by the addition of 20 μl 1-mM TPP^+. At $t = 10$ min a 5-ml portion was withdrawn and transferred to a vessel through which nitrogen gas was bubbled. ●: control; ○: bubbling started at the arrow. In a parallel run bubbling with nitrogen was started 10 min before adding TPP^+ (□). ($n = 4$)

neous efflux of the ion occurred after 20–30 min, probably because TPP^+ is toxic (Heinz, Geck & Pietrzyk, 1975). The accumulation ratios of both ions never differed by more than a factor of 3 (Table 1). A membrane potential (negative inside) can be inferred from the accumulation of these cations within the cells.

If this is true, conditions known to depolarize biological membranes (Slayman & Slayman, 1974) should decrease the uptake of TPP^+ and $TPMP^+$. Adding uncouplers caused either an efflux of accumulated phosphonium ions (Figs. 1 and 2) or an inhibition of ion uptake (not shown). Anaerobiosis gave a comparable inhibition, although without marked efflux of TPP^+ (Fig. 3).

Effects of Diffusible Cations

Addition of diffusible cations causes an additional influx of positive charges into the cells and thereby depolarizes the membrane potential. Figure 4 shows that not only alkaline and alkaline earth ions, but also

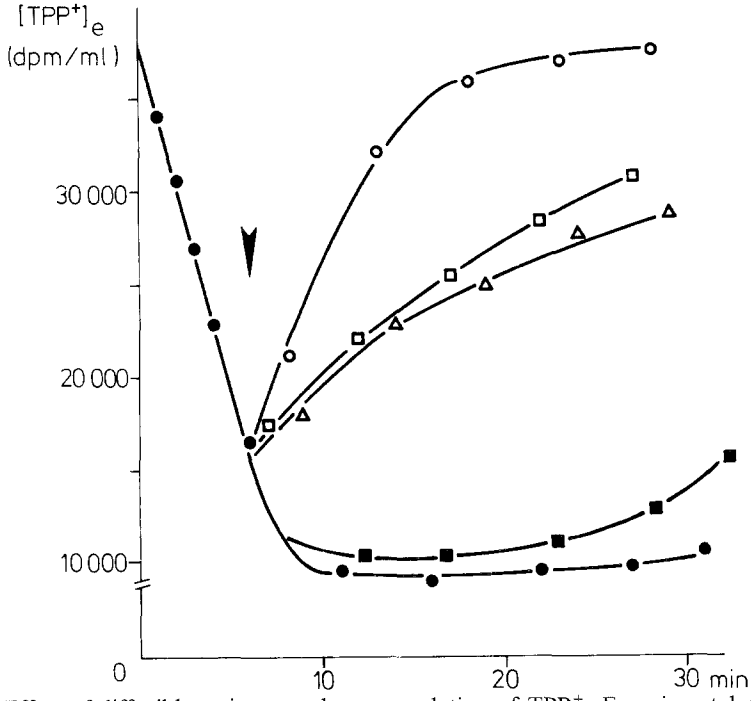

Fig. 4. Effect of diffusible cations on the accumulation of TPP^+. Experimental conditions were as in Fig. 1 except that the 4-ml portions were transferred to vessels containing solid KCl, $CaCl_2$, or choline chloride to give a final concentration of 100 mM. In one vessel the cell suspension was titrated with 0.1 M HCl to pH of 3.0. ●: control; △: KCl; □: $CaCl_2$; ■: choline chloride; ○: HCl. ($n=4$)

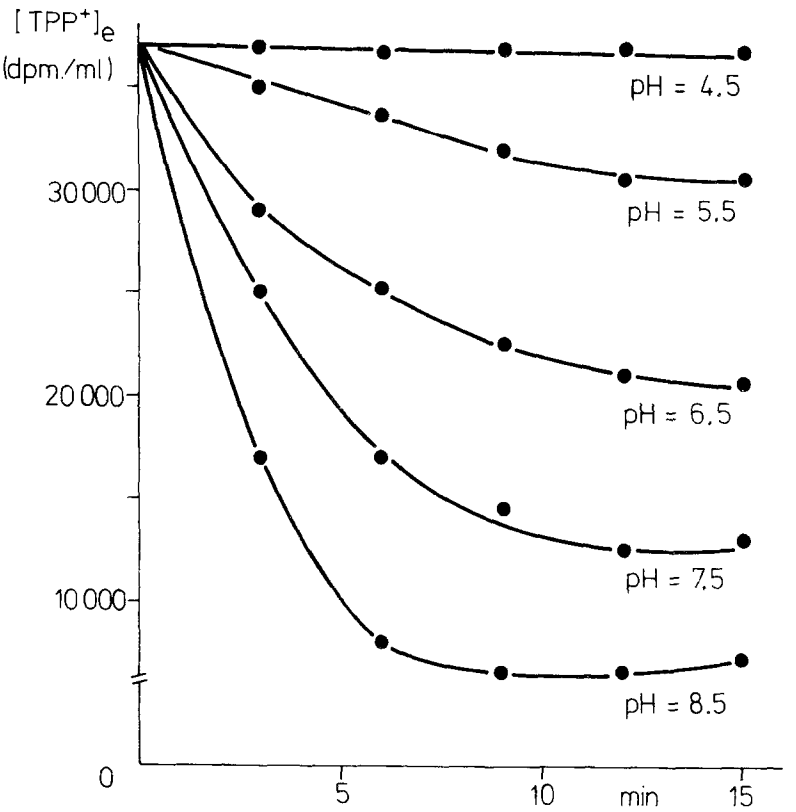

Fig. 5. Dependence of the accumulation of TPP^+ on the extracellular pH. Experimental conditions were as in Fig. 1. ($n=5$)

protons, induce an efflux of TPP^+. The effect of protons is suggestive of an increase of the membrane potential with increasing pH (Fig. 5).

The membrane potential can also be affected by the indicator ions themselves. With increasing concentrations of $TPMP^+$ or TPP^+, their accumulation ratio was reduced (Table 1).

Effect of Nystatin

The polyene antibiotic nystatin forms unselective pores in membranes containing sterols (Finkelstein & Holz, 1973). This is true for *Rh. gracilis*, for which 10 μM nystatin causes efflux of small ions and neutral molecules, such as K^+, inorganic phosphate, and sugars, as well as the uptake of protons (H. Huh & M. Höfer, *unpublished*).

The addition of 10 μM nystatin to intact cells of *Rh. gracilis* resulted in an immediate equilibration of charges and a short circuiting of the membrane, allowing a rapid efflux of TPP^+ (Fig. 6).

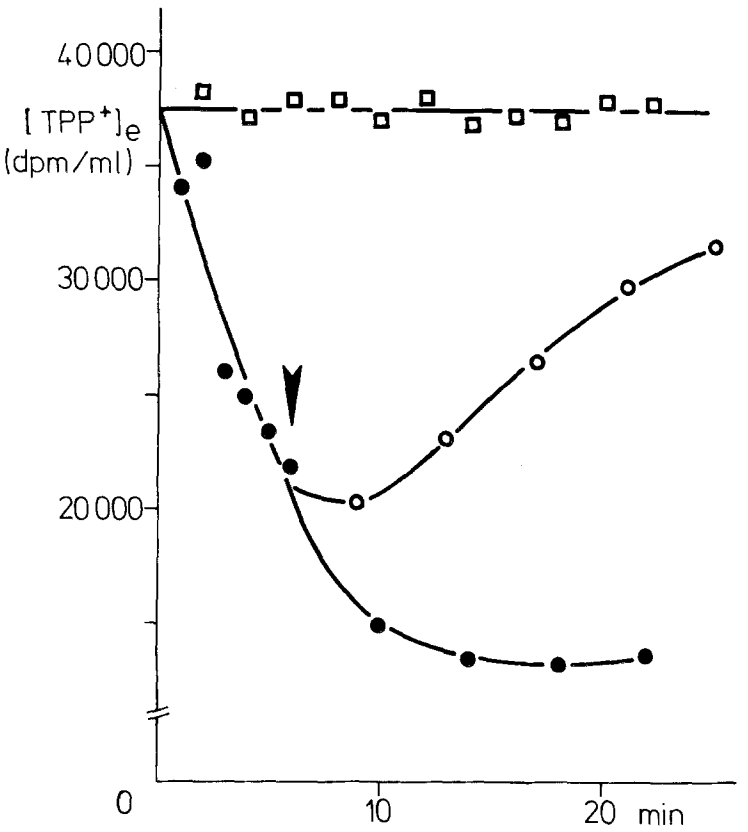

Fig. 6. Efflux of TPP^+ from preloaded cells produced by adding nystatin. Experimental conditions were as in Fig. 1. ●: control; ○: 10 μM nystatin added at the arrow. In a parallel run the cells were pretreated with 10 μM nystatin for 2 min before adding TPP^+ (□). ($n=5$)

Effect of Sugars on the Membrane Potential

Substrates transported by the monosaccharide system, such as D-xylose, D-galactose, D-fructose, D-glucose or D-arabinose, decrease the accumulation of TPP^+ (Fig. 7). Controls with D-ribose and sucrose, which are not taken up by this system (Horak & Kotyk, 1969; Janda & von Hedenström, 1974), had no effect. Similar results were obtained with $TPMP^+$ (not shown). These observations demonstrate that the influx of monosaccharides coupled to the movement of protons is electrogenic. The close coupling of monosaccharide transport and depolarization is shown in Figure 8. The depolarization was enhanced by increasing the extracellular sugar concentration. The degree of depolarization displayed saturation kinetics. It may be described by a half-saturation constant

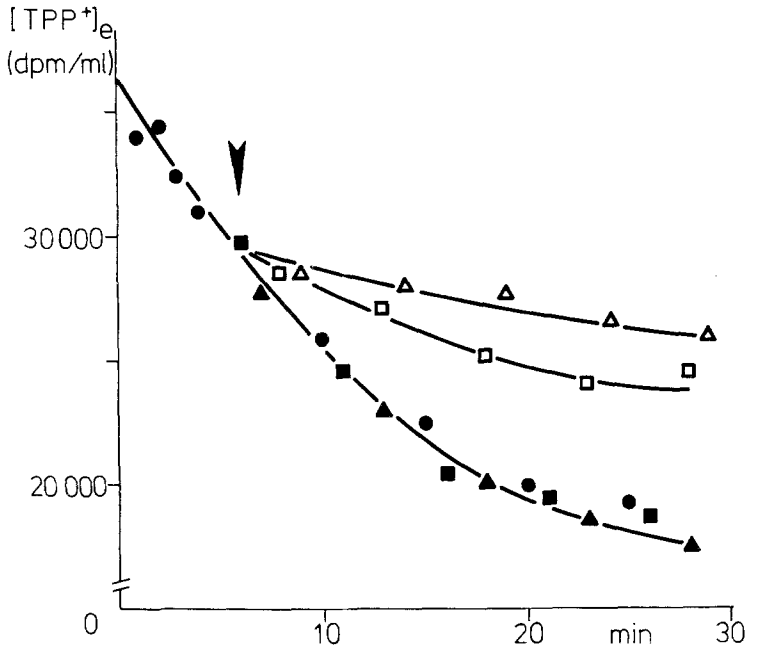

Fig. 7. Effect of various sugars on the uptake of TPP^+. Experimental conditions were as in Fig. 4. The final sugar concentration was 20 mM. ●: control; □: D-xylose; △: D-galactose; ■: D-ribose; ▲: sucrose. ($n=20$)

of depolarization which corresponds to that of sugar uptake (Table 2). These experiments were carried out at pH 7.5, since the depolarization was expected to be more pronounced at a high membrane potential. At this pH, the activity of the protonated carrier (pK 6.8; Höfer & Misra, 1978) is still sufficiently high. Later experiments showed this effect over the entire pH range from 5.5 to 8.5 (results not shown).

Association of Changes in the Membrane Potential with Active Transport

All manipulations affecting the membrane potential ought to show parallel effects on active transport. This is true of all uncouplers used and of anaerobic conditions which are potent inhibitors of the active monosaccharide transport (Höfer & Kotyk, 1968; Höfer, 1971).

In addition, since uncouplers are weak acids working optimally at low pH, a concomitant decrease of their efficiency to depolarize the

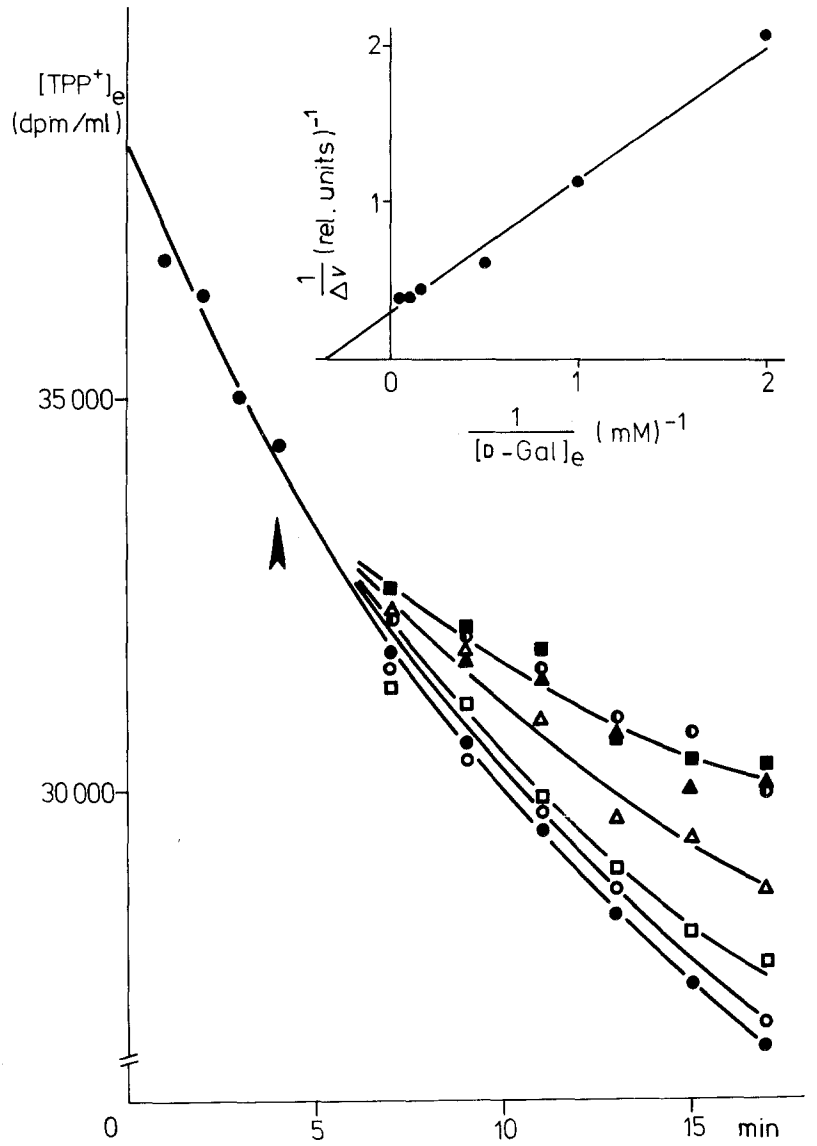

Fig. 8. Uptake of TPP^+ as a function of D-galactose concentration. Experimental conditions were as in Fig. 4. After adding substrate, the differences in the rate of TPP^+ uptake (Δv) relative to the control were assumed to be linearly related to the degree of depolarization. The Δv-values were plotted against the sugar concentration (inset), as by Lineweaver and Burk (1934). ●: control; ○: 0.5 mM; □: 1 mM; △: 2 mM; ▲: 10 mM; ■: 20 mM; ◑: 50 mM D-galactose. ($n=4$)

electrical potential difference and to suppress active transport should be found with increasing pH. At pH 5.0 both 2,4-dinitrophenol (DNP) and CCCP inhibited transport and depolarized the membrane potential

Table 2. Comparison of the half-saturation constants (K_T) of sugar transport and of depolarization of the membrane potential[a]

Effect studied	K_T (mM)	
	D-Galactose	D-Xylose
Transport	1 –3	0.8–2
Depolarization	0.6–3	0.6–2

[a] Experimental conditions as in Fig. 1. The numbers shown give the range of values from at least three experiments. The transport results were taken from Höfer & Misra (1978).

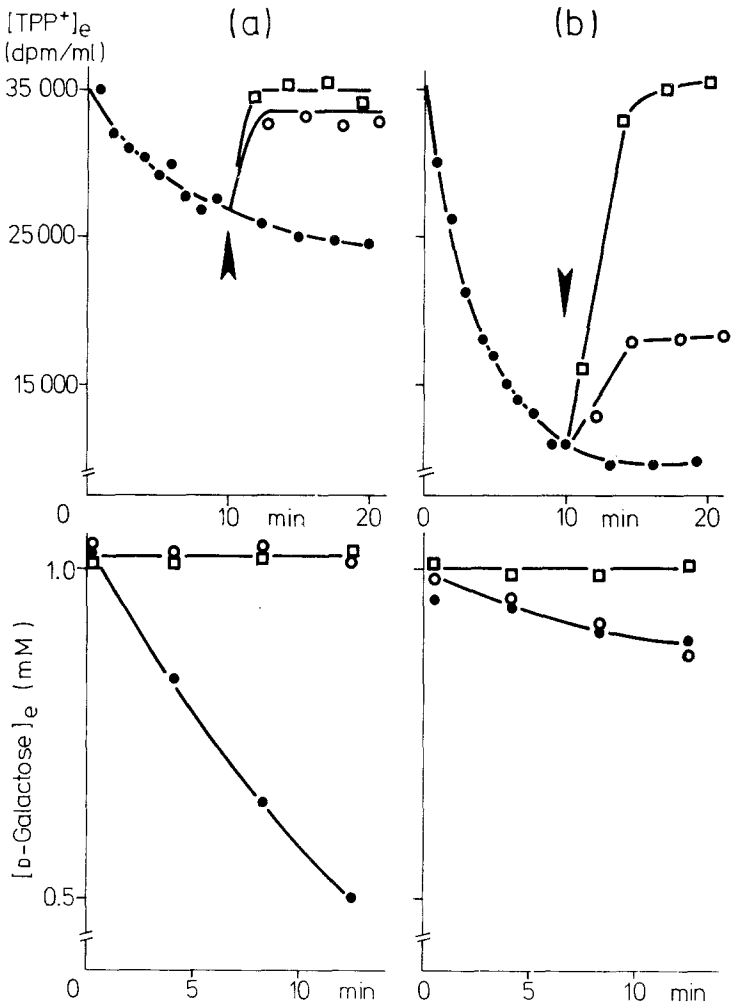

Fig. 9. Effect of extracellular pH on the efficiency of uncouplers to inhibit the accumulation of TPP^+ (upper part) and D-galactose transport (lower part). (*a*) pH = 5.0; (*b*) pH = 7.5. Experimental conditions: for TPP^+ uptake, as in Fig. 1; for D-galactose uptake, ^{14}C-labeled sugar (final concentration 1 mM, 6000 cpm/μmol) was added in parallel runs instead of TPP^+. ●: control; □: 10 μM CCCP; ○: 100 μM DNP. ($n = 3$)

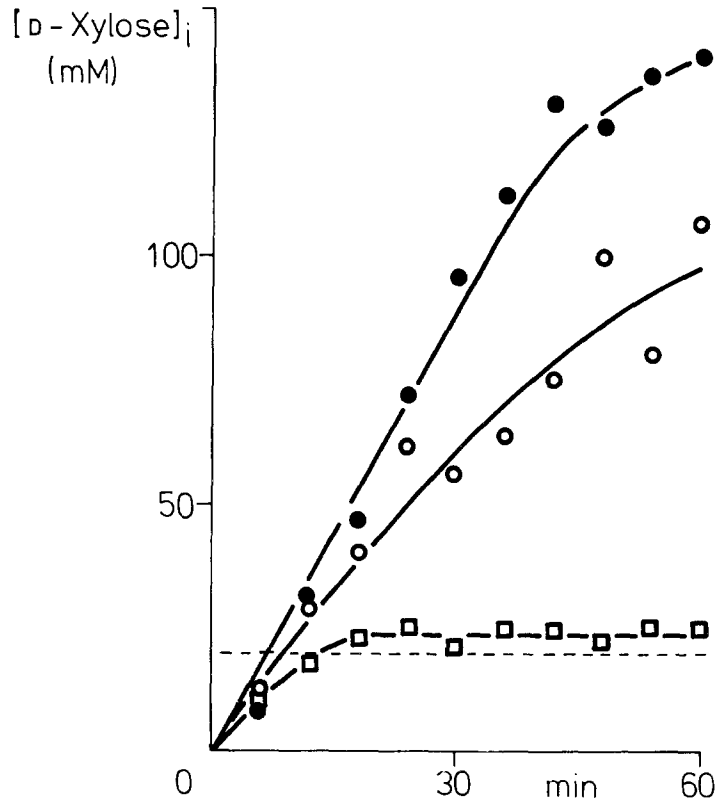

Fig. 10. Inhibition of D-xylose accumulation by diffusible cations. 5.5 ml of 5% (wet wt/vol) yeast suspension were incubated with an equal volume of Tris/citric acid buffer at pH 6.0. The experiment was started by the addition of 20 mM D-xylose. ●: control; ○: 100 mM KCl; □: 10 mM TPP^+. The broken line corresponds to the diffusion equilibrium. ($n=3$)

completely. As a consequence of its lower pK value, DNP was much less efficient at pH 7.5 (Fig. 9).

Figure 10 depicts the predicted inhibition of monosaccharide uptake under conditions of a depolarized membrane potential due to the presence of TPP^+ or potassium ions.

Discussion

Many people have used the lipophilic cations, introduced by Liberman and Topali (1969), to measure electrical potential differences across membranes enclosing small compartments (for fungi *see* Miller & Budd, 1976). The usefulness of these cations as qualitative indicators is generally

accepted, although the results they give often differ from those obtained with direct or other indirect methods (e.g., Azzone *et al.*, 1976; Laris, Pershadsingh & Johnstone, 1976). Similarly, in our experiments the accumulation of either TPP^+ or $TPMP^+$ varied by as much as a factor of 3 (Table 1). Even the highest value of the membrane potential calculated from our results was considerably lower than those found in other eukaryotic microorganisms, such as in *Chlorella vulgaris*, 150 mV (Komor & Tanner, 1976), or in *Neurospora crassa*, up to 250 mV (Slayman & Slayman, 1974). So, our measurements of the membrane potential are probably underestimates, and hence the uptake of phosphonium ions must be interpreted only qualitatively.

The two kinds of lipid-soluble cation gave comparable results. TPP^+ was accumulated to a higher degree, as compared to $TPMP^+$. However, with prolonged incubation, TPP^+ appeared toxic to cells (*cf.* Heinz *et al.*, 1975). Both ions fulfill the requirements of an indicator of the membrane potential. (i) They are accumulated against their concentration gradient, indicating a negative potential difference across the plasmalemma, which seems to be general for living cells. (ii) Their accumulation is prevented or reversed by uncouplers which depolarize membrane potentials. (iii) Their intracellular content is depleted by diffusion potentials of opposite sign. (iv) Addition of 10 μM nystatin, which abolishes the potential difference by increasing the ion permeability of the cell membrane, produces a complete efflux of these ions from the cells. So the plasmalemma itself and, for the following reasons, not the mitochondria must be mainly responsible for the observed accumulation: (i) 100 μM nystatin did not affect mitochondrial respiration (von Hedenström, 1976) although it caused a complete loss of accumulated phosphonium ions. (ii) The increase of the extracellular proton activity resulted in a marked efflux of the phosphonium ions without affecting the rate of respiration (Höfer & Misra, 1978). (iii) An increase in concentration of potassium ions chased the phosphonium ions from the cells without markedly increasing the intracellular K^+ concentration (G. Uhlemann & M. Höfer, *unpublished*). Hence TPP^+ and $TPMP^+$ were suitable for investigating membrane potentials in our yeast.

Monosaccharide-proton cotransport decreased the accumulation of lipophilic cations, the half saturation constant of the degree of depolarization equaling that of transport. These findings demonstrate a direct coupling between the movement of sugars and the driving force of the potential difference across the membrane, so sugar transport in *Rh. gracilis* must be electrogenic.

Interference with the membrane potential would be expected to modify the activity of an electrogenic transport system and, indeed, such modifications of monosaccharide uptake were observed in *Rh. gracilis*. All alterations of the electrical potential difference changed the initial velocity of monosaccharide uptake and the accumulation ratio in the expected direction. The only exception was observed when comparing the influence of pH on the membrane potential and monosaccharide uptake. A decrease of the proton activity in the medium increases the former but inhibits the latter (Höfer & Misra, 1978; *cf.* also Fig. 9). This apparent anomaly can be explained by an increase of the pH giving a decrease of the pH gradient, the second source of energy for transport.

Whether the energy conserved in the electrochemical proton gradient is sufficient to account for the observed accumulation ratios of sugars can only be judged after simultaneous and more direct measurements of the pH gradient, the membrane potential, and the accumulation ratio in a given population of cells.

This work has been supported by the Deutsche Forschungsgemeinschaft (grant No. Ho 555). The financial support to R.H. by the Studienstiftung des Deutschen Volkes is gratefully acknowledged.

References

Azzone, G.F., Bragadin, M., Pozzan, T., Dell'Antone, P. 1976. Proton electrochemical potential in steady state rat liver mitochondria. *Biochim. Biophys. Acta* **439**:90

Brewer, J.M., Pesce, A.J., Ashworth, R.B. 1974. Experimental Techniques in Biochemistry. p. 299. Prentice-Hall, Englewood Cliffs

Deshusses, J., Reber, G. 1977. Transport of cyclitols by a proton symport in *Klebsiella aerogenes*. *Eur. J. Biochem.* **72**:87

Finkelstein, A., Holz, R. 1973. Aqueous pores created in thin lipid membrane by the polyene antibiotics nystatin and filipin. *In:* Membranes. Vol. 2. Lipid Bilayers and Antibiotics. G. Eisenman, editor. p. 377. Dekker, New York

Giacquinta, R. 1977. Phloem loading of sucrose. *Plant Physiol.* **59**:750

Hedenström, M. von 1976. Untersuchungen an Protoplasten der obligat aeroben Hefe *Rhodotorula gracilis*. Ph.D. Thesis. University of Bonn, Germany

Heinz, E., Geck, P., Pietrzyk, C. 1975. Driving forces of amino acid transport in animal cells. *Ann. N. Y. Acad. Sci.* **264**:428

Heller, K., Höfer, M. 1975. Temperature dependence of the energy-linked monosaccharide transport across the cell membrane of *Rhodotorula gracilis*. *J. Membrane Biol.* **21**:261

Hoeberichts, J.A., Borst-Pauwels, G.W.F.H. 1975. Effect of tetraphenylboron upon the uptake of the lipophilic cation dibenzyldimethylammonium by yeast cells. *Biochim. Biophys. Acta* **413**:248

Höfer, M. 1971. Transport of monosaccharides in *Rhodotorula gracilis* in the absence of metabolic energy. *Arch. Mikrobiol.* **80**:50

Höfer, M., Kotyk, A. 1968. Tight coupling of monosaccharide transport and metabolism in *Rhodotorula gracilis*. *Folia Microbiol. Prague* **13**:197

Höfer, M., Misra, P.C. 1978. Evidence for a H^+-sugar symport in the yeast *Rhodotorula gracilis* (*glutinis*). *Biochem. J.* **172**:15

Horak, J., Kotyk, A. 1969. Anomalous uptake of D-ribose by *Rhodotorula gracilis*. *Folia Microbiol. Prague* **14**:291

Janda, S., Hedenström, M. von 1974. Uptake of disaccharides by the aerobic yeast *Rhodotorula glutinis*. *Arch. Microbiol.* **101**:273

Komor, E., Rotter, M., Tanner, W. 1977. A proton-cotransport system in a higher plant: Sucrose transport in *Ricinus communis*. *Plant Sci. Lett.* **9**:153

Komor, E., Tanner, W. 1976. The determination of the membrane potential of *Chlorella vulgaris*. *Eur. J. Biochem.* **70**:197

Kotyk, A., Höfer, M. 1965. Uphill transport of sugars in the yeast *Rhodotorula gracilis*. *Biochim. Biophys. Acta* **102**:410

Lagarde, A., Haddock, B.A. 1977. Proton uptake linked to the 3-deoxy-2-oxo-D-gluconate-transport system of *Escherichia coli*. *Biochem. J.* **162**:183

Laris, P.C., Pershadsingh, H.A., Johnstone, R.M. 1976. Monitoring membrane potentials in Ehrlich ascites tumor cells by means of a fluorescent dye. *Biochim. Biophys. Acta* **436**:475

Liberman, E.A., Topali, V.P. 1969. Permeability of biomolecular phospholipid membranes for lipid-soluble ions. *Biofizika* **14**:452

Lineweaver, H., Burk, D. 1934. The determination of enzyme dissociation constants. *J. Am. Chem. Soc.* **56**:658

Lombardi, R.J., Reeves, J.P., Kaback, H.R. 1973. Mechanisms of active transport in isolated bacterial vesicles. *J. Biol. Chem.* **248**:3551

Miller, A.G., Budd, K. 1976. Evidence for a negative membrane potential for movement of Cl^- against its electrochemical gradient in the Ascomycete *Neocosmospora vasinfecta*. *J. Bacteriol.* **132**:741

Misra, P.C., Höfer, M. 1975. An energy-linked proton extrusion across the cell membrane of *Rhodotorula gracilis*. *FEBS Lett.* **52**:95

Racusen, R.H., Galston, A.W. 1977. Electrical evidence for rhythmic changes in the cotransport of sucrose and hydrogen ions in *Samanea pulvini*. *Planta* **135**:57

Seaston, A., Inkson, C., Eddy, A.A. 1973. The absorption of protons with specific amino acids and carbohydrates by yeast. *Biochem. J.* **134**:1031

Slayman, C.L., Slayman, C.W. 1974. Depolarization of the plasma membrane of *Neurospora* during active transport of glucose: Evidence for a proton-dependent cotransport system. *Proc. Nat. Acad. Sci. USA* **71**:1935

West, I.C., Mitchell, P. 1972. Proton-coupled β-galactoside translocation in non-metabolizing *Escherichia coli*. *J. Bioenerg.* **3**:445

West, I.C., Mitchell, P. 1973. Stoichiometry of lactose-H^+ symport across the plasma membrane of *Escherichia coli*. *Biochem. J.* **132**:587