J. Membrane Biol. 49, 21–55 (1979)

Amine Uniport at the Plasmalemma of Charophyte Cells: I. Current-Voltage Curves, Saturation Kinetics, and Effects of Unstirred Layers

N.A. Walker, M.J. Beilby, and F.A. Smith*

Biophysics Laboratory, A12, School of Biological Sciences, University of Sydney, Sydney, New South Wales, Australia, 2006

Received 9 January 1979

Summary. In voltage-clamped cells of the algae *Chara* and *Nitella* an inward current of positive charge occurs when NH_4^+ or $CH_3NH_3^+$ is added to the external medium. There is a simultaneous increase in membrane conductance, agreeing with earlier evidence that the current represents the inward uniport of the amine ion.

We have obtained current-voltage curves for this uniport, and show the effect on their shape caused by the unstirred layer of solution adjacent to the cell membrane. The current-voltage curves for $CH_3NH_3^+$, which are less affected by the unstirred layer, are concave towards the current axis, show no saturation with membrane PD in the range -100 to -300 mV, and show saturation as the concentration is raised. The dependence of current on concentration follows a Michaelis-Menten relation, the parameters having the following values at -200 mV:

$$V_m = \text{up to } 200 \text{ mA m}^{-2} \text{ for both substrates}$$
$$K_M = 3 \text{ μM for } NH_4^+ \text{ and } 200 \text{ μM for } CH_3NH_3^+.$$

Both V_m and K_M depend on membrane potential, approximately as $\exp(-F\psi/6RT)$ and $\exp(F\psi/3RT)$, respectively.

The results suggest a transport channel with a single, selective binding site below the membrane surface and a single potential energy barrier at the center of the membrane.

The rate of transport falls as the cell takes up amine, and also varies markedly from culture to culture. The significance of this transport for the biology of the charophyte plant is discussed.

It has been shown (Smith, Walker & Raven, 1977; Smith & Walker, 1978; Smith, Raven & Jayasuriya, 1978) that ammonia and methylamine almost certainly enter *Chara* and *Hydrodictyon* as ions rather than as neutral molecules, over a considerable range of pH – approximately 5 to 9. This entry of NH_4^+ or $CH_3NH_3^+$ can be detected by the depolarization

* Department of Botany, University of Adelaide, Adelaide, South Australia, 5001.

0022-2631/79/0049-0021 $07.00

of the cell membrane, observed by Barr, Koh and Ryan (1974) and Jayasuriya (1975). It is better, however, for the quantitative study of transport rates to measure the change in membrane current in cells under voltage clamp (Smith & Walker, 1976, 1978). The change in membrane current when amine is presented to the cell can be studied as a function of the membrane potential difference (PD) and the amine concentration. This gives valuable information on the kinetic properties of the transport system. It is also possible to study the effects of, for example, pH, without consequent unwanted changes in PD.

When NH_4^+ and $CH_3NH_3^+$ are present at the same time, the observed change in current is less than the sum of the changes produced by the amines separately. This observation led us to postulate that the two amines enter *Chara* by the same transport system (Smith & Walker, 1978). Tracer studies which support this conclusion will be published shortly; they show that NH_4^+ strongly inhibits the entry of $CH_3NH_3^+$ when the membrane PD is held constant.

Since the ions in question have a single positive charge, the normal membrane PD would lead to their accumulation in the interior of the cell. The largest concentration ratios so far observed are consistent with this mechanism of accumulation, the simplest reaction being:

$$(RNH_3^+)_{out} \rightleftarrows (RNH_3^+)_{in} \tag{1}$$

for which the flux is equal to the current (through the transport system) divided by the Faraday. It is assumed that this is the reaction being studied. This postulate is confirmed by the tracer studies to be published, which show a ratio of electric charge to quantity of CH_3NH_2 which is close to 1.0 between pH 5.7 and 8.5. The ratio falls at higher pH, a finding consistent with a permeability for the free amine of about 10^{-5} m sec^{-1}. The rate of entry of free amine is a significant fraction of the total rate of entry at pH 9.5 and above, but is not significant at pH 7.5.

This paper reports the study of the current-voltage curves for amine transport, using both NH_4^+ and $CH_3NH_3^+$, in both *Chara* and *Nitella*. The concentration dependence of the current is also reported, and a channel model is described which has properties similar to those observed. The effects of unstirred layers of solution outside the cell membrane are demonstrated, and taken into account in the kinetic analysis. The pH dependence of the transport is briefly described.

There is no agreement in the literature on the conductance of the tonoplast in charophyte cells or on the appropriate method of measuring it (Coster & Smith, 1977; Skierczynska *et al.*, 1972, 1977). Since this question is of some importance in the interpretation of the results

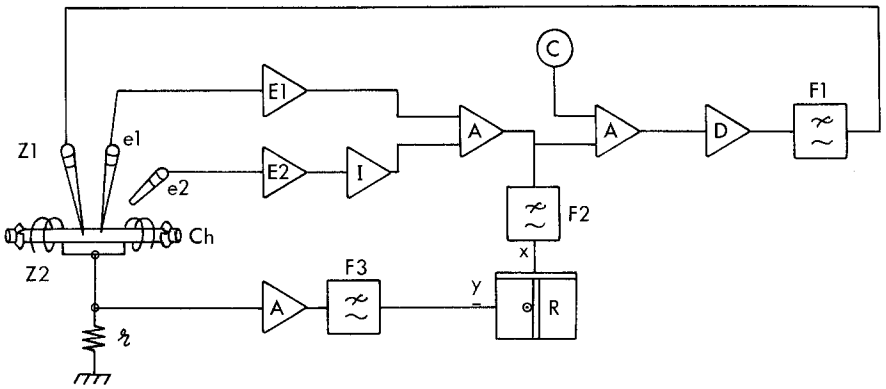


Fig. 1. Apparatus for measurement of current-voltage curves. *A*: summing amplifier; *C*: source of command potential; *Ch*: charophyte internodal cell; *D*: driving amplifier; *E*1, *E*2: unity-gain electrometer amplifiers of Keithley Model 604; *e*1, *e*2: electrodes for potential differences measurement; *F*1, *F*2, *F*3: low-pass filters; *I*: unity-gain inverter; *R*: *X* − *Y* recorder; *r*: switched resistors for current measurement; *x*, *y*: inputs to recorder; *Z*1, *Z*2: electrodes for passing current

presented here, we have used a new method based on the phenomenon of punch-through (*see* Coster, 1965) to obtain the minimum conductance of the tonoplast.

Materials and Methods

Chara corallina (= *C. australis*), originally collected at various sites in New South Wales, and *Nitella clavata*, gift of Dr. C.E. Barr, were grown in the laboratory as previously described (Hope & Walker, 1975), at about 20 °C, with a day-length of 12 h. The standard experimental medium (SW) contained (in mM): NaCl, 1; K_2SO_4, 0.1; $CaSO_4$, 0.5; zwitterionic buffer, 5; NaOH, as required to adjust pH. The buffers used, each at or near its pK, were MES[1], MOPS, TAPS and CHES, obtained from Calbiochem. Amines were added to the solutions just before each experiment as salts of MES acid. The stock amine solutions were made by adding ammonia vapor or liquid methylamine to a solution of the buffer acid. The final amine concentration was calculated from the measured pH, the pK of the buffer, and its known concentration. When the addition of stock amine might have changed the pH of the experimental solution, we prepared several stocks of different pH. All solutions were made with glass-distilled water: except in the experiments described first (*see* Fig. 3), the water was scrubbed after distillation by passage through a column of Dowex-50 cation exchange resin, in the acid form.

Internodal cells shorter than 26 mm were chosen for the electrical experiments: their characteristic cable length was 10 to 20 mm. The isolated internodes were mounted in a plastic chamber in which they were held along the axis of a helical Ag − AgCl current electrode (Fig. 1). Microelectrodes were inserted into the vacuole as already described (Smith & Walker, 1978) for measurement of PD and for injection of current.

[1] *Abbreviations:* MES, 2-(N-morpholino)ethanesulphonic acid; MOPS, 3-(N-morpholino)propanesulphonic acid; TAPS, N-tris-(hydroxymethyl)methyl-3-amino-propanesulphonic acid; and CHES, cyclohexylaminoethanesulphonic acid.

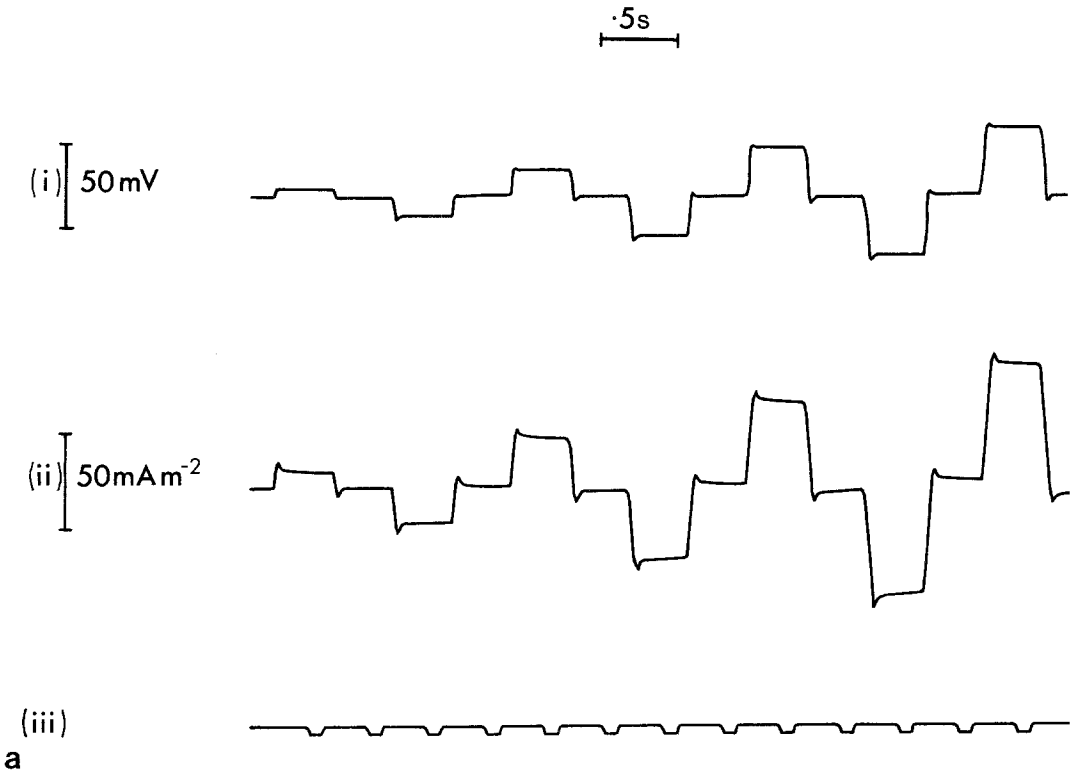
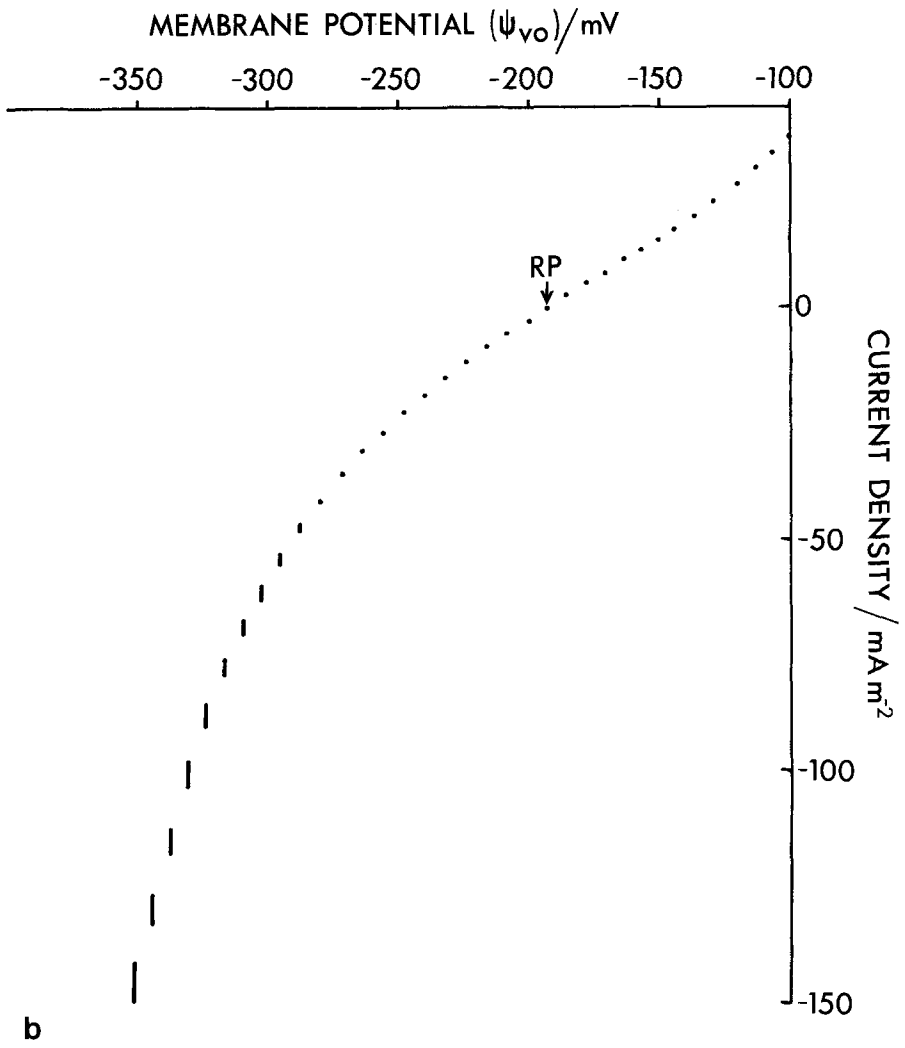


Fig. 2. (*a*): Waveforms used in obtaining current-voltage curves. (*i*) Bipolar staircase of pulses added to command potential; (*ii*) resulting change in membrane current; (*iii*) signal to recorder pen (down puts pen down). Pulse rate bout 2.8 Hz. (*b*): Typical current-voltage curve for a charophyte cell, obtained with the apparatus described (Fig. 1) and the waveforms of *a*. *RP*, resting potential

The electrical apparatus was set up as shown in Fig. 1. The unity-gain outputs of Keithley Model 604 electrometer, which measured the membrane PD, were connected to the *x*-axis input of a fast *x*, *y*-recorder. An EAI hybrid analog-digital computer was used to set up the command potential, to subtract the membrane PD from it, and amplify the result. An amplifier with an output swing of ± 35 V accepted this signal and drove the current through the inserted electrode. The current path included a known resistor, the PD across it being taken as a measure of the current. Current and voltage changes were measured with an accuracy of about 1 %; absolute membrane PD with an accuracy of about 5 mV.

The command potential was a steady potential, on which could be superimposed a bipolar staircase of pulses: this produced corresponding changes in the membrane PD of a cell under voltage clamp and consequent changes in membrane current density (Fig. 2*a*). The pen of the *x*, *y*-recorder was down only during the third quarter of each pulse and space, when the current was not changing rapidly (Fig. 2*a*). This gave a dotted current-voltage curve (Fig. 2*b*) having about 15–30 points lying between -100 and



−300 mV. An excitation was always produced if the membrane PD was driven more positive than −90 mV, and this altered the shape of the current-voltage curve for some time; if the PD was taken more negative than about −300 mV there was usually a very rapid rise in current, the "punch-through" phenomenon of Coster (1965). These effects set the limits of the normal experiment. A continuous current-voltage curve was interpolated by hand, to get readings at fixed voltages or for display (e.g., Figs. 5, 6).

The current-voltage curve was determined before, during, and after a brief exposure of the cell to amine at a particular concentration; subtraction of the zero-concentration curve gave the curve for inward transport of amine. An exposure of about 30 sec did not usually alter the current-voltage curve, except in the punch-through region. If there were significant changes in the working region, the experiment was discontinued. The results were discarded if at the end of the series of concentrations a repeat of the first concentration gave a result different by 20% or more.

The current-voltage curves obtained by this method gave correct values of membrane current, but the voltage measured was that across plasmalemma and tonoplast in series

(ψ_{vo}). This could be corrected to give the desired PD across the plasmalemma (ψ_{co}) by:

$$\psi_{co} = \psi_{vo} - \psi_{vc} \tag{2}$$

where the PD across the tonoplast (ψ_{vc}) could be represented, over a limited voltage range, as the sum of the resting value (ψ^0_{vc}) and the ohmic potential drop:

$$\psi_{vc} = \psi^0_{vc} + g_r i \tag{3}$$

where the conductance of the tonoplast is g_r and the current density is i.

The minimum value of g_r was determined by measuring the current-voltage curve of the cell beyond -300 mV, the voltage being increased until a steep, constant slope was obtained. The beginning of such a slope is seen in Fig. 2*b*. This slope gave the conductance of the tonoplast in series with that of the punched-through plasmalemma. The latter is expected to be small, but this cannot be established in any way that avoids the objections of Skierczynska *et al.* (1977). The values so obtained were corrected for cable-effect by solving for g, the conductance of the two membranes in series, the following equation (*see* Cole & Hodgkin, 1938):

$$G = 2\pi d\lambda g \tanh(l/2\lambda) \tag{4}$$

where G is the measured conductance, d is the diameter of the cell, l is the length of the cell, and λ is given by $2\lambda = \sqrt{d/g\rho}$ where ρ is the electric resistivity of the cell interior. This correction was necessary because the conductance of the punched-through cell was much higher than its normal conductance.

Theoretical equations were fitted to sets of the experimental data. Initially, each set contained the values of the change in current density at a particular voltage, as a function of amine concentration. Later, each set was the set of current-voltage curves at all concentrations. The values of the parameters were chosen to minimize the sum of squared differences between observed and calculated current density. This seems the most reasonable procedure: if the errors are only in the measurements of current density, and if they are normally distributed, this method gives the maximum likelihood values of the parameters. The actual choice of values was made by a program in Fortran IV using a subroutine, written by M. Bell, based on the pattern-search method of Hooke & Jeeves (1961) as described by Colquhoun (1971). The program was run on either a C.D.C. Cyber 72 or a Cromemco Z2–D.

Results

Effect of Flow-Rate on Membrane Current

Solutions such as SW, when freshly prepared from glass-distilled water, caused a depolarizing (inward positive) current to flow through the membrane of a voltage-clamped cell; the current increased with increasing flow rate. When flow of the solution was stopped, the current returned to the zero-flow value with a characteristic time-course (Fig. 3*i*). The effect was much smaller if the solution was made from water scrubbed, by passage through a column packed with Dowex-50, after distillation (Fig. 3*ii*). It was also small when medium from a *Chara*

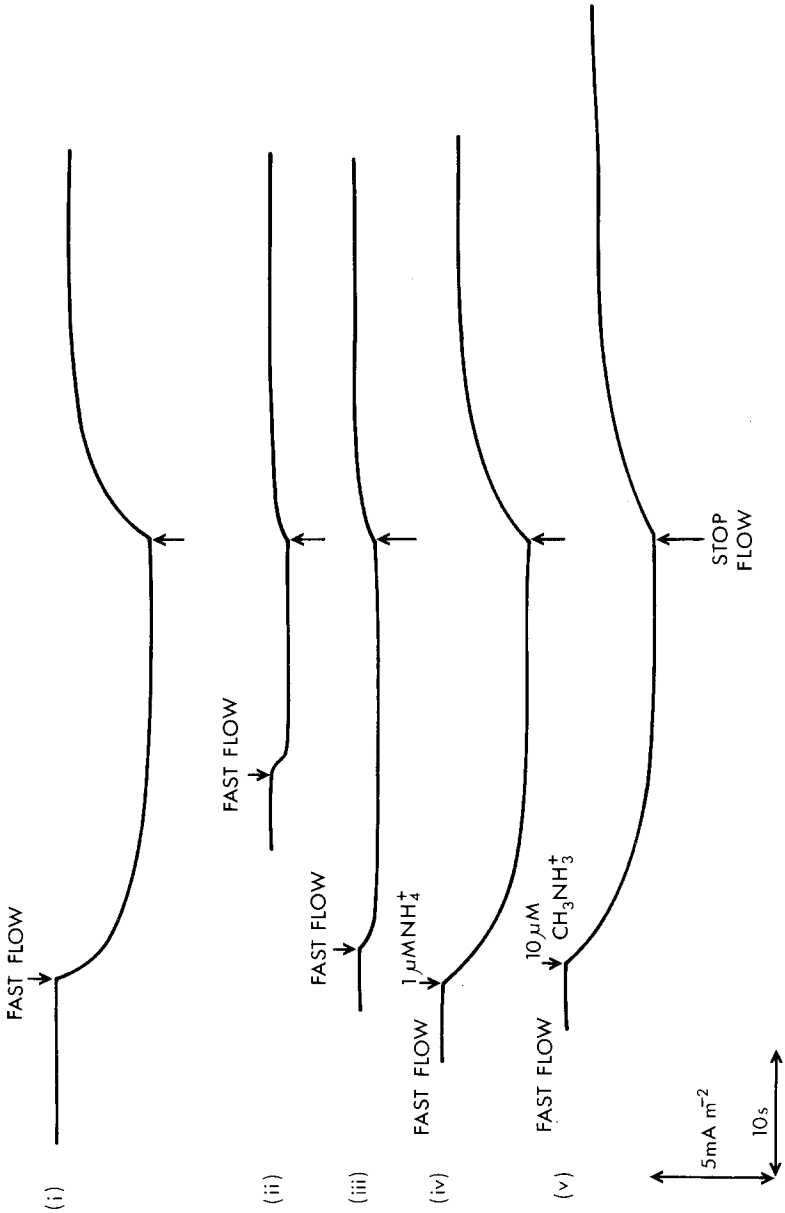


Fig. 3. Time-course of membrane current, showing effect of initiating rapid flow or adding amine to flowing solution, followed by stopping of the flow. (*i*) flow and stop of freshly-made SW medium, using glass-distilled water; (*ii*) as (*i*), but using distilled water passed through deionizing column, (*iii*) flow and stop of medium removed from *Chara* culture tanks; (*iv*) introduction of 1 μM NH_4^+ into fast-flowing medium, followed by stopping of flow; (*v*) as (*iv*), but using 10 μM $CH_3NH_3^+$

culture tank was used to bathe the experimental cell (Fig. 3*iii*). It was found that medium made from distilled water that had not been scrubbed showed little flow-depolarization after standing for a week or two.

The addition of a low concentration of NH_4^+ to a scrubbed water medium increased the flow-depolarization (Fig. 3*iv*), as did a higher concentration of $CH_3NH_3^+$ (Fig. 3*v*). The time course of current when flow was stopped was the same for media with added NH_4^+ and for

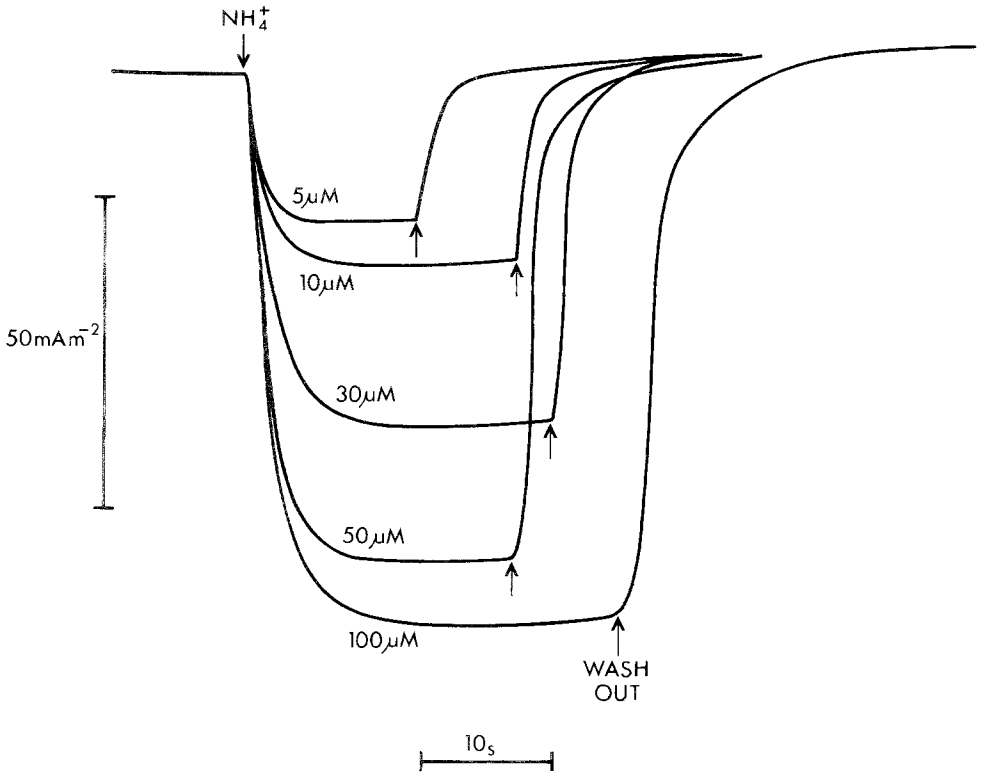


Fig. 4. Time-course of membrane current, showing effect of introducing NH_4^+ into the fast-flowing medium, followed by its removal

media made with unscrubbed water. These results suggested that fresh glass-distilled water contained low concentrations of NH_4^+. Measurements with an Orion NH_4^+ electrode showed that the glass-distilled water contained up to 3 μM volatile base (the electrode is not specific for NH_4^+), while the scrubbed distilled water contained 0.2–0.5 μM. The latter value was obtained by extrapolation from calibration points at higher (known) concentrations, assuming a slope of 58 mV per log unit.

Effect of Amine at High Flow-Rate

In experiments using media made from scrubbed water, at flow rates of 5–10 mm sec^{-1}, a change in the external medium to one containing added amine produced a definite and reproducible change in the membrane current density (Fig. 4). These changes were rapid when the flow of medium was rapid, and after the change was established the current was

then constant for a minute or more. The washing out of the amine produced a rapid return of the current to its former value (Fig. 4). A later section of the results deals with the relatively irreversible effects of longer exposure to amine solutions.

At all concentrations used, the current change conformed to the pattern shown (Fig. 4), happening as rapidly as the new concentration was established in the chamber, being rapidly reversible, and showing only the slow time-dependence reported below.

Current-Voltage Curves

With the electrical apparatus described, we measured the current-voltage curves of *Chara* and *Nitella* cells before, during, and after the presentation of amine solution to the cell. With the cell membrane PD clamped, it proved possible to make measurements at a number of amine concentrations on each cell, and often to use both substrates. The limiting factor was the slow fall in transport rate as the cell took up amine (discussed below).

Figure 5 shows typical families of current-voltage curves for a single *Chara* internode, with various concentration of both substrates. If the zero-concentration curve is subtracted from the curve for each concentration, one obtains a family of current-voltage curves for the amine transport system. This procedure gives, for example, the curves of Fig. 6, derived from the results in Fig. 5.

The current-voltage curves obtained by the addition of NH_4^+ were consistently flatter and of lower slope than those for $CH_3NH_3^+$, as Fig. 6 indicates. The curves for $CH_3NH_3^+$ were concave towards the current axis, as the same figure shows. This concavity is taken to be characteristic of the transport system, since the curves for NH_4^+ can be made to bend back further towards the voltage axis by slowing the flow of medium (Fig. 7). This is the result of increasing the thickness of the unstirred layer of medium at the cell surface, since transport across this layer will depend on concentration difference, and will be independent of membrane properties when membrane transport is not rate limiting.

Effect of Concentration on Current

At any selected value of PD, a set of curves such as those of Fig. 6 will give points on a curve of current against concentration. We took

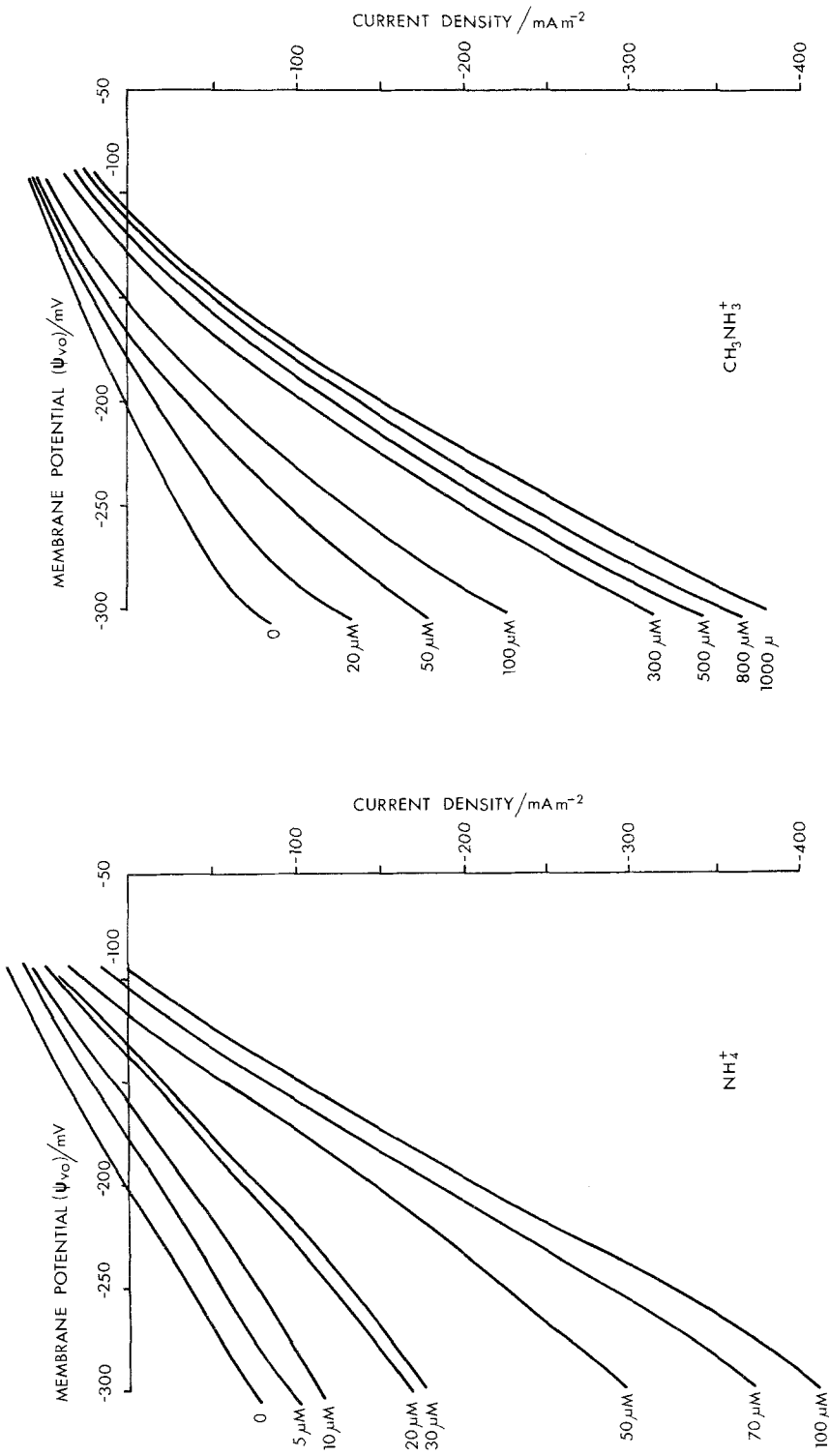


Fig. 5. Current-voltage curves for *Chara* internodal cell in the presence of the stated concentration of amine. (*a*): NH_4^+; (*b*): $CH_3NH_3^+$. Curves for the same cell at different times, pH 7.5

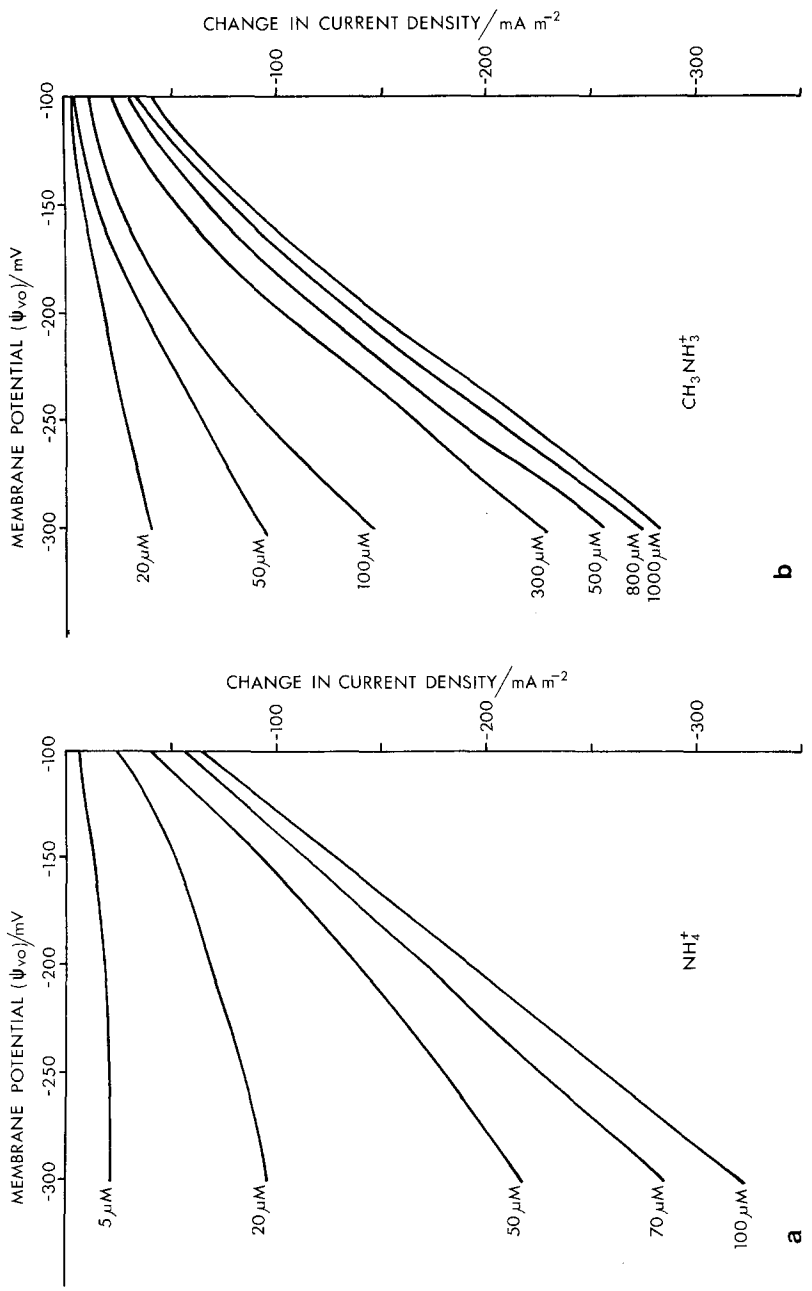


Fig. 6. Current-voltage curves for inward transport of amine in the *Chara* internode of Fig. 5, obtained by subtraction of the curve for zero amino concentration

such data from the current-voltage curves at intervals of 50 mV, a typical set of results appearing in Fig. 8. Here Fig. 8*a* contains results for a *Chara* cell with NH_4^+, Fig. 8*b* results for a *Chara* cell with $CH_3NH_3^+$, and Fig. 8*c* results for a *Nitella* cell with NH_4^+. The points show clearly that the current saturates as the concentration rises. The curves in Fig. 8

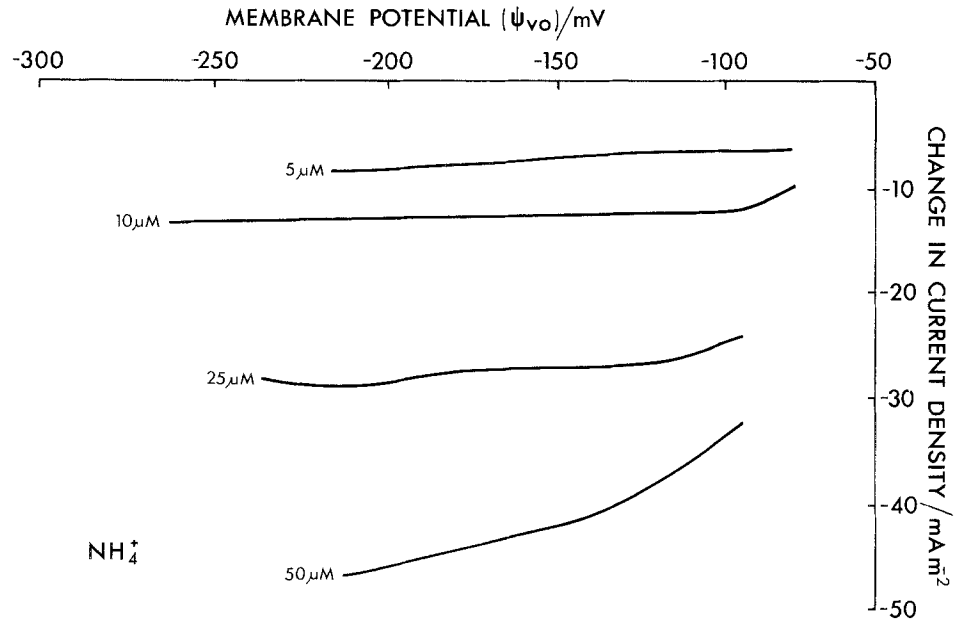


Fig. 7. Current-voltage curves for the inward transport of NH_4^+ in a *Chara* internode, showing the effect on the shape of the curve when flow of the bathing medium is quite slow

are calculated from the equation for the rate of a process showing Michaelis-Menten kinetics, in series with a diffusion resistance due, for example, to an unstirred layer (Hill & Whittingham, 1957). This equation is:

$$i=\tfrac{1}{2}\{(K_M FP_u+cFP_u+V_m)-[(K_M FP_u+cFP_u+V_m)^2-4cFP_u V_m]^{\frac{1}{2}}\} \quad (5)$$

where i is the current density, K_M is the Michaelis constant, P_u is the permeability of the diffusion barrier ($P_u=D/d$, if d is the thickness), c is the concentration and V_m is the maximum rate of the reaction, in electrical units. Equation (5) was fitted to the data by least squares, with the constraints that P_u should have the same value for all the data on each cell, while K_M and V_m could vary with PD and substrate, but not, of course, with concentration. It was generally possible to get satisfactory fits, with residual sums of squares less than 10% of the total sum of squares. The equation seems to represent the data well; it fits the data for NH_4^+ much better than a simple Michaelis-Menten equation does, and the data for $CH_3NH_3^+$ somewhat better. This point can be readily appreciated if one compares the shapes of the curves in Fig. 8*b*, which are essentially hyperbolic, with the shapes in Fig. 8*a*, which have rather

straight sections at low and high concentrations, with a sharpish shoulder joining them.

The values of P_u giving best fit varied from experiment to experiment, with a median of 5×10^{-5} m sec^{-1}, corresponding to that of a layer of water about 40 μm thick. In Fig. 7 the voltage-independent currents can be used to calculate P_u as 1.3×10^{-5} m sec^{-1}, corresponding to 150 μm of water.

The values of K_M for NH_4^+ were also quite variable, particularly in the *Chara* experiments. Study of the residual sum of squares as a function of P_u and K_m showed that in most cases there was an elongated valley, with low residuals available from many pairs of values of the two parameters. If Eq. (5) is differentiated we find that the initial slope of i against c is determined by $(F^{-1} P_u^{-1} + K_M/V_m)^{-1}$, while only the curvature is determined by the relative magnitudes of FP_u and V_m/K_M. Clearly the present experiments will not give accurate values of K_M for NH_4^+, at least in *Chara*. The value of $(FP_u)^{-1} + K_M/V_m$ is quite well determined.

Both K_M and V_m were found to depend on membrane PD as well as on the nature of the substrate – these effects are dealt with below.

Effect of Membrane PD on Kinetic Parameters

It is clear from inspection of the curves in Fig. 8 that V_m will vary with membrane PD in the range -100 to -300 mV. This variation is shown explicitly in Fig. 9, which contains mean values for a number of *Chara* cells and for both substrates (Fig. 9*a*) and mean values for a few *Nitella* cells and one *Chara* for NH_4^+ (Fig. 9*b*). Figure 9*a* shows the mean values from a rather heterogeneous collection of experiments, at flow rates of 5–10 mm sec^{-1}; the results for the single *Chara* cell in Fig. 9*b* were taken at the higher rate of 20 mm sec^{-1}. The curves in Fig. 9 are based on the equation:

$$V_m = V_{-200} \exp\left(\frac{\psi_{vo} + 200\,\text{mV}}{-B}\right) \tag{6}$$

where V_{-200} and B are chosen for best fit. The values used are:

Alga	Substrate	Fig.	V_{-200} (mA m^{-2})	B (mV)
Chara	NH_4^+	9*a*	83.9	139
Chara	$CH_3NH_3^+$	9*a*	66.8	143
Chara	NH_4^+	9*b*	47.5	160
Nitella	NH_4^+	9*b*	64.2	190

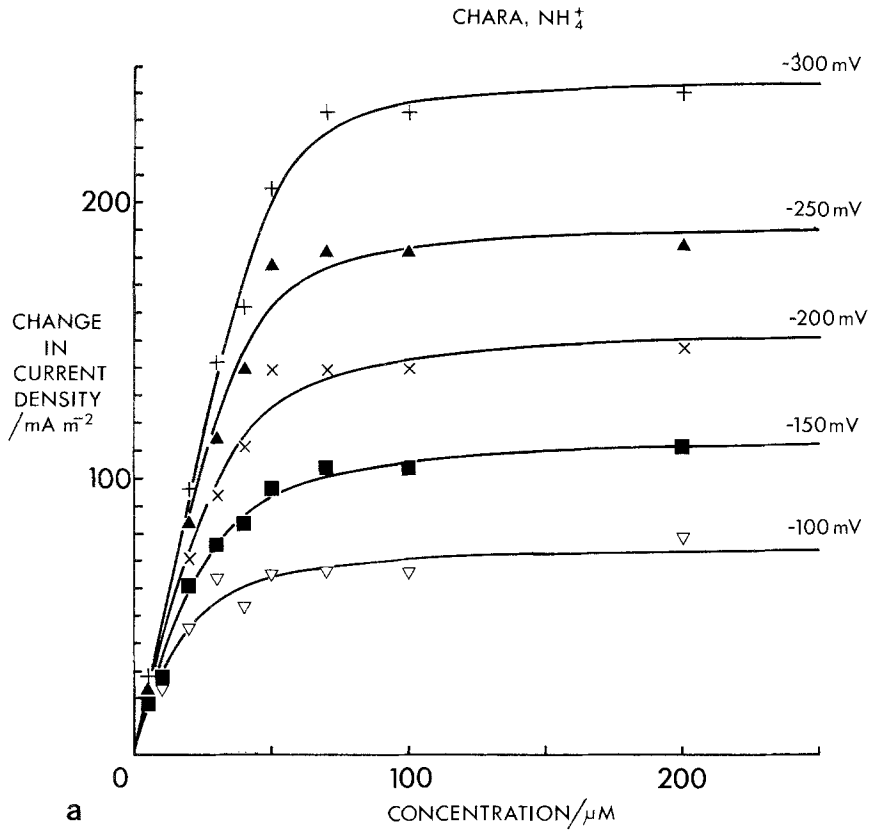


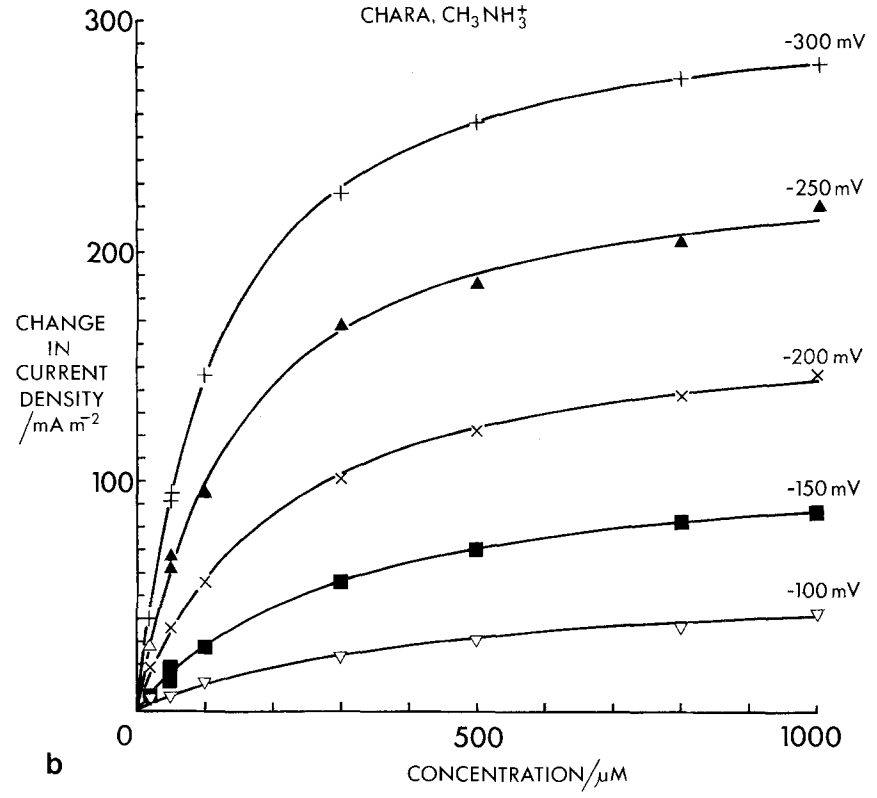
Fig. 8. Current-concentration curves for the inward transport of amine in charophyte internodes, showing the effect of rapid changes in membrane potential. Curves obtained directly by replotting current-voltage curves such as those of Fig. 6. (*a*): NH_4^+ in *Chara*; (*b*): $CH_3NH_3^+$ in *Chara*; (*c*): NH_4^+ in *Nitella*. All at pH 7.5

The dependence of K_M on ψ_{vo} in the same experiments is shown in Fig. 10, except that the K_M values for NH_4^+ in *Chara* at low flow rates have been omitted. These were unreliable because they were affected by the unstirred layer. The other measurements show a dependence of K_M on ψ_{vo} that is also fitted by an exponential:

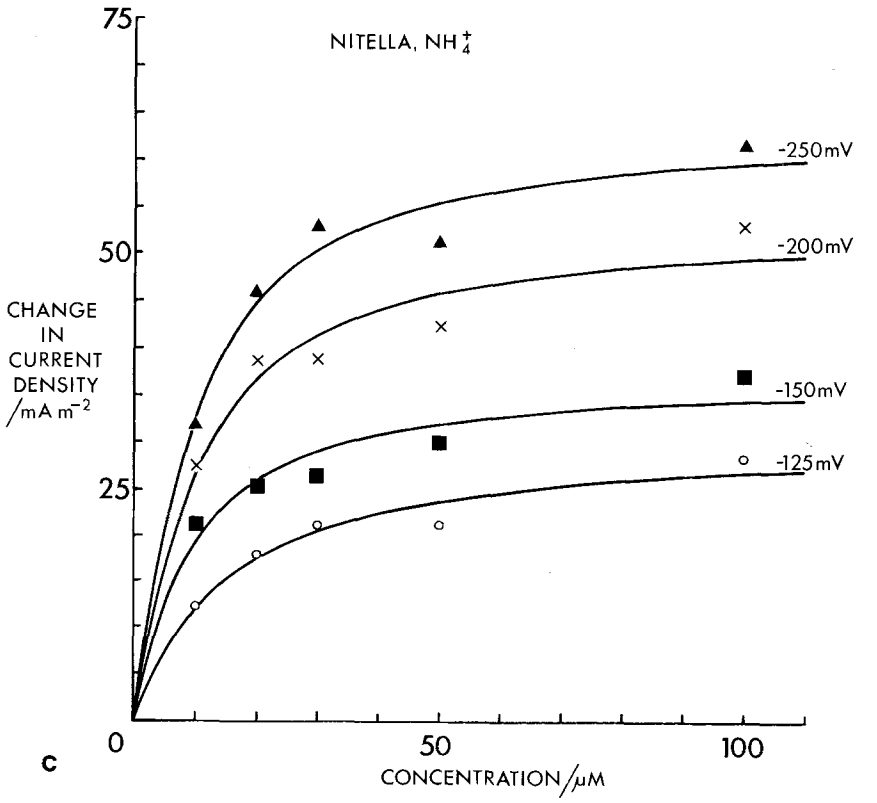
$$K_M = K_{-200} \exp\left(\frac{\psi_{vo} + 200\,\text{mV}}{A}\right) \tag{7}$$

where K_{-200} and A are chosen for best fit. The values used are:

Alga	Substrate	Fig.	K_{-200} (μM)	A (mV)
Chara	$CH_3NH_3^+$	10*a*	180	100
Chara	NH_4^+	10*b*	3.0	62
Nitella	NH_4^+	10*b*	6.2	74


CHARA, $CH_3NH_3^+$
300
200
100
0
500
1000
CHANGE IN CURRENT DENSITY /mA m^{-2}
CONCENTRATION/μM
-300 mV
-250 mV
-200 mV
-150 mV
-100 mV

b


NITELLA, NH_4^+
75
50
25
0
50
100
CHANGE IN CURRENT DENSITY /mA m^{-2}
CONCENTRATION/μM
-250mV
-200mV
-150mV
-125mV

c

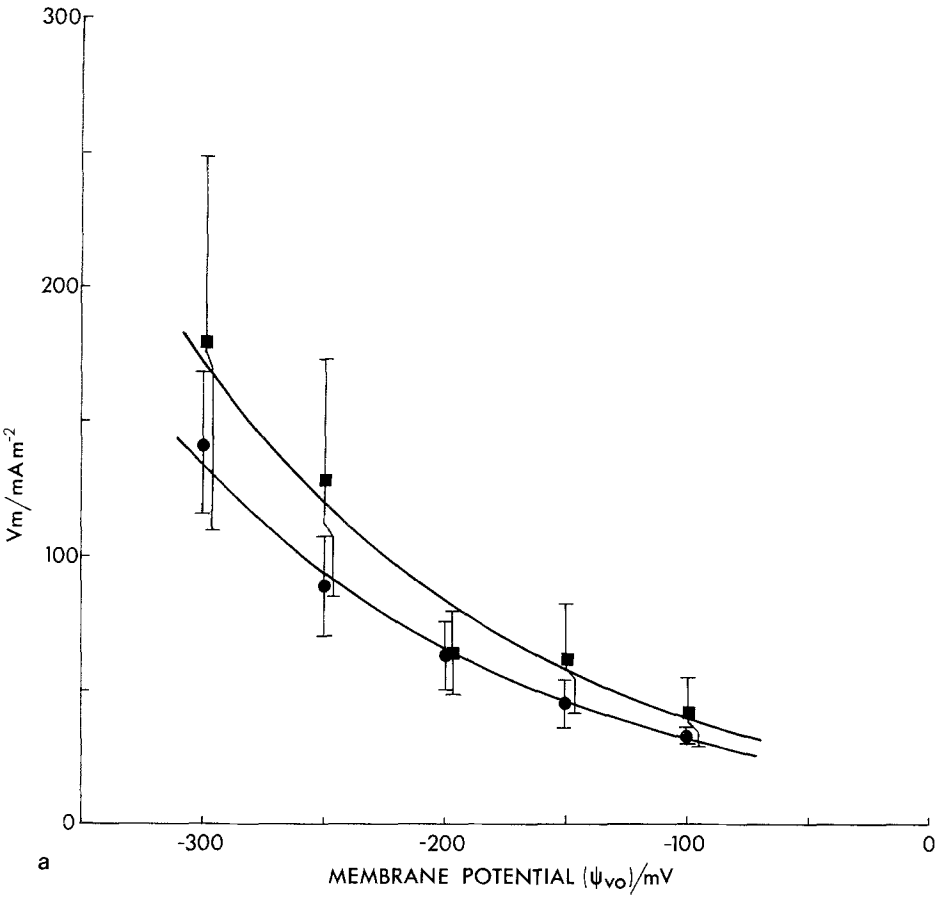
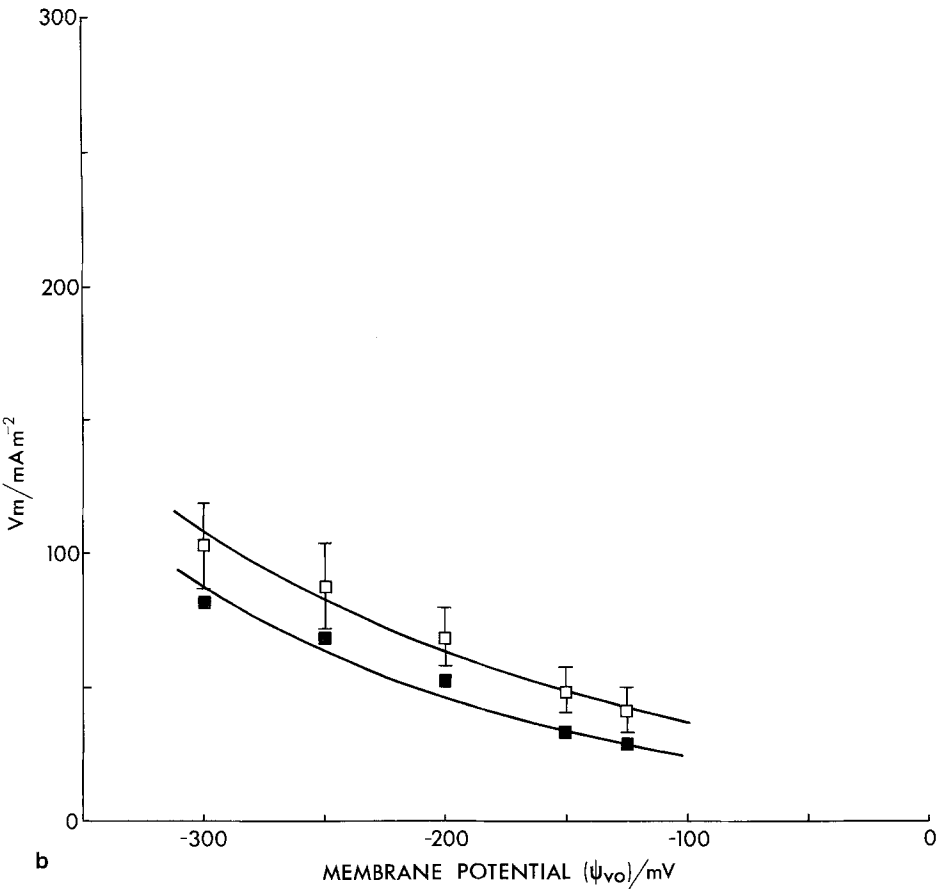


Fig. 9. Voltage dependence of the parameter V_m obtained by least-squares fitting of data such as that of Fig. 8 to the modified Michaelis-Menten equation (Eq. 5). Symbols represent mean and SEM for a heterogeneous group of cells. Squares, NH_4^+; circles, $CH_3NH_3^+$; filled symbols, *Chara;* open symbols, *Nitella.* (*a*): *Chara*, intermediate flow-rate, means of 9 (NH_4^+) and 13 ($CH_3NH_3^+$) cells; (*b*): *Chara*, one cell at high flow-rate and *Nitella*, intermediate flow-rate, means of 2 cells

It is seen that similar values of V_m are found for the two substrates, the values of V_{-200} and B for $CH_3NH_3^+$ falling within the ranges for NH_4^+. The values of K_{-200} are, however, quite different. Each value should be within a factor of 2 of the true value, so we regard this difference as highly significant.

Reconstructing the Current-Voltage Curves for $CH_3NH_3^+$

A better representation of the current-voltage curves for the amine transport system should be given by the data for $CH_3NH_3^+$, which is less



affected by the unstirred layer. Accordingly, we carried out the following steps: (i) the data from experiments on $CH_3NH_3^+$ were read at intervals of 25 mV from -300 mV to -100 mV; (ii) the whole set of such data for all concentrations on each cell was fitted by a single search, using the Hill-Whittingham equation (5) in which both V_m and K_M were voltage-dependent (Eqs. (6) and (7)).

The parameters available for fitting the curves were P_u, K_{-200}, A, V_{-200} and B; usually there were 6 or 7 different concentrations with about 50 points in all. The residual sums of squares were typically 2% of the total sums of squares. One such fit is illustrated in Fig. 11. The best fits for 10 experiments are shown in Table 1.

The estimates of P_u and of K_{-200} are linked, as already discussed; the variability of these parameters is believed to result from the failure of the data to provide a clear means of distinguishing them. The scatter of

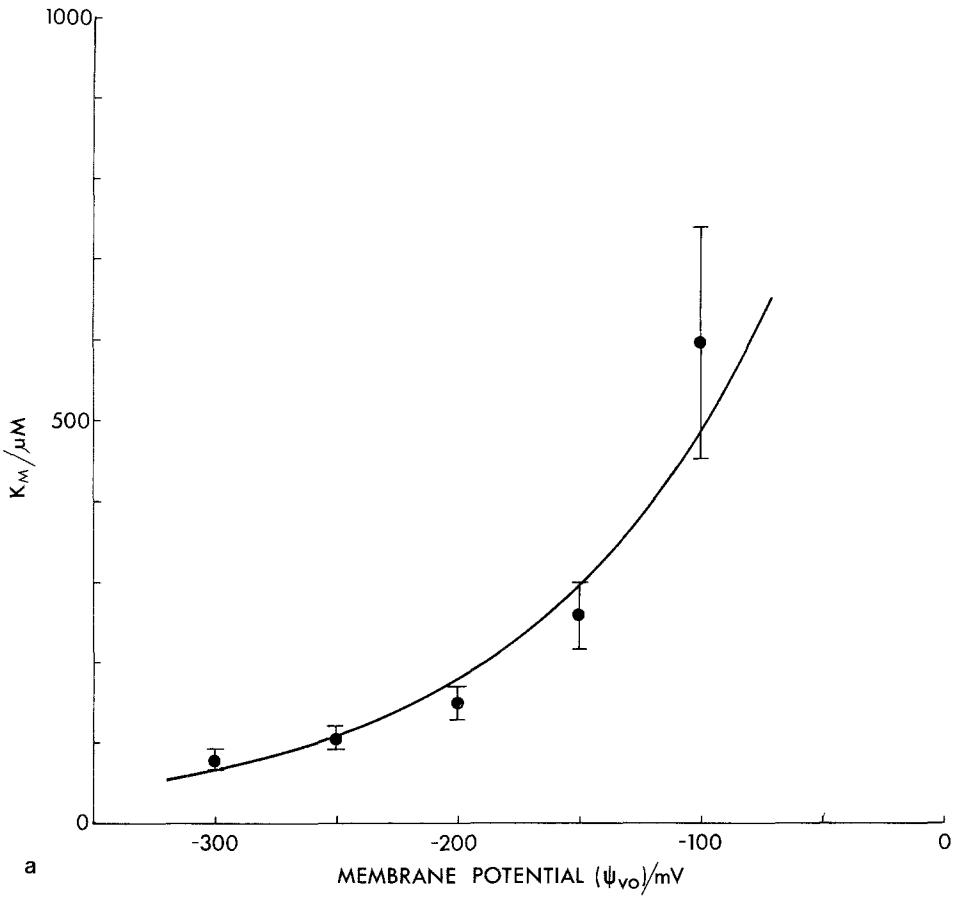
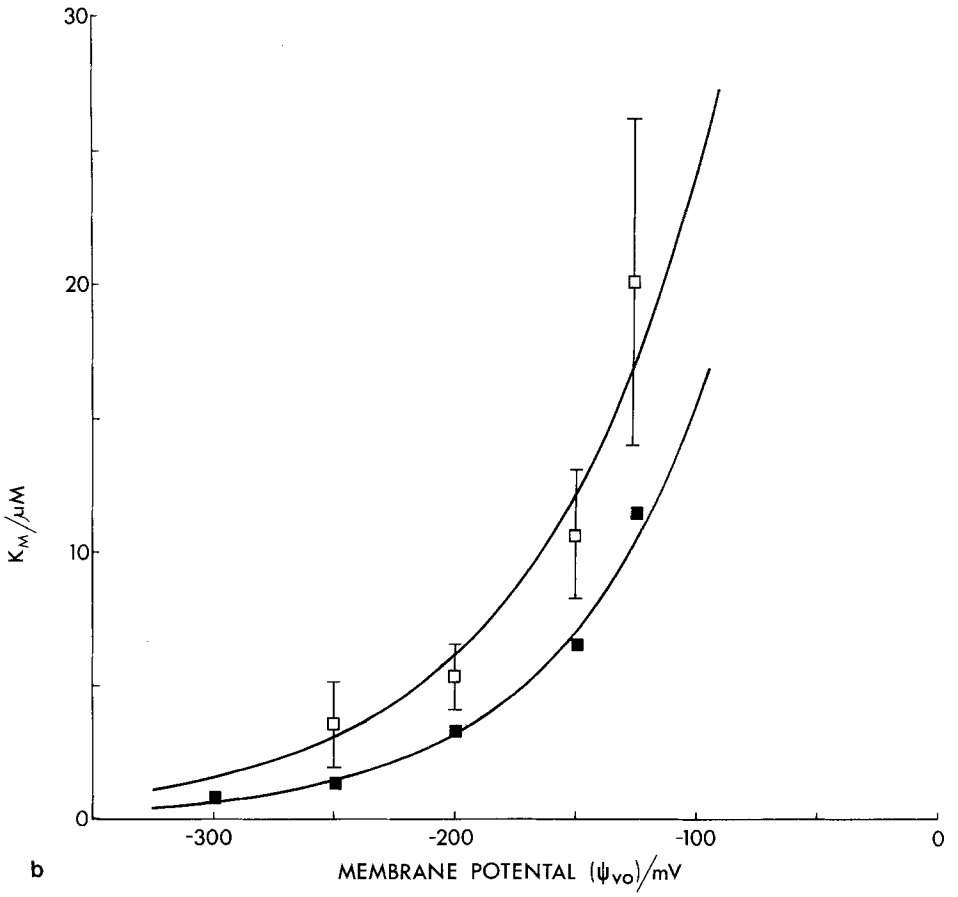


Fig. 10. Voltage dependence of the parameter K_M obtained by least-squares as described for V_m (Fig. 9). Symbols as Fig. 9. (*a*): *Chara*, $CH_3NH_3^+$, means of 13 cells; (*b*): *Chara*, NH_4^+, one cell, same as that of Fig. 9*b*, and *Nitella*, NH_4^+, means of 3 cells

values of A and B also reflects the difficulty of finding these parameters accurately when the data is confined to the range -300 to -100 mV. By contrast, the values of V_{-200} are closely confined by the data, and the various values in Table 1 give a good picture of the variability of this parameter between cells at different times and from different cultures.

Correction for Conductance of the Tonoplast

We made 16 determinations on 7 cells similar to those used in the experiments described above; the median value was 11 S m^{-2}, with 97% confidence limits of 31 and 8 S m^{-2}.



In order to estimate the effect of such a conductance, the data for the experiments of Table 1 were corrected, point by point, for a nominal tonoplast conductance of 10 S m^{-2} and PD of -12 mV. The curve fitting was then repeated for each set of data. There was no significant percentage change in the value of any parameter except B, whose value was reduced by a mean of $13 \pm 4\%$. We conclude that no serious error arises from the neglect of the conductance of the tonoplast in the results presented here.

Effect of pH on Kinetic Parameters

Since the exposure of each cell to amine had to be limited, we were not able to obtain a complete set of results for each pH on one cell. As a preliminary to a more complete study we investigated the effect of pH on

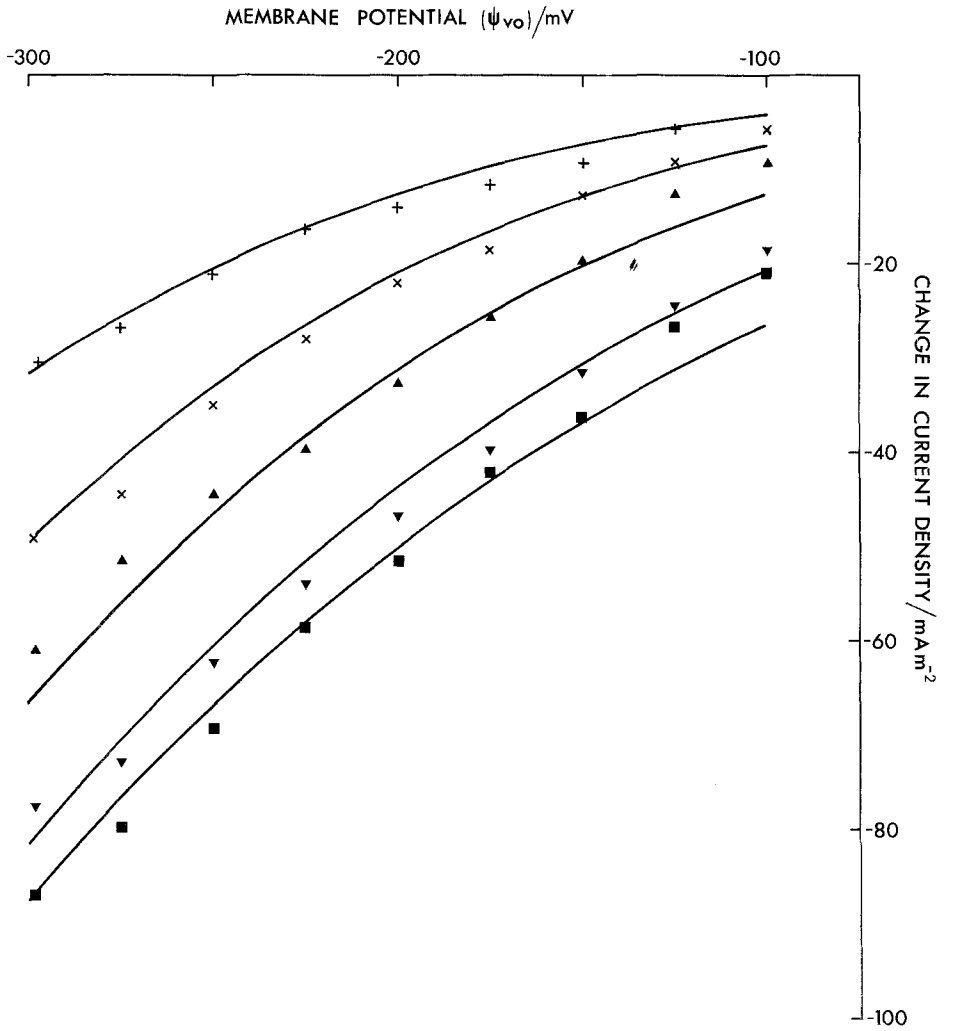


Fig. 11. Current-voltage curves for inward transport of $CH_3NH_3^+$ in a *Chara* internode. Points are readings from the original chart record; lines are best fit of Eq. (9), with the following values: $P_u = 2.2 \times 10^{-5}$ m sec^{-1}; $K_M = 158$ μm; $A = 121$ mV; $V_f = 58$ mA m^{-2}; $B = 210$ mV. Fitting done by least-squares (*see* text), with "regression coefficient" of 0.994. Experiment 77.12.01

the current change produced by a constant concentration of each substrate, with results like those shown in Fig. 12. There was always a marked decline in the response at pH below 7.5, as the figure shows. Other experiments suggest that this fall results from a fall in V_m, but an effect of pH on K_M is not ruled out.

Table 1. Kinetic parameters for $CH_3NH_3^+$ transport by *Chara*

Cell	$10^6 P_u$ (m sec^{-1})	K_{-200} (μM)	A (mV)	V_{-200} (mA m^{-2})	B (mV)	$(A^{-1}+B^{-1})^{-1}$ (mV[a])	$R.C.$[b]
pH 7.5							
77.05.05	9	67	76	18.4	142	49.5	0.990
77.06.07	30	267	69	125	173	49.6	0.998
77.12.01	22	158	121	58	210	76.7	0.994
77.06.29.1	30	224	59	182	212	46.2	0.997
77.05.02	2	125	78	13.8	139	49.7	0.987
Median	22	158	76	–	173	49.6	–
pH 5.5							
77.06.29.2	4	109	60	20	85	35.2	0.992
77.08.09	3	106	81	30	141	51.4	0.987
pH 9.5							
77.06.30	16	221	91	62	183	59.1	0.989
77.06.16	8	210	113	130	178	69.2	0.990
77.08.04	45	277	69	127	195	51.1	0.993
Median	16	221	91	–	183	59.1	–

[a] This "total voltage dependence" is discussed later.
[b] The "regression coefficient" was calculated as
$\sqrt{(\text{total sum of squares} - \text{residual s.sq.})/\text{total s.sq.}}$

Effect of Long Exposure to Amine

During the course of each experiment the current change produced by a given concentration fell off, and a few trials showed that cells left overnight in 50 μM NH_4^+ gave no measureable current change next day. Figure 13 shows the results of exposing cells of *Chara* to 100 μM NH_4^+ at pH 7.5, while the membrane PD was clamped at −250 mV. The filled and open symbols distinguish two different experiments whose results, taken separately, can be roughly represented by falling exponentials with half-times of 500 and 900 sec.

Discussion

The Cause of the Current

The inward positive current in the presence of low concentrations of amine has in the past been explained in two different ways. It was

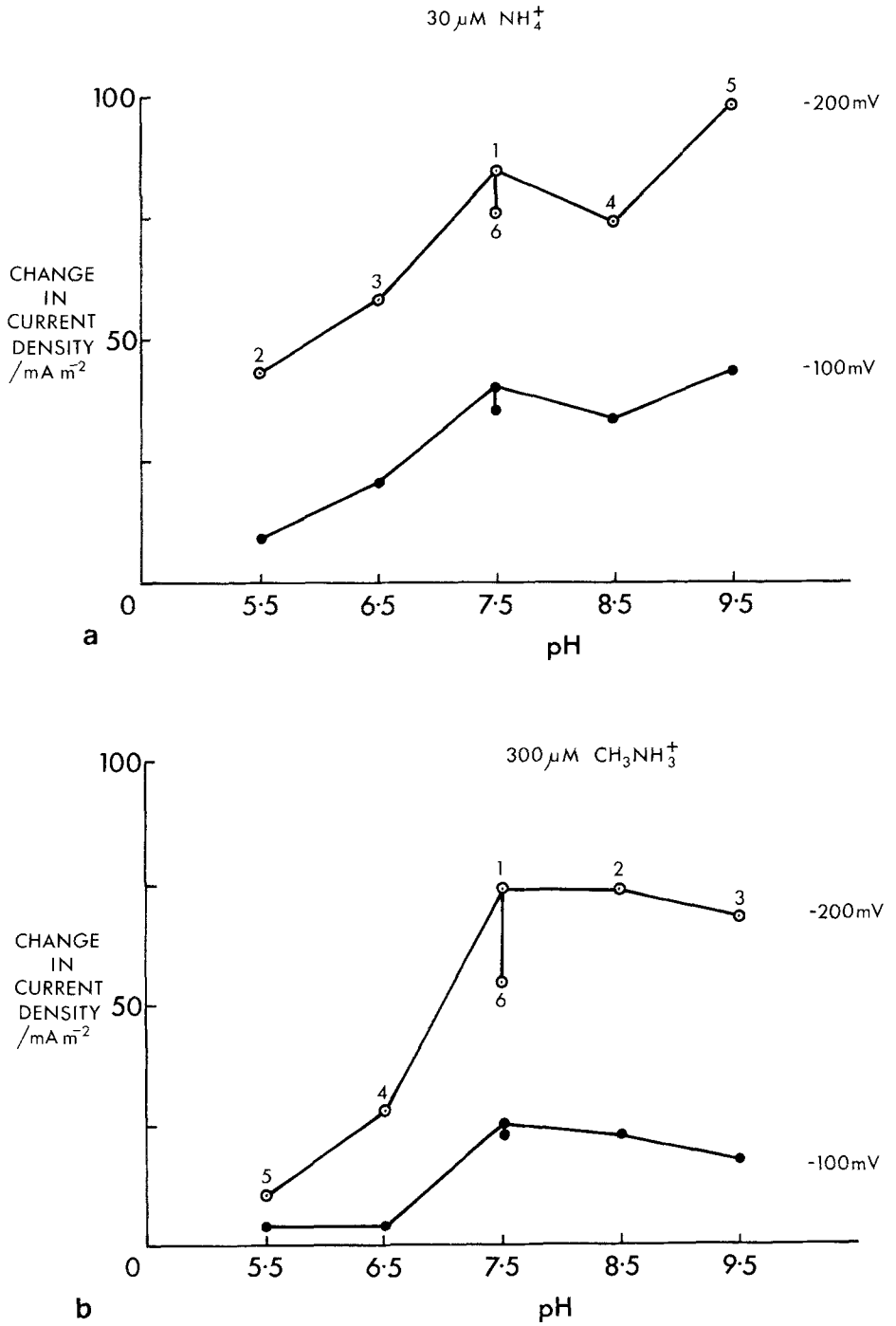


Fig. 12. Effect of pH on the change in membrane current produced by amine at constant membrane potential. (*a*): On current produced by 30 μM NH_4^+ at −100 and −200 mV; (*b*): On current produced by 300 μM $CH_3NH_3^+$ at −100 and −200 mV

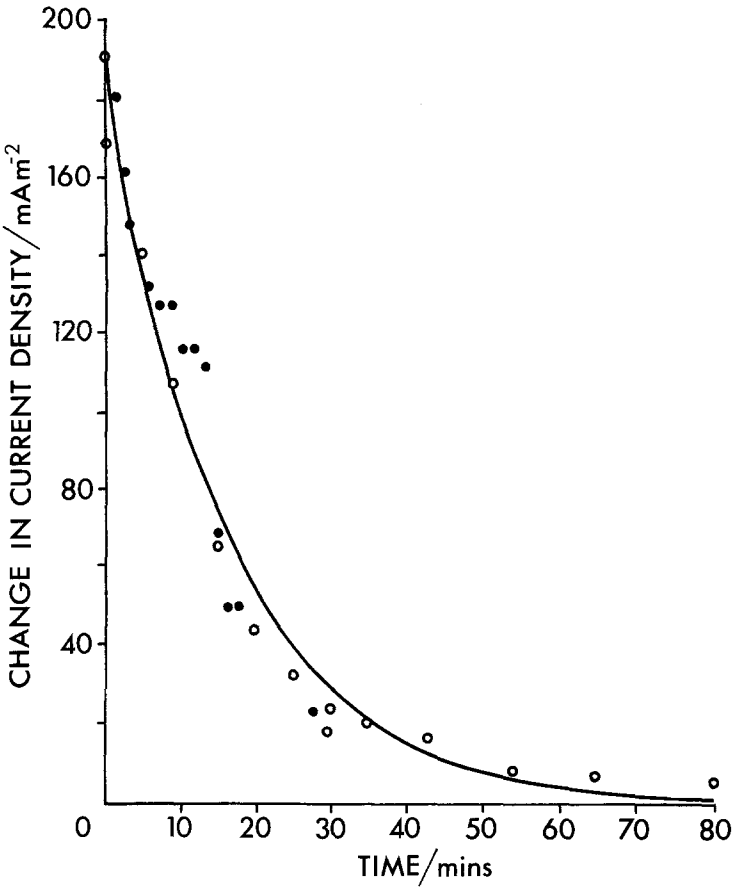


Fig. 13. Time-course of change in membrane current produced by 100 μM NH_4^+, showing the effect of continuous exposure to 100 μM NH_4^+ between tests: *Chara* membrane PD clamped at −200 mV, pH 7.5. Filled and open symbols distinguish two different experiments

suggested that NH_3 is the form entering the cell (Barr *et al.*, 1974; Jayasuriya, 1975) and that after entry it inhibits the outward (active) transport of H^+. Alternatively, it has been held that the current represents the charge carried by NH_4^+ itself (Smith *et al.*, 1977; Smith & Walker, 1978).

The first explanation involves the postulate that the H^+ pump is inhibited by micromolar concentrations of NH_4^+, for which there is no evidence at all. Such an inhibition is an unlikely "design specification" for an H^+ pump in the plasmalemma of a free-living eucaryote. The plasmalemma ATPase of *Neurospora* is in fact stimulated by millimolar NH_4^+ (Bowman *et al.*, 1978). Indeed, the reasonable strategy for a cell

such as *Chara* or *Neurospora* is the uptake of NH_4^+, which will generally be present at much higher concentrations than NH_3.

A more convincing argument for NH_4^+ entry is the one we have already given (Smith & Walker, 1978): that the inhibition of an ATPase would cause an inward positive current and a fall in membrane conductance (*cf.* Spanswick, 1973, 1974), while the inward transport of a cation should cause a current in the same direction but a rise in membrane conductance. With the data presented here the conclusion in favor of NH_4^+ transport is considerably strengthened, since the increase in conductance when amine is added is often greater than 0.2 S m^{-2}, and may be as large as 1.5 S m^{-2} (as in Fig. 6).

Although the current-voltage curves are available only between -100 and -300 mV, they increase our confidence in this conclusion, since they show the increase in conductance to occur at all PDs in that range. An equivalent statement of the same argument is that the observed change in current is biggest at -300 mV, where an inward current of cations would be expected to be biggest, and where the effect of inhibiting an H^+ efflux pump would be expected to be smallest.

That the current change happens as fast as we can change the concentration of amine in the medium is important here, for a delay in the effect would indicate that the equilibration of a phase (e.g., the cytoplasm) must take place between the application of the amine and the appearance of the change in current.

We conclude that the changes in current shown here are the direct expression of the inward transport of amine ions, with the reaction given by Eq. (1). We refer to such transport as passive uniport (Mitchell, 1968). Having already discounted, as a mechanism, the simple diffusion of the ions in question through the lipid bilayer of the membrane (Smith & Walker, 1978), we expect it to involve a specific channel or carrier. Its specificity has not yet been systematically explored; it is indicated by the 60-fold difference in K_M produced by the addition of one $-CH_2$-group to the NH_4^+ ion, and by the ability of the system to respond to micromolar NH_4^+ in the presence of 200 μM K^+ and 1 mM Na^+.

It is important to notice that we have tried to limit our observations to the change in current associated with the sudden addition of amine to the outside medium, at zero or low internal concentration. Thus in most of the experiments reported we believe that we have measured the current change that is directly due to a change in the influx of amine ion. An exception is the experiment shown in Fig. 13, where the change in current at later times could represent the effect of changes in external concentration on the net flux.

Variability of the Effect

The current produced by a given concentration of amine at a particular PD was found to vary from culture to culture, and from week to week with the same culture (Table 1). In the present study we looked for cultures that gave large current changes, and as a result report many values of i and V_m some ten times bigger than those described previously (Smith & Walker, 1978). Indeed V_m can have values ranging from zero to the largest reported here. The experiment shown in Fig. 13 shows the inhibition of transport as ammonium is taken up, a phenomenon which could well provide the basis of the observed variability of V_m both between different cells and during measurements on one cell.

The cultures from which cells were taken for these experiments would be subject to variations in NH_4^+ concentrations as the medium became depleted by plant growth. Since the cultures were not axenic, the NH_4^+ supply would also fluctuate as a result of growth and death of micro-algae and animals. The small size of the depolarization produced by rapid flow of culture medium (Fig. 3) suggests that the medium must have contained less than 1 μM NH_4^+, with the consequence that cells from those cultures might well be nitrogen-starved, and would certainly be ammonium-starved. In *Neurospora*, de-repression of the fast ammonium transport system follows ammonium starvation, even in the presence of NO_3 (C.L. Slayman and N.A. Walker, *unpublished*).

The Magnitude of the Current

The current changes reported here are very large, with instantaneous values as high as 350 mA m^{-2} or 3.5 μmol/m^2 · sec (Fig. 6); at this current the electrical term[1] in the membrane dissipation ($i \cdot \psi_{co}$) is about 100 mW m^{-2}. For comparison with this figure, respiration in *Chara* makes available an energy flow of about 30 mW m^{-2} through ATP (data of R.J. Reid, in Hope & Walker, 1975). So large a dissipation is not likely to occur in nature, it is observed in the laboratory only by means of the voltage clamp, which holds ψ_{co} at a high value, providing electrical energy which is dissipated by the transport system. Under natural conditions the membrane depolarizes if a significant concentration of

[1] Unless the internal concentration of NH_4^+ is large, or there is an unsuspected linkage of amine transport to some other reaction, the electrical term will be the dominant one, i.e., it will represent the actual dissipation of energy by the amine transport system.

amine is present; this also reduces i, so that the dissipation will be much lower than the maximum. At the reduced membrane PD, it would be expected that the energy supply for amine transport would come directly from the putative H^+-ATPase proposed by Spanswick (1973), or from the K^+ concentration difference, as discussed by Walker and Smith (1977*a*).

The values of V_m that are reported here indicate the maximum capacity of the amine system; in this it ranks with the HCO_3^- transport system and its associated H^+ uniport (Lucas, 1976; Lucas, Ferrier & Dainty, 1977; Walker & Smith, 1977*b*). The only transport systems in *Chara* that were previously known to carry such large current densities are those associated with the action potential (Findlay & Hope, 1964; Beilby & Coster, 1976).

Current densities of the size reported here do not necessarily result from densely-packed transporters. If we take the maximum conductance of the system to be 2 S m^{-2}, and the maximum conductance of a single channel to be 10^{-10} S (the value for gramicidin – Sandblom, Eisenman & Neher, 1977), the channel density need only be 2×10^{10} m^{-2} – comparable with a square array with 7 μm spacing. No doubt the density is greater than this minimum. The high current density need not imply a serious temperature rise in the conductors. If they are represented as spheres of radius 2 nm in water, with heat generated uniformly throughout their volume, the result of Carslaw and Jaeger (1959) gives a temperature rise of 10^{-3} K after 200 sec.

Location of the Internal Amine

At the end of the experiments shown in Fig. 13, the cells would have contained a calculated 5 mM NH_4^+, assuming that NH_4^+ metabolism was relatively slow. Earlier experiments (Smith & Walker, 1978) have already indicated that the cytoplasmic concentration is normally lower than the vacuolar; the present result implies this, since NH_4^+ is an effective uncoupler of photosynthesis at such concentrations.

Unstirred Layers and Amine Storage

The presence of an unstirred layer of solution affects the shape of the current-voltage curve, as Figs. 6*a* and 7 show. In the absence of flow, the

rate of entry of NH_4^+ can fall to a small fraction of that observed in flowing medium (Fig. 3). In nature the rate of uptake of NH_4^+ must usually be limited by the rate of diffusion across the unstirred layer, as would be expected for CO_2 and HCO_3^-.

The influx of NH_4^+ needed to support growth of charophyte plants, even in the absence of NO_3^- assimilation, is comparatively small. With a photosynthetic rate of say 200 nmol $m^{-2} sec^{-1}$ (Smith, 1967; Lucas, 1976) and an N/C ratio of about 0.05 (Raven, 1976), the NH_4^+ influx required is only 5 nmol m^{-2} sec^{-1}, corresponding to 0.5 mA m^{-2}. The fact that the transport system has a much larger capacity, up to 200 mA m^{-2}, suggests an opportunistic approach to N nutrition on the part of these plants. The storage of NH_4^+ in the vacuole (Barr *et al.*, 1974; Smith & Walker, 1978) makes this a reasonable view. If this is correct, the opposite conclusions of Mercer and Mercer (1971), who found no N in the *Chara* vacuole, must be attributed to their use of cells that came from N-limited plants. The suggested "opportunism" would be an advantage in any medium whose ratio of NH_4^+ to HCO_3^- was normally less than 0.05, if there were occasional transient increases in NH_4^+, caused for example by the decomposition of an animal body nearby.

The Shape of the Current-Voltage Curves

The effect of the unstirred layer on the shape of the current-voltage curves for NH_4^+ (Figs. 6*a* and 7) makes it seem likely that the curves for $CH_3NH_3^+$ (Figs. 6*b* and 11) represent better the characteristics of the system. At all concentrations, there is a steady increase in i with increasingly negative ψ_{vo}, the curves being concave towards the current axis. There is no sign of saturation in the voltage dependence at the most negative potential studied. The constant-field equation (Goldman, 1943):

$$i_j = -P_j \frac{F^2 \psi}{RT} \cdot \frac{c_{oj}}{(1-\exp(-F\psi/RT))} \tag{8}$$

has often been used to calculate the current flow through ion uniport, with varying success. For these results it cannot be used, since it does not give current-voltage curves concave towards the current axis, nor currents that saturate as the concentration is raised. At a constant PD the current is well represented by the Hill-Whittingham Equation (5); when the membrane PD is allowed to vary, both K_M and V_m are found to be

functions of ψ. The use of this equation with variable parameters is not as arbitrary as it may seem, since it appears that they can both be represented by the simple exponential functions given in Eqs. (6) and (7), and that these functions can be given a simple theoretical basis. The complete equation for transport through the amine system, isolated from the unstirred layer, is thus:

$$i = \frac{c_o V_{-200} \exp\left(-\dfrac{\psi + 200\text{ mV}}{B}\right)}{c_o + K_{-200} \exp\left(\dfrac{\psi + 200\text{ mV}}{A}\right)} \tag{9}$$

with the parameters having values such as:

	V_{-200} (mA m^{-2})	B (mV)	K_{-200} (μM)	A (mV)
$CH_3NH_3^+$:	150	175	250	75
NH_4^+:	150	150	3	70

At first sight the dependence of V_m and K_M on ψ seems rather weak compared with that of, e.g., an ion equilibrium concentration ratio, which would vary as $\exp(\psi/25\text{ mV})$.

A transport system described by Eq. (9) will show its maximum voltage-dependence at low concentrations, with the current density having the limiting value:

$$i = c_o(V_{-200}/K_{-200}) \exp\left[-(\psi + 200\text{ mV})\left(\frac{1}{A} + \frac{1}{B}\right)\right] \tag{10}$$

which can be regarded as defining a limiting permeability P_a for amine transport:

$$P_a = \frac{V_{-200}}{FK_{-200}} \exp\left[-(\psi + 200\text{ mV})\left(\frac{1}{A} + \frac{1}{B}\right)\right]. \tag{11}$$

This is of course only the limiting permeability for small outside concentrations at zero inside concentration. This limiting permeability exhibits the total voltage dependence of the amine influx, whose value is shown in Table 1. For $CH_3NH_3^+$ the constant $\left(\frac{1}{A} + \frac{1}{B}\right)^{-1}$ has the value 50 mV (Table 1), while for NH_4^+ it is 45 mV (Figs. 9*b* and 10*b*), neither value being significantly different from $2RT/F$.

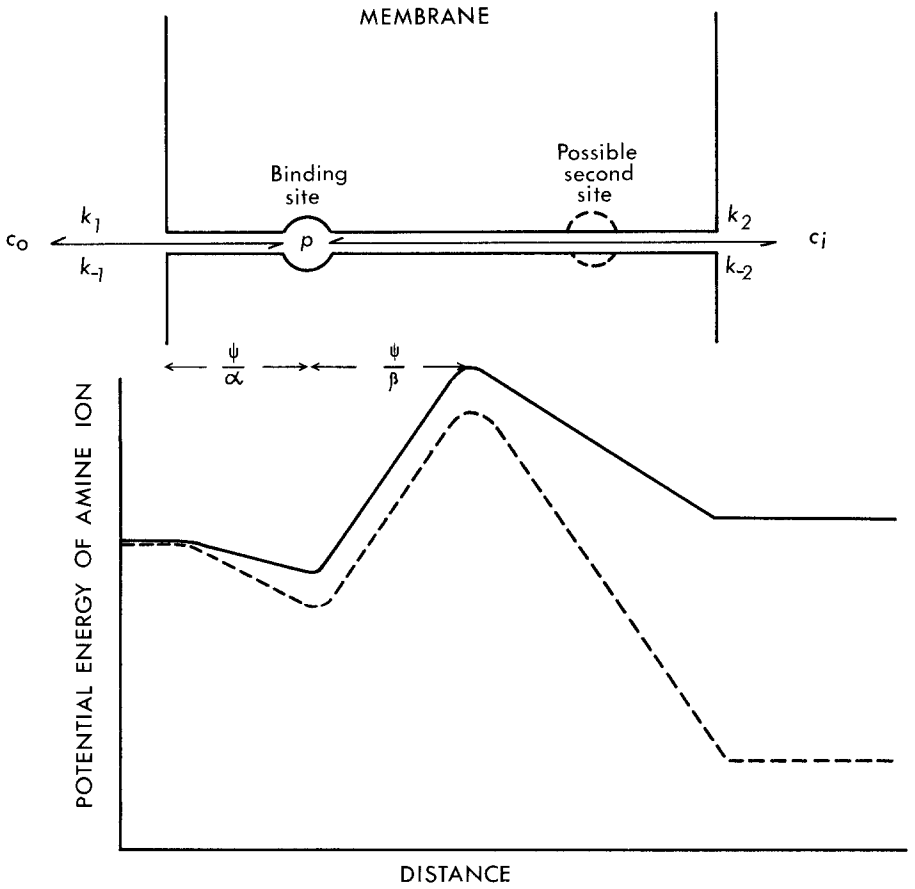


Fig. 14. Model of amine transport channel with kinetic properties indicated by the present data. Above, schematic diagram of channel with selective binding site below the membrane surface. ψ, membrane PD; ψ/α, fraction of membrane PD appearing between surface and site; ψ/β, fraction of PD appearing between site and peak of potential energy profile. Below, assumed potential energy profile for amine ion

The Channel Model

Many authors have derived equations for the flow of electric current through an ion-conducting channel in a membrane, and in particular, Hladky & Harris (1967), Läuger (1973, 1976), and Sandblom *et al.* (1977) have dealt with the phenomenon of saturation. We have given here (Fig. 14) a model channel which seems the simplest that will exhibit the properties reported above, i.e., a current described by Eq. (9). The appendix shows that Eq. (9) describes the influx through the model, the voltage dependence of K_M resulting from the binding site being located below the

membrane surface. It also shows that very similar conclusions can be drawn from the simplification of Läuger's equation (Eq. (21) of 1973), which is based on a very similar model.

It seems necessary therefore to assume a site about 1/3 of the membrane potential away from the potential of the medium. We do not translate this in any strict way into a fraction of the membrane thickness – in this sense Fig. 14 is very diagrammatic.

The voltage dependence of P_a is close to $F\psi/2RT$, suggesting a symmetrically placed peak in the potential energy profile. We can derive no information about the inner half of the profile from the present measurements; efflux measurements will allow a decision to be made about the possible presence of an inner binding site, at present demanded only by aesthetic considerations.

Effects of pH

We found previously (Smith & Walker, 1978) that the change in current density produced by NH_4^+ and $CH_3NH_3^+$ decreased greatly as the external pH was lowered from 7.5 to 5.5. In those experiments the cells were clamped at their resting PD for each pH, and this PD falls with pH in the range used (Richards & Hope, 1974; Smith & Walker, 1976). Accordingly, it was not possible to separate the effects of pH from those of PD. This was also the case in the measurements of $CH_3NH_3^+$ influx with ^{14}C (Smith & Walker, 1978).

The present work shows that the amine current falls with decreasing pH in the range 7.5 to 5.5 when the membrane PD is held constant (Fig. 12). Between pH 7.5 and 9.5 there is little effect of pH on the amine current or on V_m or K_M (Table 1). If the binding site is at $\psi/3$ or -65 mV from the potential of the bathing medium, the fall-off in current at pH 6.5 to 5.5 may suggest a pK in the range of 4.5–5.5. These effects need further investigation if they are to contribute to an understanding of the mechanism of amine transport.

Depolarization by Flowing Medium

That rapidly flowing media tend to depolarize cells of free-living eucaryotes has been observed before, by M.J. Beilby with *Chara* and by C.L. Slayman and N.A. Walker with *Neurospora* (*unpublished data* in both cases). The natural inference was that the effect was due to mechanical damage caused by the relative movement of cell and mi-

croelectrode. Our results make that explanation unnecessary, since the effect (Fig. 3) is seen to be due to a very permeable contaminant present in the distilled water, a substance largely removable by a cation exchange resin. Since NH_4^+ is known to occur as a common contaminant of distilled water, there seems no reason to doubt that this is the correct explanation.

The depolarizing effect of NH_4^+ should be guarded against in all experiments on membrane PD in free-living autotrophs, since a difference between contaminating NH_4^+ concentrations in different solutions (resulting, e.g., from their having aged for different times) could be mistaken for another effect being studied by membrane electrical properties.

Conclusions

The charophytes appear to have the ability to rapidly transport NH_4^+ ions by means of a selective transport system of low ($\sim 3\,\mu M$) K_M. Both K_M and maximum rate depend on membrane potential; the maximum rate also depends on the amount of amine taken into the cell and on that cell's history. In these properties charophyte plants strongly resemble *Neurospora*, which appears to have a de-repressible transport system for NH_4^+ (Slayman & Walker, in Slayman, 1977). The *Neurospora* system has a comparable maximum velocity, of the order of $100\,mA\,m^{-2}$ as judged from membrane depolarization, and a similarly low K_M, of the order of $7\,\mu M$ (Slayman, 1977). Physical difficulties in working with *Neurospora*, together with the very rapid repression of uptake by added NH_4^+, make *Chara* a very much better organism for the study of transport kinetics. It is not, however, easy to demonstrate the de-repression of amine transport in *Chara*, while *Neurospora* is ideal for this type of study. The amine transport system of charophyte plants, especially with methylammonium as substrate, should prove an interesting subject from the point of view of kinetic models.

This work was supported by the Australian Research Grants Committee to whom F.A.S. and N.A.W. are grateful for grants. We thank many colleagues for their comments, especially C.L. Slayman, M.G. Pitman, and M.A. Bisson.

Appendix

Let solutions o and i be separated by a membrane-containing channels through which amines permeate. The simplest channel exhibiting

Michaelis-Menten kinetics would contain a single binding site which can bind a single substrate ion (Fig. 14). A simplifying – but not necessary – assumption is that this site is essentially in equilibrium with the medium at o, the forward and backward rate-constants being k_1 and k_{-1}; and that the rate-limiting step is movement from the site to the medium at i, for which the rate constants are k_2 and k_{-2}. Let the probability that the site is occupied be p, and the density of the channels be s.

Then, for equilibrium:

$$sc_o k_1(1-p)=sk_{-1}p \tag{A1}$$

from which we get:

$$p=c_o k_1/(c_o k_1+k_{-1}). \tag{A2}$$

The current through the channel will be:

$$i=Fs(pk_2-c_i k_{-2}), \tag{A3}$$

and if $c_i \to 0$:

$$i \to Fspk_2. \tag{A4}$$

From (A2) and (A4) we have:

$$i=Fsk_2 c_o/(c_o+k_1/k_{-1}) \tag{A5}$$

and if we write V_1 for Fsk_2 and K_1 for k_1/k_{-1} this becomes

$$i=c_o V_1/(c_o+K_1) \tag{A6}$$

which is the Michaelis-Menten equation. The voltage dependence of V_1 and K_1 can be argued, using a rate-theory approach (e.g., *see* Johnson, Eyring & Polissar, 1954): if the site is at a potential of ψ/α with respect to the medium at o, the equilibrium binding constant K_1 will be given by:

$$K_1=k_1/k_{-1}=(k_{1,0}/k_{-1,0})\exp(F\psi/\alpha RT). \tag{A7}$$

However, rate constant k_2 will be given by:

$$k_2=k_{2,0}\exp(-F\psi/\beta RT) \tag{A8}$$

where $1/\beta$ is the fraction of the membrane PD appearing between the site and the peak of the energy profile. If the energy profile is symmetrical, and the electrical potential profile is also, we will find that $\psi/\alpha+\psi/\beta=\psi/2$. Writing A for $\alpha RT/F$; B for $\beta RT/F$; K_0 for $k_{1,0}/k_{-1,0}$; and V_0 for $Fsk_{2,0}$ we obtain from (A5), (A7), and (A8):

$$i = \frac{c_o V_0 \exp(-\psi/B)}{c_o + K_0 \exp(\psi/A)} \tag{A9}$$

which has the required form, *cf.* Eq. (9). It is apparent that for a symmetrical energy profile, $1/A + 1/B = F/2RT$, in agreement with the results.

A similar conclusion is reached if one takes the model of Läuger (1973), containing an arbitrary number of sites, and simplifies it by letting the number of sites be 1, and c_i be 0. His Eq. (21) then reduces to a form of the Michaelis-Menten equation, in which K_M and V_m are voltage dependent:

$$V_1 = V_0 \exp\left(-\frac{\psi}{B}\right) \tag{A10}$$

and

$$K_1 = K_0 \exp\left(\frac{\psi}{A}\right) \cdot \left(1 + \exp\left(f - \frac{\psi}{C}\right)\right) \tag{A11}$$

where f depends on the relative heights of the barriers between the site and the two solutions, and A^{-1}, B^{-1} and C^{-1} represent, as before, fractions of the membrane PD which appear between energy maxima and minima in the model. When more, and more accurate, data become available it will be of interest to compare them with Eqs. (A8) and (A11). This is not warranted at present. However, comparison of Eq. (A11) with the present results again indicates that the energy maximum between the binding site and the interior solution must occur at $\frac{1}{2}$ or less of the potential difference, as measured from the outside solution. This is thus true independently of the assumption made initially that the site equilibrates with the outside solution.

References

Barr, C.E., Koh, M.S., Ryan, T.E. 1974. NH_3 efflux as a means for measuring H^+ extrusion in *Nitella. In:* Membrane Transport in Plants. U. Zimmermann and J. Dainty, editors. pp. 180–185. Springer-Verlag, Berlin

Beilby, M.J., Coster, H.G.L. 1976. The action potential in *Chara corallina:* Effect of temperature. *Aust. J. Plant Physiol.* **3**:275

Bowman, B.J., Mainzer, S.E., Allen, K.E., Slayman, C.W. 1978. Effects of inhibitors on the plasma-membrane and mitochondrial adenosine triphophatases of *Neurospora crassa. Biochim. Biophys. Acta* **512**:13

Carslaw, H.S., Jaeger, J.C. 1959. Conduction of Heat in Solids. Oxford University Press, Oxford

Cole, K.S., Hodgkin, A.L. 1938. Membrane and protoplasm resistance in the squid giant axon. *J. Gen. Physiol.* **22**:671

Colquhoun, I. 1971. Lectures in Biostatistics. Clarendon Press, Oxford

Coster, H.G.L. 1965. A quantitative analysis of the voltage-current relationships of fixed charge membranes and the associated property of punchthrough. *Biophys. J.* **5**:669

Coster, H.G.L., Smith, J.R. 1977. Low-frequency impedance of *Chara corallina:* Simultaneous measurements of the separate plasmalemma and tonoplast capacitance and conductance. *Aust. J. Plant Physiol.* **4**:667

Findlay, G.P., Hope, A.B. 1964. Ionic relations of cells of *Chara australis.* IX. Analysis of transient membrane currents. *Aust. J. Biol. Sci.* **17**:400

Goldman, D.E. 1943. Potential, impedance and rectification in membranes. *J. Gen. Physiol.* **27**:37

Hill, R., Whittingham, C.P. 1957. Photosynthesis. Methuen, London

Hladky, S.B., Harris, J.D. 1967. Ion displacement membrane model. *Biophys. J.* **7**:535

Hooke, R., Jeeves, T.A. 1961. Direct search solution of numerical and statistical problems. *J. Assoc. Comput. Mach.* **8**:212

Hope, A.B., Walker, N.A. 1975. The physiology of giant algal cells. Cambridge University Press, London

Jayasuriya, H.D. 1975. Ph.D. thesis. University of Cambridge, Cambridge

Johnson, F.H., Eyring, H., Polissar, M.I. 1954. The kinetic basis of molecular biology. Wiley, New York

Läuger, P. 1973. Ion transport through pores: A rate-theory analysis. *Biochim. Biophys. Acta* **311**:423

Läuger, P. 1976. Diffusion-limited ion flow through pores. *Biochim. Biophys. Acta* **455**:493

Lucas, W.J. 1976. Plasmalemma transport of HCO_3^- and OH^- in *Chara corallina:* Non-antiporter systems. *J. Exp. Bot.* **27**:19

Lucas, W.J., Ferrier, J.M., Dainty, J. 1977. Plasmalemma transport of OH^- in *Chara corallina.* Dynamics of activation and deactivation. *J. Membrane Biol.* **32**:49

Mercer, M.J., Mercer, F.J. 1971. Studies on the comprative physiology of *Chara corallina.* III. Nitrogen relations of internodal cell components during internodal cell expansion. *Aust. J. Bot.* **19**:1

Mitchell, P.D. 1968. Chemiosmotic coupling and energy transduction. Glynn Research, Bodmin

Raven, J.A. 1976. Transport in algal cell.s *In:* Encyclopedia of Plant Physiology, N.S. U. Lüttge and M.G. Pitman, editors. Vol. IIA, pp. 129–173. Springer-Verlag, Berlin

Richards, J.L., Hope, A.B. 1974. Th role of protons in determining membrane electrical characteristics in *Chara corallina. J. Membrane Biol.* **16**:121

Sandblom, J., Eisenman, G., Neher, E. 1977. Ionic selectivity, saturation and block in gramicidin A channels. I. Theory for the electrical properties of ion selective channels having two pairs of binding sites and multiple conductance states. *J. Membrane Biol.* **31**:383

Skierczynska, J., Zarebski, W., Sielewiesiuk, J., Spiewla, E. 1977. Some methods for the measurements of tonoplast resistance of *Nitellopsis obtusa. J. Exp. Bot.* **28**:37

Skierczynska, J., Zolnierczuk, R., Spiewla, E., Bulanda, W., Przygodzka, A. 1972. Measurements of membrane resistance of characeae with external electrodes and microelectrodes. *J. Exp. Bot.* **23**:591

Slayman, C.L. 1977. Energetics and control of transport in *Neurospora. In:* Water Relations in Membrane Transport in Plants and Animals. A.M. Jungreis, editor. p. 69. Academic Press, New York

Smith, F.A. 1967. Rates of photosynthesis in characean cells. I. Photosynthetic $^{14}CO_2$ fixation by *Nitella translucens. J. Exp. Bot.* **18**:509

Smith, F.A., Raven, J.A., Jayasuriya, H.D. 1978. Uptake of methylammonium ions by *Hydrodictyon africanum. J. Exp. Bot.* **29**:121

Smith, F.A., Walker, N.A. 1976. Chloride transport in *Chara corallina* and the electrochemical potential difference for hydrogen ions. *J. Exp. Bot.* **27**:451

Smith, F.A., Walker, N.A. 1978. Entry of methylammonium and ammonium ions into *Chara* internodal cells. *J. Exp. Bot.* **29**:107

Smith, F.A., Walker, N.A., Raven, J.A. 1977. Transport of ammonium and methylammonium ions in *Chara* and *Hydrodictyon*. *Colloq. Int. C. N. R. S.* **258**:233

Spanswick, R.M. 1973. Evidence for an electrogenic ion pump in *Nitella translucens*. I. The effects of pH, K^+, Na^+, light and temperature on the membrane potential and resistance. *Biochim. Biophys. Acta* **288**:73

Spanswick, R.M. 1974. Evidence for an electrogenic ion pump in *Nitella translucens*. II. Control of the light-stimulated component of the membrane potential. *Biochim. Biophys. Acta* **332**:387

Walker, N.A., Smith, F.A. 1977*a*. The H^+ ATPase of the *Chara* cell membrane: Its role in determining membrane P.D. and cytoplasmic pH. *Colloq. Int. C. N. R. S.* **258**:255

Walker, N.A., Smith, F.A. 1977*b*. Circulating electric currents between acid and alkaline zones associated with HCO_3^- assimilation in *Chara*. *J. Exp. Bot.* **28**:1190