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Vacuolar pH Measurement in Higher Plant Cells[1]

I. EVALUATION OF THE METHYLAMINE METHOD

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ABSTRACT

[^{14}C]Methylamine is rapidly accumulated by *Acer pseudoplatanus* cells cultivated in liquid medium. The accumulation ratio of intracellular concentration to the extracellular one reaches, within 60 minutes, values as high as 3,000. This lipophilic amine appears to enter the cells through a diffusion process and is probably mainly accumulated as a cation inside the large acidic vacuolar compartment.

A large discrepancy has been observed between the vacuolar pH calculated from the distribution of methylamine molecules between the cells and their culture medium and the pH of the sap extracted from frozen-thawed cells, the pH of the sap being higher than the calculated values.

The pH estimated through the use of the methylamine method must be considered with some care as evidence has been obtained showing that the intracellular accumulation of this base depends not only on the vacuolar pH but also on: (*a*) the transplasmalemma and transtonoplast potential differences; (*b*) the relative permeabilities of these membranes to the nonionized and ionized form of the base; and (*c*) the adsorption of methylamine on cellular constituents.

MeA[2] has been used for the determination of intracellular pH of various biological systems (1, 7, 10).

The principle of the measurement is based on the penetration of the undissociated molecules which diffuse through the cell membranes. They dissociate inside and outside the cells according to the pHi and pHe and a diffusion equilibrium is established between the undissociated molecules in the cells and the incubation medium.

From the equation of Waddell and Butler (13):

$$Ci/Ce = \frac{1 + 10^{(pK'a-pHi)}}{1 + 10^{(pK'a-pHe)}} \quad (1)$$

where Ci and Ce are, respectively, the intracellular and extracellular total concentrations of MeA (undissociated and dissociated molecules), pHe the extracellular pH and pHi the intracellular pH, it is possible to calculate pHi from measurements of pHe, Ce, and Ci. Only an overall pHi value can be estimated by such a procedure which assumes that the intracellular MeA molecules are randomly distributed within the cells. However, as the plant cells have a large acidic vacuolar compartment (2, 11), one must expect that MeA would be strongly dissociated and accumulated into the vacuole. In agreement with the Waddell and Bates statement (12) about the calculation of pHi in case of inhomogeneity of H^+ distribution within the cell "calculations from the distribution of a weak base yield a pH value closer to the lower pH value in that inhomogeneous space" we can expect to get a calculated pHi close to the vacuolar pH. Indeed, a ΔpH of 1 to 3 units (11) between cytoplasm and vacuole would give a vacuolar concentration of amine 10 to 1,000 times that of the cytoplasmic compartment. Consequently, as the relative volume of the vacuolar compartment of plant cells cultivated in liquid medium is frequently rather large (about 0.8–0.9), the vacuolar concentration of MeA (Cv) would be close to the total Ci. Inasmuch as it is possible to measure this Ci, pHi calculation from equation 1 is expected to give a value very close to vacuolar pH.

It seems that MeA is potentially a good probe to measure vacuolar pH in higher plant cells and the present paper is devoted to an evaluation of this possibility.

MATERIALS AND METHODS

Plant Material and MeA Uptake. *Acer pseudoplatanus* cells were cultivated as described previously (6). For the measurement of MeA uptake, a cell suspension (between 2.10^5 and 5.10^5 cells/ml) was incubated with 0.52 μM [^{14}C]MeA (41.2 mCi/mmol, 99% radiochemical purity, CEA, France). At various times, 5 to 10 ml aliquots of the suspension were rapidly filtered on Whatman glass fiber filters (GF/A) with a vacuum intensity of about 20 cm of mercury vacuum. Different measurements (contaminating extracellular fluid retained by the filtered cells, total radioactivity associated with the filtered cells, total volume occupied by the cells) were made to calculate Ci according to the procedure already described for 5,5-dimethyloxazolidine dione (4, 5).

The initial rates of MeA uptake were measured from the slope of the evolution with time of the intracellular radioactivity. Aliquots (5 ml) of the cell suspension were pipetted every 30 to 50 s during 8 to 10 min and rapidly filtered to measure the intracellular radioactivity as described above.

To study the influence of the pH of the culture medium on the distribution of [^{14}C]MeA molecules between the cells and their culture medium and on the pH of the vacuolar sap, Mops and Tricine buffers were used. The buffers were sterilized by filtration through a Millipore filter (0.22 μm) before adding to the culture medium to a final concentrationof 50 mM, 16 h before the measurements.

The transplasmalemma and transtonoplast potential differences were measured with potential microelectrodes according to the procedure described by Rona *et al.* (8, 9).

Cell Sap Extraction and pH Measurement. To measure the pH of the vacuolar sap, the sap was extracted through a freeze-thaw technique. Aliquots of 10-ml cell suspensions were rapidly filtered; cells were scraped off the filters and immediately stored in a

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[2] Abbreviations: MeA, methylamine; pHi, intracellular pH; pHe, extracellular pH; Ci, intracellular total concentration of MeA; Ce, extracellular total concentration of MeA; pHs, cell sap pH.

precooled conical centrifuge tube and kept for 30 min at −25 C. The tubes were then plunged for 45 s in a water bath at 50 C and immediately centrifuged for 5 min (2,600*g*) in a refrigerated centrifuge. The supernatant was delicately removed with a Pasteur pipette and its pH measured with a pH glass electrode. Between 0.5 and 1 g fresh weight were needed for each pH measurement. One g fresh weight cells gave about 500 μl of "vacuolar sap."

RESULTS

Kinetics of MeA Uptake and Distribution of MeA Molecules Between the Cells and Their Culture Medium. Figure 1 shows that the rate of MeA uptake was high and that the MeA molecules were highly accumulated inside the cells. The accumulation ratio Ci:Ce reached within 60 min was about 1,800. Despite the low population density of the cell suspension used (about 100 mg fresh weight/10 ml of culture medium) the extracellular MeA concentration was greatly lowered in relation to the powerful intracellular accumulation. From equation 1, it is possible to calculate a pHi value of 3.54 from Ci:Ce = 1,810 and pHe = 6.80. Consequently, it appears that a rather highly acidic vacuole could account for the strong intracellular MeA concentration.

In agreement with the assumption that the uptake equilibrium reached within 60 min was a diffusion equilibrium, Figure 2 shows that within a large range (from 0.47–50 μM) the Ci and Ce at equilibrium were linear functions of the initial MeA concentration. The accumulation ratio Ci:Ce and the pHi value calculated from equation 1 were independent of the MeA concentration below 50 μM (Fig. 3). However, when the initial extracellular MeA concentration was increased over 50 μM, the accumulation ratio Ci:Ce was strongly decreased (Fig. 3) and the calculated pHi increased.

Sensitivity to pHe Modifications of the Distribution of MeA Molecules Between the Cells and Their Culture Medium. As shown by equation 1, the accumulation ratio Ci:Ce is dependent on pHe. If it is assumed that pHi is controlled at a constant level when the pH of the culture medium is modified, the Ci:Ce ratio must be increased by a pHe increase. The results obtained (Fig. 4) show that the accumulation ratio Ci:Ce was markedly decreased when the pH of the culture medium was increased. As a consequence, the theoretical pHi value, calculated from equation 1 was increased. This result suggested that pHi (likely the vacuolar pH) was regulated in such a way that its increase followed that of pHe.

Comparison of the pH Value Calculated from the Equilibrium Distribution of MeA Molecules to the pHs Extracted by a Freeze-thaw Technique. The pHi values calculated from the accumulation ratios of MeA (Ci:Ce) measured for different pHe were compared to the pH values directly measured on the sap extracted from the cells incubated in the same buffered medium (pHs). The pHi and pHs values obtained were plotted against pHe (Fig. 5). In the range of pH studied, pHi and pHs appeared to increase linearly with pHe increase and reasonable adjustments to straight lines were obtained. A strong difference between pHi and pHs was observed, pHi being about 2.5 units lower than pHs for a pHe around 6 and only 1 unit lower than pHs when pHe was increased around 8. Consequently, pHi calculated from the distribution of MeA molecules appeared to increase with pHe, more rapidly than pHs.

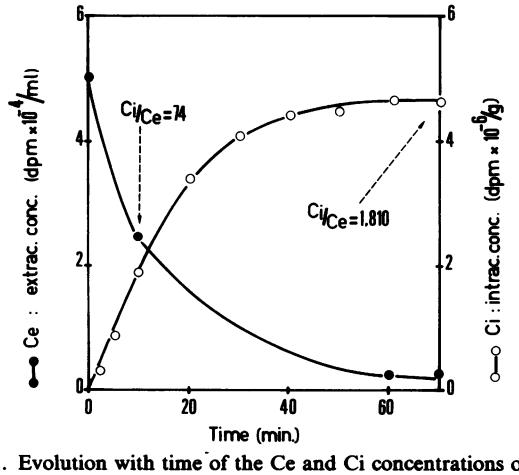

FIG. 1. Evolution with time of the Ce and Ci concentrations of MeA. *A. pseudoplatanus* cells were incubated (about 100 mg fresh weight/10 ml of culture medium) with 0.52 μM [^{14}C]MeA. The measurements of extracellular and intracellular radioactivities were made at intervals as indicated.

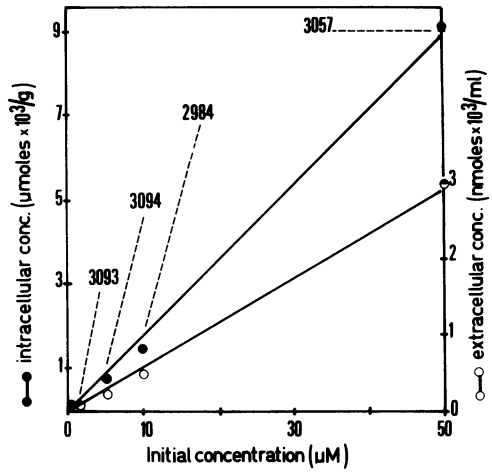

FIG. 2. Influence of the initial extracellular methylamine concentration on the intracellular and extracellular concentrations at equilibrium. Cells were incubated with [^{14}C]MeA at various concentrations. After 90 min, when the uptake equilibrium was reached, the intracellular and extracellular radioactivities were measured and the corresponding concentrations calculated. The calculated Ci:Ce ratios are indicated on the curves.

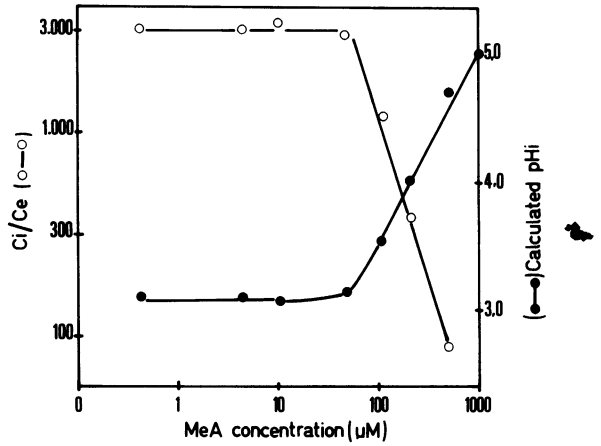

FIG. 3. Influence of the initial Ce on the distribution of the molecules of this base between the cells and their culture medium. Cells were incubated with MeA solutions at various concentrations but containing the same concentration (0.52 μM) of [^{14}C]MeA (50 mCi/mmol). The Ci and Ce at equilibrium were measured as indicated. From the Ci:Ce and pHe values, the theoretical pHi values were calculated from equation 1.

DISCUSSION

Accumulation of MeA Molecules by *A. pseudoplatanus* Cells. The results obtained show that MeA uptake is very fast and leads to a large intracellular accumulation within 60 min (Fig. 1). The maximum uptake reached at equilibrium is proportional to the

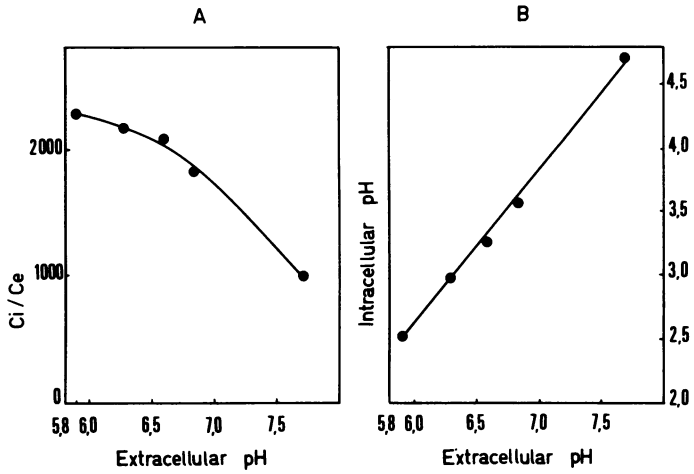

FIG. 4. Influence of pHe on the theoretical pHi values (B) calculated from the Ci:Ce distribution ratio of MeA molecules between the cells and their culture medium (A). Cells were incubated 16 h before adding [^{14}C]MeA in the culture medium buffered at different pH. The Ci:Ce values (A) were measured as indicated and the theoretical pHi values calculated from equation 1.

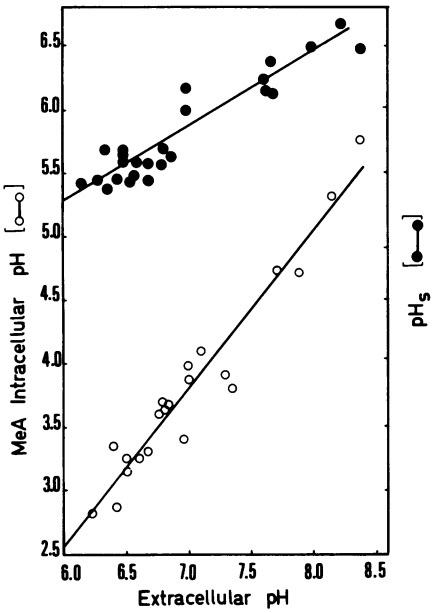

FIG. 5. Influence of pHe on the calculated pHi value and on the measured pH of the cell sap. Cells were incubated with culture medium buffered at different pH. The pHi MeA values were calculated from the measured accumulation ratios of [^{14}C]MeA and the pHs values were measured as indicated. The fitting of the experimental results to linear adjustments gave, respectively, pHi MeA = 1.23 pHe − 4.83 (r = 0.97) and pHs = 0.58 pHe + 1.81 (r = 0.94).

MeA concentration over a wide range. These results are in good agreement with already published models (1, 7, 10), assuming that the uptake equilibrium of MeA is a diffusion equilibrium of the undissociated form. The decrease of the accumulation ratio when the MeA concentration in the culture medium was increased over 50 to 100 μM (Fig. 3) remains to be explained because such a result is contradictory to the existence of a diffusion process. We have described such a situation for tryptamine uptake by *Catharanthus roseus* cells (3).

If it is assumed that the permeability constants of the plasmalemma (PB) and the tonoplast (P'B) for the nonionized MeA molecules are much higher than those corresponding to the ion, the organic base is strongly accumulated as a cation inside the acidic vacuolar compartment. This cation accumulation induces a vacuolar alkalinization as the undissociated base crossing the tonoplast is ionized inside the vacuole. It seems that a possible explanation of the nonlinearity of intracellular MeA accumulation when the concentration of the base was increased over 50 to 100 μM would be that the accumulation of the base overcomes the buffering capacity of the vacuole. The increase of vacuolar pH reducing the pH difference between the culture medium and the vacuole would induce a decrease of MeA accumulation. To check this hypothesis, the influence of various initial extracellular MeA concentrations on pHs and the distribution of this base between the cells and their culture medium was studied. Table I shows that the accumulation ratio Ci:Ce was decreased for MeA concentrations higher than 0.1 mM. Most interesting was the fact that pHs was increased by 0.7 to 0.8 units when MeA concentration was increased from 0.52 μM to 5 mM. The pH difference between the culture medium and the cell sap was lowered to about zero when the MeA concentration reached 1 mM. At the same time the accumulation ratios were lowered by 50 to 70 times. The same action of high concentrations of tryptamine on pHs of *C. roseus* cells has been observed (3).

We concluded that the nonlinear increase in the intracellular concentration of MeA when the extracellular concentration of this base was increased over 50 to 100 μM is not contradictory to an uptake of this base through a diffusion process but is due to an increase of the vacuolar pH.

Possible Significance of the pH of the Cell sap Extracted by a Freeze-thaw Technique. As the vacuolar volume of *A. pseudoplatanus* cells cultivated in liquid medium represents about 80 to 90% of the total cell volume, the cell sap extracted was considered as representative of vacuolar sap. The extent of modifications of the vacuolar sap pH by the intercellular culture medium and cytoplasmic molecules or ions that contaminated the sap during its extraction had to be estimated.

We have shown (4, 5) that the percentage of contaminating water which stays in the intercellular spaces of the filtered cell suspension was 12.5% of the total fresh weight. To be sure that this amount of water (and, in some experiments, of buffer) did not

Table I. *Influence of the Initial Extracellular MeA Concentration on pHs and the Distribution of [^{14}C]MeA Molecules Between the Cells and Their Culture Medium*

The pHs values and the accumulation ratios Ci:Ce were measured as indicated under "Material and Methods." The cells were incubated with 0.52 μM [^{14}C]MeA and with the amount of unlabeled MeA necessary to reach the concentrations indicated in the Table.

Initial MeA concn	pHe	Accumulation Ratio Ci:Ce	pHs
mM			
Experiment A			
0.00052	6.60	2,239	5.76
0.1	6.60	2,398	5.96
0.5	6.54	1,259	6.07
1.0	6.45	34	6.45
2.5	6.44	17	6.40
5.0	6.44	9	6.46
Experiment B			
0.00052	6.50	1,995	5.84
0.1	6.49	1,862	6.10
0.5	6.45	912	6.25
0.66	6.43	676	6.30
0.8	6.43	275	6.36
1.0	6.49	36	6.44

change the pHs values obtained, we made the following experiment. Tricine buffer (1 M, pH 9.0) was injected in the culture (50 mM final concentration) just before the cell suspension was filtered and the cells frozen for the pHs measurement. During the filtration, the cells were rapidly washed to minimize the effect of the buffer contaminating the intercellular spaces. For the cell sap extracted from cells issued from the culture medium, pH values were respectively, 5.78 and 5.73, for pHe values 6.57 and 6.54. In case of cells treated with the Tricine buffer, the pHs values measured were 5.77 and 5.81, for pHe values 8.50 and 8.58. It seems clear that pHs were not significantly modified by the contaminating intercellular fluid.

With respect to the possible influence of a contamination of the vacuolar sap by constituents from the cytoplasm and the cell wall, the following experiment was done. Cells were frozen-thawed as quickly as possible and after centrifugation and measurement of pHs, the pellet of the frozen-thawed cells was resuspended in the vacuolar sap and the suspension was sonicated 2 or 3 min to insure the maximum intermixing of vacuolar and cytoplasmic constituents, and centrifuged again. The pHs values obtained before resuspending the pellet and after 2 or 3 min sonication were, respectively, 5.00, 5.02, and 5.07. This is an argument in favor of the fact that the pH of the vacuolar sap is probably not modified to a large extent by cytoplasmic constituents released from the cytoplasm during the freeze-thaw process. When 0.5 ml of cell sap with an initial pH of 5.23 were titrated with 1 mM NaOH, no pH variation was obtained for 100 μl of the base solution added. This indicated a very strong buffering power and we have shown (unpublished results) that this property is linked to the accumulation of large amounts of malate, citrate, and potassium ions.

From the results obtained, the pHs value measured through the technique described was considered as a rather good estimate of the vacuolar pH.

Analysis of the Differences between Values of the pHs and the pHi Value Estimated by the MeA Method. The large difference observed between pHs and pHi calculated (Fig. 5) has to be explained. If the pHs value obtained from the freeze-thaw method is taken as a reference, it follows that the pHi value calculated from the MeA method is too low by 1 to 2 pH units (Fig. 5). As the pHi values were calculated from the simplified form of relation (equation 1) $Ci:Ce = 10^{(pHe-pHi)}$ the difference between pHi and pHs means that the experimentally measured accumulation ratios of MeA were 10 to 100 times higher than those that could be calculated from the theoretical relation: $Ci:Ce = 10^{(pHe-pHs)}$. We have to assume that factors other than the simple pH difference between the cells and their culture medium drive the intracellular accumulation of MeA molecules. The same situation has been observed for tryptamine accumulation by *C. roseus* cells (3).

Several hypotheses can be presented to account for the intracellular accumulation of MeA over that which could be predicted from the simple law of Waddell and Butler (13).

According to the first one, the adsorption of MeA on intracellular sites would give a total intracellular concentration higher than the real intracellular concentration of free MeA molecules and ions. Some evidence that a part of MeA molecules associated with the filtered cells are probably adsorbed to cell wall or intracellular sites has been obtained. The adsorption of MeA by frozen-thawed cells was studied through the following procedure. Cells from a population grown on 0.2 μM 2,4-D were filtered, frozen-thawed, and washed three times with distilled H_2O buffered at pH 7 with Tricine 50 mM. These frozen-thawed cells were incubated (about 50 mg fresh weight filtered cells/ml) with 0.52 μM [^{14}C]MeA. After 60 min incubation, aliquots of the suspension were pipetted out and filtered and the radioactivity of free MeA remaining in the incubation medium was measured. The ratio of bound *versus* unbound MeA was calculated to be 0.58 in the conditions described. This ratio was reduced, respectively, to 0.19 and 0.09 when cold 1 mM or 5 mM MeA was added to the incubation medium. These results show that cellular sites with a low affinity for MeA are able to bind this base and consequently modify the accumulation ratio.

The second hypothesis is that, as shown for tryptamine (3), if the plasmalemma permeability to the methylammonium ions is not negligible compared to that of the undissociated molecule, in some conditions, the accumulation ratio Ci:Ce can be drastically increased. The accumulation ratio is dependent not only on the pH differences between the culture medium and the cell compartments but also on the transmembrane potential differences across the plasmalemma (Vm) and the tonoplast (V'm) and on the relative permeabilities of these two membranes to the nonionized (P_B and P'_B) and protonated forms (P_{B^+} and P'_{B^+}) of the base (3). The distribution, at equilibrium, of MeA between the cytoplasm and the extracellular medium (Cc:Ce) can be calculated from equation 2:

$$Cc/Ce = \frac{H^+i + K'a}{H^+e + K'a} \times \frac{\frac{PB}{PB^+} - \frac{H^+e}{K'a} \times \frac{FVm}{RT\,(1 - e^{FVm/RT})}}{\frac{PB}{PB^+} - \frac{H^+i}{K'a} \times \frac{FVm \times e^{FVm/RT}}{RT\,(1 - e^{FVm/RT})}} \quad (2)$$

In that case, $PB:PB^+$ and Vm refer to plasmalemma properties. The distribution of MeA between the cytoplasm and the vacuole (Cc:Cv) can be calculated from the properties V'm and $P'B:P'B^+$ of the tonoplast. Such a model can account for an intracellular accumulation of MeA over that which can be predicted from the simple pH differences according to the relation (equation 1) of Waddell and Butler (13) if it is assumed (*a*) that the permeability of the plasmalemma and the tonoplast to the methylammonium ions is much lower than that to the nonionized molecules; and (*b*) that the relative permeability of the plasmalemma to the methylammonium ion ($PB^+:PB$) is much higher than that of the tonoplast ($P'B^+:P'B$). Figure 6 shows that if properties (*a*) and (*b*) are satisfied, it is possible to obtain calculated pHi values much lower than the vacuolar pH. This is dependent on the fact that MeA accumulation in the cytoplasm is driven both by the pH and transplasmalemma potential differences between the cytoplasm and the culture medium and, consequently, much dependent on the relative permeability of the plasmalemma to methylammonium ions. The vacuolar accumulation is mainly driven by the pH difference across the tonoplast if it is assumed that this membrane is relatively impermeable to methylammonium ions. Coming back to the large discrepancy observed between pHs and the pHi values calculated from the distribution of MeA molecules (Fig. 5), theoretical calculations (Fig. 6) show that the accumulation induced by the entry of MeA cation driven by the potential difference across the plasmalemma can be so high to give a calculated pHi about 3 units lower than pHs. This process can quantitatively account for the difference we observed between pHs and the calculated pHi.

Some evidence for the uptake of methylammonium ions by the cells has been obtained. The influence of pHe on the initial rate of uptake of MeA was studied (Table II). It seems that the rate of uptake was not affected by the pH of the culture medium between 6.2 and 7.6 suggesting that the uptake was largely dependent on the extracellular concentration of methylammonium ions. Furthermore, when *A. pseudoplatanus* cells incubated with [^{14}C]MeA were treated by 70 mM KCl, which is known to depolarize the cells, the intracellular accumulation of MeA was reduced (Fig. 7) showing that the transplasmalemma potential difference was taking part in the accumulation of the base.

Therefore, it appears that the over accumulation of MeA corresponding to the (pHs − pHi) difference is due to both phenomena, the adsorption of MeA molecules on cell walls and/or intra-

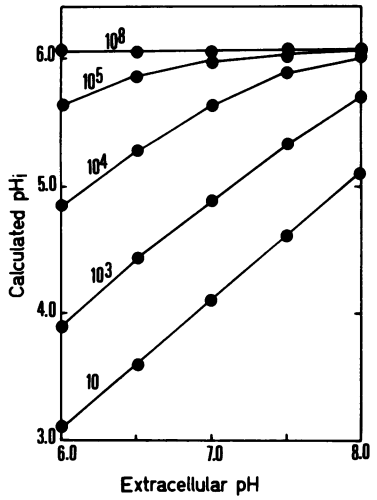

FIG. 6. Theoretical study of the influence of the relative permeability of the plasmalemma to methylammonium ions and undissociated MeA molecules on the difference between pHs and the calculated pHi. Calculations were made from equation 2 assuming cytoplasmic and vacuolar pH of 7.8 and 6.0 and a relative vacuolar volume of 0.9. Transplasmalemma (Vm) and transtonoplast (V'm) potential differences were measured according to the techniques described for plant cells cultivated in liquid medium (8, 9). Vm and V'm values used for calculations were, respectively, −75 mv and +10 mv. The PB:PB$^+$ ratio, which is the relative permeability of the plasmalemma to undissociated MeA molecules compared to methylammonium ions, was given different values from 10 to 10^8. The P'B:P'B$^+$ ratio was taken as 10^8 which means that it was assumed that the tonoplast was not permeable to methylammonium ions.

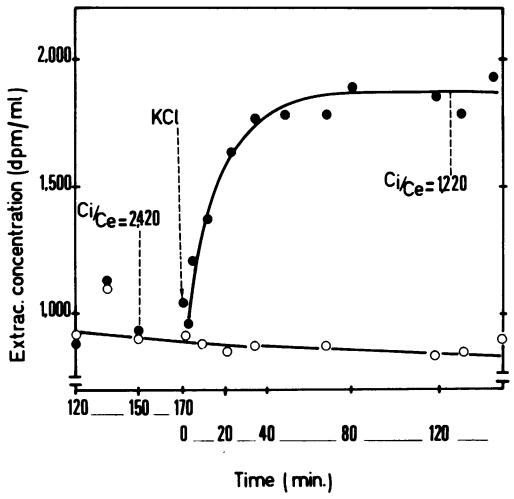

FIG. 7. Influence of 70 mM KCl on the distribution of MeA molecules between the cells and their culture medium. Cells were incubated on Mops buffered medium (Mops 25 mM, pH 6.78—about 260 mg fresh weight/10 ml of culture medium) with [^{14}C]MeA. After 3 h, KCl was added to a final concentration of 70 mM and the evolution with time of the extracellular radioactivity was followed in control (○——○) and treated (●——●) suspensions. The accumulation ratio Ci:Ce was measured before adding KCl and after reaching a new equilibrium.

Table II. *Influence of the Extracellular pH on the Initial Rate of Uptake of MeA*

Cells (about 60 mg/5 ml) were incubated with 0.52 μM MeA. The initial rates of uptake (V_i) were measured from the slopes of the intracellular radioactivity *versus* time. Aliquots of the cell suspension (5 ml) were pipetted every 30 to 50 s during 8 to 10 min and rapidly filtered to measure the intracellular radioactivity as described. The extracellular concentrations of the undissociated methylamine were calculated from pK'a = 10.6 and pHe values.

pHe	V_i	Concn of Undissociated Form
	nmol/g fresh wt·min	*nM*
6.20	4.7	0.02
6.48	5.6	0.04
6.70	4.8	0.07
6.85	5.7	0.09
7.10	4.9	0.16
7.60	5.1	0.52

cellular sites and the direct uptake of methylammonium ions. The accumulation of free molecules and ions is for a large part determined by the pH gradient between the vacuole and the extracellular medium but is also sensitive to the transmembrane potential differences, especially at low pHe for which the relative importance of the ion uptake is high compared to that of the undissociated base.

We conclude that the pHi value calculated from the distribution of MeA between the cells and their culture medium must be considered with care for two reasons: (*a*) the permeability of the cells to the methylammonium ions cannot be neglected; (*b*) some MeA molecules associated with the cells do not participate in the diffusion equilibrium but are bound to cell constituents. Compared to the MeA method, measuring pHs seems to be a better way to estimate the vacuolar pH of these cells. The selection of other lipophilic organic bases as pH probes (for which the relative permeability of the cell membranes to the cationic form could be much lower than that to methylammonium ions) and the comparison of the pH values obtained through their use of pHs is reported in the next paper of this series.

LITERATURE CITED

1. BORON WF, ROOS A 1976 Comparison of micro electrodes, DMO and methylamine methods for measuring intracellular pH. Am J Physiol 231: 799–809
2. CALDWELL PC 1956 Intracellular pH. Int Rev Cytol 5: 229–277
3. COURTOIS D, KURKDJIAN A, GUERN J 1980 Tryptamine uptake and accumulation by *Catharanthus roseus* cells cultivated in liquid medium. Plant Sci Lett 18: 85–96
4. KURKDJIAN A, GUERN J 1978 Intracellular pH in higher plant cells. I. Improvements in the use of 5,5-dimethyloxazolidine-2-^{14}C, 4-dione distribution technique. Plant Sci Lett 11: 337–344
5. KURKDJIAN A, LEGUAY JJ, GUERN J 1978 Measurement of intracellular pH and aspects of its control in higher plant cells cultivated in liquid medium. Resp Physiol 33: 75–89
6. LEGUAY JJ, GUERN J 1975 Quantitative effects of 2,4-dichlorophenoxyacetic acid on growth of suspension cultured *Acer pseudoplatanus* cells. Plant Physiol 56: 356–359
7. MEIZEL S, DEAMER DW 1978 The pH of the Hamster sperm acrosome. J Histochem Cytochem 26: 98–105
8. RONA JP, CORNEL D 1979 Résistances électriques chez les cellules libres, les protoplastes et les vacuoles isolées d'*Acer pseudoplatanus* L. Physiol Vég 17: 1–11
9. RONA JP, CORNEL D, HELLER R 1976 Determination and interpretation of the electrical profile of free cells of *Acer pseudoplatanus* L. *In* M Thellier, A Monnier, M Demarty, J Dainty, eds, Transmembrane Ionic Exchanges in Plants. Colloque International du C.N.R.S., Paris, pp 349–356
10. ROTTENBERG H, GRUNWALD T, AVRON M 1972 Determination of ΔpH in chloroplasts. I. Distribution of ^{14}C-methylamine. Eur J Biochem 25: 54–63
11. SMITH FA, RAVEN JA 1979 Intracellular pH and its regulation. Annu Rev Plant Physiol 30: 289–311
12. WADDELL WJ, BATES RG 1969 Intracellular pH. Physiol Rev 49: 285–328
13. WADDELL WJ, BUTLER TC 1959 Calculation of intracellular pH from the distribution of DMO. Application to skeletal muscle of the dog. J Clin Invest 38: 720–729