directed proton pump at the plasma membrane has been obtained for a number of bacteria (Harold & Papineau, 1972; Padan et al., 1976) and cyanobacteria (Scholes et al., 1969).

The present investigation was undertaken to establish the relative importance of the electrical and chemical components of the proton gradient ($\Delta \psi$ and $\Delta \phi$ respectively) in the filamentous heterocystous cyanobacterium *Anabaena variabilis* over a range of external pH.

Measurements of membrane potential were obtained by using the lipophilic cation triphenylmethylphosphonium, which is accumulated within cells in accordance with the Nernst equation (Grinius et al., 1970). Intracellular pH was determined by measuring the distribution of 5,5-dimethyl-2,4-oxazolidinedione in accordance with the equations described by Heldt et al. (1973). Cells were harvested during exponential growth and incubated in a medium containing either 3H-labelled triphenyl-dione; silicone-oil micro-centrifugation was employed to separate cells from the bathing medium. Results were converted in direct response to $\psi_m^*$, although some of the data are not wholly consistent with such a mechanism (Raven, 1980). The present study was undertaken to establish the primary characteristics of $K^+$ transport in *Anabaena variabilis* in order to assess the validity of the chemiosmotic theory as applied to cation transport in cyanobacteria.

Experiments were performed with radioisotopic $K^+$ to follow the uptake and loss of $K^+$ in cells of *A. variabilis*. Efflux determinations involved the incubation of cells in medium containing $3^4$KCl together with $3^4$KCl for known lengths of time; incubation was terminated by silicone-oil micro-centrifugation. Effluxes were performed with pre-loaded cells that were subsequently transferred to unlabelled medium and subjected to silicone-oil micro-centrifugation after a fixed time interval. All samples were assayed by liquid-scintillation spectrometry.

Initial experiments with cells maintained in a medium containing $5^1$KCl at 5 mol m$^{-3}$ external concentration demonstrated that efflux of $K^+$ was drastically inhibited when $3^4$KCl was removed from the bathing medium. There are a number of

Potassium ion transport and electrogenesis in *Anabaena variabilis*

ROBERT H. REED, PETER ROWELL and WILLIAM D. P. STEWART

Department of Biological Sciences, University of Dundee, Dundee DD1 4HN, Scotland, U.K.

The chemiosmotic theory of Mitchell (1966) is frequently invoked to explain the observed distribution of $K^+$ within microbial cells. Basically, the mechanism comprises the generation of an interior-negative membrane potential, $\psi_m$, via an electrogenic proton pump that transfers net positive charge out of the cell; this subsequently leads to intracellular accumulation of $K^+$ by passive unport (cf. active transport) in a manner dependent on $\psi_m$. Such a mechanism demands that $\psi_m$ be more negative than, or at least equal to, the Nernst equilibrium potential for $K^+$, $\psi_m$. This has been shown to be feasible in some bacteria (Harold & Papineau, 1972) and fungi (Slayman & Slayman, 1968), but may not be true of microalgal cells, e.g. *Chlorella* (Tromballa, 1980). Paschinger (1977) has suggested that $K^+$ is accumulated within cells of the cyanobacterium *Anacystis nidulans* in direct response to $\psi_m^*$, although some of the data are not wholly consistent with such a mechanism (Raven, 1980). The present study was undertaken to establish the primary characteristics of $K^+$ transport in *Anabaena variabilis* in order to assess the validity of the chemiosmotic theory as applied to cation transport in cyanobacteria.

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Values for the membrane potential, $\psi_m$, obtained from triphenylmethylphosphonium accumulation studies are also shown. All values are shown as the mean (three replicates per treatment; standard error associated with each value is less than 5% of the mean in each case).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$[K^+]_{I-p}$ (mol·m$^{-3}$)</th>
<th>$[K^+]_{I+}$ (mol·m$^{-3}$)</th>
<th>$\psi_m$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.0</td>
<td>196.0</td>
<td>-94.0</td>
</tr>
<tr>
<td>Dicyclohexylcarbodi-imide (0.1 mol·m$^{-3}$)</td>
<td>5.0</td>
<td>126.5</td>
<td>-82.8</td>
</tr>
</tbody>
</table>

Table 1. Internal $K^+$ concentrations [$K^+]_I$ and Nernst equilibrium potentials ($\psi_m$) in control and dicyclohexylcarbodi-imide-treated cells (incubation period in inhibitor 100 min)

We are grateful to the staff of the Scottish Universities Reactor Centre for providing $\delta^6$Rb$^+$ and to the Agricultural Research Council for financial support.

ROBERT H. REED, PETER ROWELL and WILLIAM D. P. STEWART

Discrimination between rubidium and potassium ions in Anabaena variabilis

The elements rubidium (Rb) and potassium (K) are adjacent members of the alkali-metal series, displaying similar chemical, electrical and physical properties (Relman, 1956). The radioisotope $\delta^6$Rb$^+$ has frequently been used as a convenient tracer for K$^+$ in biological systems, since it has a lower rate of decay than $\delta^4$K$^+$ (the half-life of these isotopes being 19.5 days and 12.36 hours respectively). Use of $\delta^6$Rb$^+$ facilitates longer-term experimental design and counting procedures. However, in comparative studies of Rb$^+$ and K$^+$ transport in several microbial and plant cell types the findings are frequently equivocal. Some workers have concluded that $\delta^6$Rb$^+$ is an adequate tracer for K$^+$ (e.g. Laüchli & Epstein, 1970), whereas others suggest that $\delta^6$Rb$^+$ may underestimate (Jacoby & Nissen, 1977) or overestimate (Maas & Leggett, 1968) K$^+$ exchange. A small number of studies show that $\delta^6$Rb$^+$ exchange may be qualitatively and quantitatively dissimilar to $\delta^4$K$^+$ exchange in selected cell types (Ramani & Kannan, 1976). The present study was undertaken during an investigation of K$^+$-transport processes in Anabaena variabilis (Reed et al., 1980), the initial stimulus for this research being provided by the data of Paschinger (1977), which suggest that $\delta^6$Rb$^+$ may not equilibrate at the same electrochemical potential as K$^+$.

Uptake and loss of both $\delta^6$Rb$^+$ and $\delta^4$K$^+$ were determined by using double-labelling techniques to minimize the possibility of spurious results due to sample variation. Samples were counted at $t_1 = 0.00$ h and $t_2 = 12.36$ h; the difference between the count rate at $t_2$ and $t_1$ should therefore be half of the original count rate for $\delta^4$K$^+$. The amount of $\delta^6$Rb$^+$ can then be simply calculated by subtracting the counts due to $\delta^4$K$^+$ from the total count rate at $t_2$. Decay of $\delta^6$Rb$^+$ during this time period is minimal and constitutes a possible error of less than 2%.

Table 1 gives data for K$^+$ flux rates estimated by means of $\delta^6$Rb$^+$ and $\delta^4$K$^+$ at low (0.01–0.05 mol·m$^{-3}$) and high (1.0–5.0 mol·m$^{-3}$) external K$^+$ concentrations; these values cover the two transport systems (high-affinity and low-affinity respectively) associated with K$^+$ transport in A. variabilis. It is clear that $\delta^6$Rb$^+$ flux rates are lower than the corresponding $\delta^4$K$^+$