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MEMBRANE POTENTIAL IN YEAST CELLS MEASURED BY DIRECT AND INDIRECT METHODS

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Summary

The membrane potential, $\Delta\psi$, of various yeasts estimated from the distribution of tetraphenylphosphonium cations ranged from —50 to —120 mV, depending on species, incubation conditions and technique of measurement. Values obtained directly with a microelectrode in *Endomyces magnusii* were consistently lower than those determined indirectly.

Until recently the direct measurement of membrane potentials ($\Delta\psi$) in microbial cells has been impeded by the small dimensions of the object, rigidity of the cell wall and difficulties in holding the cell for microelectrode insertion. Indirect methods, such as the change in absorbance or fluorescence of various probes with changes of the membrane potential, or the distribution of lipophilic cations or anions, have therefore been mostly used for this purpose.

The first measurements of this kind in yeasts were carried out with tetra[^{3}H]-phenylphosphonium (TPP^+) and tri[^{3}H]phenylmethylphosphonium ($TPMP^+$) cations which equilibrate at various rates according to their passive permeation through the membrane [1].

Membrane potential values calculated from TPP^+ distribution in our experiments lie within the range of —50 to —120 mV (see Fig. 1). The yeast *Saccharomyces cerevisiae* was studied in some detail with respect to the effect of some factors affecting the membrane potential.

Abbreviations: CCCP, carbonyl cyanide m-chlorophenylhydrazone; TPP^+, tetraphenylphosphonium ion; $TPMP^+$, triphenylmethylphosphonium ion.

The membrane was depolarized, according to the observed changes in the TPP^+ distribution ratio (Fig. 2), by the proton conductors 2,4-dinitrophenol and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP). It was also slightly depolarized by the addition of glucose, which brings about rapid acidification of the extracellular medium [2]. Both these results would be expected if the operation of a proton pump played a role in the generation of the membrane potential. CCCP would 'short-circuit' the pump and thus reduce the (negative) potential, and the low external pH consequent on the addition of glucose would lead to an increase in the ΔpH component of the proton electrochemical potential difference and therefore to a decrease in the $\Delta\psi$ component. The fact that addition of KCl enhances the depolarization produced by glucose might reflect its effect on the diffusion component of the membrane potential or, alternatively, a rapid uptake of K^+ through the proton pump itself.

To be sure, as found in our experiments and pointed out in Ref. 1 the results of indirect measurements of membrane potentials in the yeast *S. cerevisiae* should be interpreted with caution for the following reasons: (i) the uptake of TPP^+ by the cells has often a very fast initial component probably reflecting physical absorption; (ii) the attainment of equilibrium is slow and the response to fast processes, such as acidification bursts, will necessarily be distorted; (iii) the distribution ratio may not reflect changes in the membrane potential alone since the lipophilic cation may be taken up by means of the thiamine transport system [3] and may possibly interfere with other membrane systems including the H^+-transporting ones.

These drawbacks make it imperative to try to measure the potential directly. Since the small size of the *S. cerevisiae* cells and the rigidity of the cell wall made the direct impalement of the cells with a microelectrode very difficult, a suitable substitute was found in the yeast *Endomyces magnusii*. Its cells are several times bigger than those of *S. cerevisiae* (15 × 30 μm versus 6 × 8 μm) but similar in their metabolic pattern and transport systems.

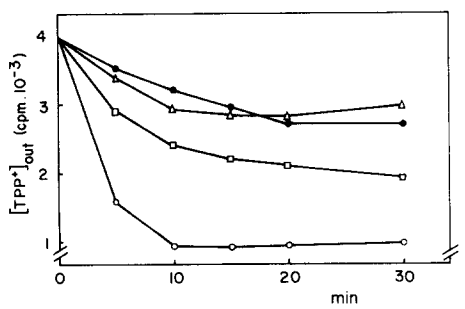

Fig. 1. TPP^+ concentration observed in the external buffered medium (Tris/succinate, 0.3 M, pH 7.5) of: △———△, *Saccharomyces bayanus* (dry weight 20 mg/ml; membrane potential after 30 min —49 mV); ●———●, *Endomyces magnusii* (30 mg/ml; —52 mV), □———□, *Saccharomyces cerevisiae* (38 mg/ml; —71 mV); ○———○, *Rhodotorula glutinis* (36 mg/ml; —112 mV). Initial TPP^+ concentration was 160 μM.

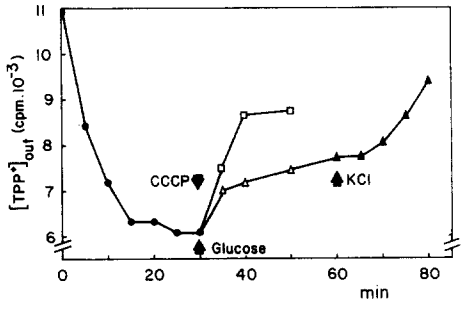

Fig. 2. TPP^+ concentration in the external medium of a *S. cerevisiae* suspension after adding CCCP (□———□), glucose (△———△) and KCl (▲———▲). Effect of CCCP measured in buffered medium (Tris/succinate, 0.1 M, pH 7.2). Cell dry weight 22 mg/ml, 22.5 μm TPP^+, 11 μM CCCP, 60 mM glucose, 100 mM KCl. The descending part of the TPP^+ equilibration curve (●———●) preceded all three treatments.

The microelectrode measurement of the membrane potential of *E. magnusii* was performed using glass microelectrodes drawn from Pyrex capillaries filled with 3 M KCl (resistance several tens of MΩ) and connected with a reference calomel electrode by an agar bridge. The cells were grown for 20 h as described in Ref. 4, aerated for 3 h to bring them to a metabolic state standard for all experiments, suspended in artificial pond water (0.50 mM NaCl, 0.51 mM KH_2PO_4, 0.14 mM K_3PO_4, 0.20 mM $CaCl_2$, 0.05 mM $MgSO_4 \cdot 7H_2O$, 0.55 mM KNO_3, 0.33 mM ferric citrate) and held at a broken tip of an unfilled microelectrode connected to a vacuum pump. Potentials were recorded on a TR-1501 electrometer (Budapest, Hungary) with a recorder; micromanipulation was carried out using a KM-1 system (Pushchino, U.S.S.R.).

Experimental recordings always exhibit the same trend: the microelectrode penetration into the cell is marked by a transient sharp hyperpolarization followed by a fast drop to steady values (Fig. 3). We believe the overshoot to be of little significance for assessing the membrane potential because of the lack of exponential character of its return (as would be suggestive of a leakage of ions from the cells) and because of the relative stability of the subsequent potential level. This potential value was hence considered to correspond to the actual membrane potential.

The directly measured membrane potential of *E. magnusii* was −35 ± 5 mV (mean ± S.E., four measurements), while indirect measurement with TPP^+ yielded −73 ± 7 mV (six measurements) in water and −54 ± 7 mV (six measurements) in artificial pond water. The indirect measurement with *S. cerevisiae* gave a potential of −76 ± 5 mV (14 measurements, in water). In *E. magnusii*, microelectrode measurements are thus seen to yield membrane potential values lower than those obtained by the TPP^+ distribution. This apparent overestima-

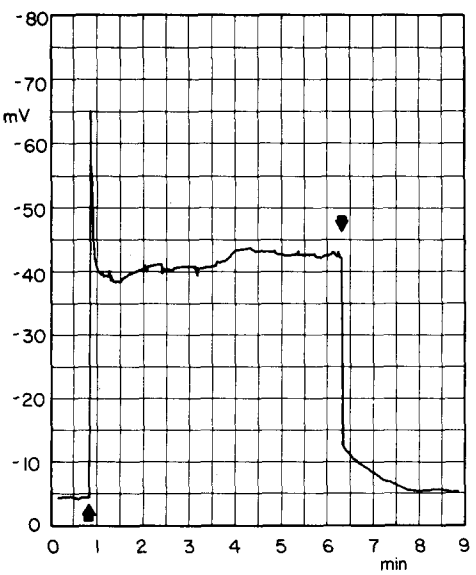

Fig. 3. Record of membrane potential of *E. magnusii* cell impaled with a microelectrode. First arrow, insertion of microelectrode; second arrow, escape of cell from the microelectrode tip.

tion of the membrane potential by the TPP^+ method may be due to the factors listed above (probe absorption, active transport). Caution should therefore be exercized when attempting to interpret, or use for further quantitative calculations, the membrane potential values obtained by the distribution techniques. Then again, direct measurements may underestimate $\Delta\psi$ in such small cells, because of damage, leakage and possibly other factors [5].

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