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Letters to the Editors

Membrane Potential in *Rhodotorula gracilis*

Received 13 March 1979

I would like to comment on the paper: "Evidence for interactions between the energy-dependent transport of sugars and the membrane potential in the yeast *Rhodotorula gracilis* (*Rhodosporidium toruloides*)" by R. Hauer and M. Höfer, published in *J. Membrane Biol.* (1978) **43**:335–349. The authors used the accumulation of the lipid-soluble cations triphenylmethylphosphonium ($TPMP^+$) and tetraphenylphosphonium (TPP^+) as a measure of the cellular membrane potential (ΔE). Table 1 in their paper states that ΔE was approximately 58 mV, as calculated from the accumulation of $TPMP^+$ and 82 mV from that of TPP^+. The authors argue that these values are too low, since other primitive eukaryotes have potentials of the order of 150 mV (in fact 120–150 mV, *Chlorella vulgaris,* Komor & Tanner, 1976) and of 250 mV (*Neurospora crassa,* Slayman & Slayman, 1974).

In Table 1 of this letter, I have recalculated ΔE from the original data of Hauer and Höfer (1978), Figs. 1, 2, 4 and 5. According to my calculations, both cations indicate considerably higher potentials than those calculated by Hauer and Höfer (Table 1 of this letter, last two columns). The discrepancy between the values of ΔE given by the two probes was also much higher. My guess is that the authors erroneously used the *total* specific activity, rather than the *actual* (left-over) external specific activity for the calculation of the accumulation ratio $[\text{cation}]_{in}/[\text{cation}]_{out}$. It is my experience that this error is often made, if the uptake data are first converted into nmol/mg cells or membranes, and subsequently into accumulation ratios (cf. Schuldiner & Kaback, 1975, Fig. 1). Therefore, I

Table 1. Membrane potential in *Rhodotorula gracilis*[a]

Fig.	Cation	cpm taken up	cpm remaining	$\frac{[\text{cation}]_{in}}{[\text{cation}]_{out}}$	ΔE calculated (mV)	ΔE reference 1 (mV)
1	TPP^+	27,000	9,000	144	125[b]	82[c]
2	$TPMP^+$	11,000	24,000	22	78	58[c]

[a] The data of the indicated figures of Hauer and Höfer (1978) were used to calculate the membrane potential ΔE. It was assumed that the cell concentration was 10 mg dry wt/ml and that the intracellular water space was 2 µl/mg dry wt (Hauer & Höfer, 1978, legend to Fig. 1 and *Materials and Methods,* respectively). The accumulation ratio, $[\text{cation}]_{in}/[\text{cation}]_{out}$, equals the ratio: $(\text{cpm}_{in}/\mu\text{l cell water})/(\text{cpm}_{out}/\mu\text{l medium})$. The membrane potential was calculated from the Nernst equation: $\Delta E = 58 \log ([\text{cation}]_{in}/[\text{cation}]_{out})$.

[b] For Figs. 4 and 5 (at pH = 7.5) values of ΔE were calculated of 126 and 116 mV, respectively.

[c] Reference 1: Hauer and Höfer, 1978.

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recommend calculating accumulation ratios and membrane potentials according to the procedure followed in Table 1 of this letter.

Thus, the cellular membrane potential of *Rhodotorula gracilis* is considerably higher than was calculated by Hauer and Höfer (1978). In fact, the values estimated from TPP^+ accumulation (116–126 mV, Table 1 of this letter) are very similar to those obtained by the same method for *Chlorella vulgaris* (120–150 mV, Komor & Tanner, 1976). However, at present it cannot be excluded that those estimates of ΔE only represent lower limits. In any case, it is clear that the accumulation ratio of $TPMP^+$, being considerably lower than that of TPP^+, did not reach the value dictated by the membrane potential (Table 1 of this letter). This finding is similar to that for intact cells of *Streptococcus faecalis,* where ΔE calculated from the $TPMP^+$ accumulation was up to 60 mV lower than ΔE determined by other methods (Bakker, 1978). The reason for this underestimation is not known. However, one should be careful with the use of $TPMP^+$ as a quantitative probe of the membrane potential (*cf.* Ramos & Kaback, 1977).

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Reply to: Membrane Potential in *Rhodotorula gracilis*

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Answering the criticism of Dr. Bakker's Letter to the Editor, we would like to point out that the membrane potentials published in Table 1 of our paper: "Evidence for interactions between the energy-dependent transport of sugars and the membrane potential in the yeast *Rhodotorula gracilis* (*Rhodosporidium toruloides*)," *J. Membrane Biol.* **43:**335–349 (1978) were calculated exactly by the same procedure as recommended by Dr. Bakker. The membrane potentials of Table 1 represent average values of 8 different experiments which were designed to compare the accumulation of TPP^+ and $TPMP^+$. The results of Figs. 1, 2, 4 and 5 show each an individual experiment with the highest accumulation of indicator ions obtained under the experimental setup given in the legends. The average value of the membrane potential in each particular set of experiments was similar to the data given in Table 1. We still feel this to be legitimate because experiments showing a high degree of accumulation are more instructive to the reader. In all experiments the response of the membrane potential to manipulations such as the addition of uncouplers was independent of the size of the membrane potential.

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