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Microautoradiographic Investigations of Sulfate Uptake by Glands and Epidermal Cells of Water Lily (*Nymphaea*) Leaves with Special Reference to the Effect of Poly-L-Lysine

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Summary. The uptake of ^{35}S-labelled sulfate ions into hydropote cells (densely cytoplasmic gland cells) and into epidermal cells (highly vacuolated cells) of *Nymphaea* leaves is dependent on metabolic energy. Only a very small fraction of the accumulated ^{35}S is incorporated into organic macromolecules during the experimental period. Both cell types exhibit a hyperbolic isotherm for ^{35}S uptake from labelled K_2SO_4 solutions over an external concentration range of 0 to 0.5 mM. Although the gland and epidermal cells behave qualitatively similarly, the glands generally absorb about twice as much ^{35}S per unit area of sections of the cells as do the epidermal cells. At 3 °C, poly-L-lysine concentrations of 10^{-8} M and up to 10^{-7} M enhance ^{35}S uptake by the epidermal and gland cells for the first 7.5 hr after application of the poly-L-lysine. Samples treated with 5×10^{-7} M poly-L-lysine are indistinguishable from the controls over the same period. After longer periods of treatment with poly-L-lysine (7.5 to 24 hr), the rates of ^{35}S uptake were reduced by all poly-L-lysine concentrations between the range 10^{-8} to 5×10^{-7} M. After 7.5 hr of ^{35}S uptake, the control samples contained the smallest amount of label, but after an uptake period of 24 hr the amount of label in the controls is considerably larger than in samples treated with poly-L-lysine. The results suggest that poly-L-lysine increases the membrane permeability and alters the metabolic uptake of sulfate in both hydropotes and epidermal cells.

The lower epidermis of the floating leaves of the water lily (*Nymphaea*) is composed of two types of cells: normal epidermal cells with a thin cytoplasmic coating along the cell walls and large central vacuoles, and gland-like structures, the hydropotes. In fully developed leaves, the hydropotes

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are formed by two gland cells which are densely filled with cytoplasr containing a high proportion (by volume) of mitochondria. These cell contain only small vacuolar vesicles but no central vacuole. Their ultra structure has been described in detail elsewhere (Lüttge & Krapf, 1969)

In a recent publication, Kristen (1969) concluded that the hydropote in the *Nymphaeaceae* are improperly named because their primary functio is not water and salt uptake but secretion of slime during the early stage of leaf development. This is certainly correct to some extent, as degradatio of the outer cell of the *Nymphaea* gland complex observed after unfoldin of leaves (Lüttge & Krapf, 1969) may be accompanied by slime production However, in all our uptake experiments, we used fully mature leaves wit the glands in a structural state as described by Lüttge and Krapf (1969) In addition, earlier investigations have also shown that the hydropote accumulate much more labelled sulfate in short-term experiments than d the adjacent epidermal cells, and that this accumulation is dependent o metabolic energy (Lüttge, 1964). More details on this process are reported here

Both the structural and physiological features make the lower epidermi of *Nymphaea* leaves attractive as an experimental material for a com parative study of ion uptake between cells which are largely cytoplasmi and those which are highly vacuolated. Similar attempts have been mad by Torii and Laties (1966) using meristematic tips and vacuolated proxima sections of maize roots. Unlike roots, in the *Nymphaea* epidermis the tw types of cells cannot be separated mechanically, and quantitative micro autoradiography was the only possible analytic approach at hand. Th advantage of the hydropotes over the root tips is that the hydropotes ar biochemically more stable. Although there are slow structural changes i the cytoplasm of the gland during the growth period of the leaves (Lüttge & Krapf, 1969; *see* also Fig. 4), the hydropote cells do not divide or grov rapidly.

It has been the aim of this study to further elucidate the characteristic of ion uptake by the two systems, i.e. non-vacuolated and vacuolated cell of the *Nymphaea* epidermis, by using poly-L-lysine as a tool for manipulatin membrane properties. Poly-L-lysine, a homopolymer of L-lysine, and othe basic polymers have been shown to alter the characteristics of mitochondri (Schwartz, 1965; Johnson, Mauritzen, Starbuck & Schwartz, 1967) an chloroplasts (Dilley, 1968) mainly by changing their membrane permeabili ties to ions such as H^+ and K^+. Osmond and Laties (1970) pointed ou that poly-L-lysine may be a useful tool for the investigation of the role o the plasmalemma and tonoplast membranes in determining the ionic rela tions of plant cells.

Materials and Methods

Fully mature leaves of water lily (different cultivars of *Nymphaea*) were collected from ponds in the Botanic Garden, Technische Hochschule Darmstadt. Thin sections of the lower surface of the leaves were cut parallel to the surface using a sharp razor blade. The sections were 3–5 mm in diameter. The epidermal cells and cells of the gland complex were the only remaining intact cells in the thin sections. For any one experiment, only a single *Nymphaea* leaf was used, thus giving uniform material.

In most experiments, sections were washed for ca. 48 hr in a large volume of aerated 10^{-4} M $CaSO_4$ at room temperature. For the determination of the time course of SO_4^{--} uptake and dependence of uptake on external SO_4^{--} concentration, a small number of the thin sections were transferred into a loading solution of high specific activity varying between 6,000 mc ^{35}S/mmole SO_4^{--} at a SO_4^{--}-concentration of 0.01 mM and 4 mc/mmole SO_4^{--} at 10 mM SO_4^{--}. $^{35}SO_4^{--}$ stock solution was supplied by Radiochemical Centre (Amersham, England).

For the investigation of the effect of poly-L-lysine, sections were washed for 15 min in aerated 0.5 mM $CaSO_4$ and were then pretreated for 3 days in a large volume of aerated solution (0.1 mM K_2SO_4 + 0.5 mM $CaCl_2$) at 3 °C. The time course of sulfate uptake was determined at 3 °C using 0.1 mM $K_2\,^{35}SO_4$ + 0.5 mM $CaCl_2$ in the presence of different concentrations of poly-L-lysine (molecular weight 51,000). The volume of the loading solution was 20 ml corresponding to approximately 250 ml per g of tissue fresh weight. Specific activity of these solutions varied between 22.5 and 30.7 mc ^{35}S/mmole SO_4^{--}.

At the end of all uptake experiments, sections were washed twice for 15 min in ice-cooled unlabelled solutions and then placed on microscopic glass slides with the epidermal surface facing upwards (Fig. 1a). These slides were then transferred to the darkroom. In a water-bath at 24 °C, Kodak AR 10 stripping film was stripped over a blank microscopic slide with the sensitive photoemulsion facing upwards (Fig. 1b). The two slides, one bearing the sections and the other bearing the film, were gently pressed together to give good contact between the film and the tissue (Fig. 1c). The edges of the film were then moved around from the blank slide to the slide bearing the tissue as shown in Fig. 1d, and the blank slide was removed (Fig. 1e). Two of these slides were tightly pressed together back to back (Fig. 1f). The "sandwiches" obtained were placed in a rack and immediately transferred to a deep freeze at −25 °C. Time elapsing between arranging the sections on the slides and freezing of the assembled microautoradiographic sandwich was usually on the order of 1 min or less. This time is negligible compared with the duration of free-space washing.

This procedure, which we developed for autoradiographic work with thin, aged sections of leaves or with moss leaves of a thickness of only one cell layer does not involve any sectioning at the end of an experiment. This procedure saves time and helps to avoid the problems and artifacts which occur in the preparation when using the usual methods of microautoradiography of diffusible substances (*cf.* Lüttge & Weigl, 1965).

Exposure time of the autoradiographs was from 20 days up to but not more than two half-times of radiation decay ($t_{1/2}$ for ^{35}S is 87.2 days) depending on experimental requirements. After photographic development (Kodak D 19b developer) and fixing at 17 °C, the slides were carefully washed in tap water and dried at room temperature.

The dry film with the microautoradiograms was separated from the tissue using fine needles, forceps and a razor blade. The autoradiograms were placed on a glass slide in a droplet of lens immersion oil and covered with a thin coverslip. Autoradiograms of hydropote glands can be easily distinguished from those of epidermal cells, because the glands usually accumulate more label than the epidermal cells, and hydropotes have a

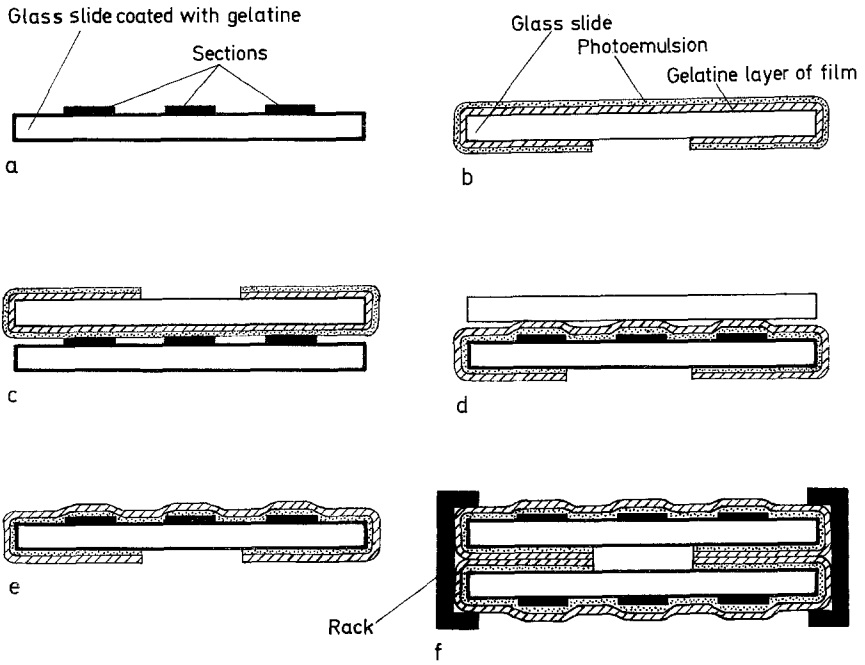

Fig. 1a–f. Schematic representation of autoradiographic procedures. (a) Sections are placed on gelatine-coated slides. (b)–(f) Dark room procedures: (b) a blank glass slide is coated with stripping film; (c) the slide with the sections and the slide with the film are brought in contact; (d) the edges of the film are stripped around the slide with the sections; (e) after removal of the blank slide, the sections are properly coated with the film; and (f) microautoradiographic sandwich is stored in a rack for exposure and for later photographic development and fixation

circular circumference in a face-on view (for microphotographs, *see* Lüttge, 1964, and Lüttge & Krapf, 1969). This method allows quantitative microdensitometer measurements of the autoradiograms without interference of light absorption by the tissue itself.

Densitometer measurements were described in detail in an earlier report (Läuchli & Lüttge, 1968). Ion uptake into epidermal and gland cells was calculated as follows:

$$\text{Uptake} = \frac{1}{S} \log_{10} \frac{B}{A} \tag{1}$$

where $S[\mu c \cdot \mu mole^{-1}]$ is the specific activity of the uptake solution, B is the transmission of light by a blank part of the film (i.e. where the film was not in contact with the tissue), and A is the transmission by the autoradiogram. Since $\log_{10} \frac{B}{A} = \text{extinction}$, the relative units for uptake obtained by this method are:

$$\frac{\text{extinction}}{\mu c} \times \mu mole = F_d \times \mu mole \tag{2}$$

(Läuchli & Lüttge, 1969). F_d (d stands for "densitometer") depends on a number of factors, including exposure time and conditions at photographic development which

affects the yield of silver grains on the autoradiogram (*see* Boyd, 1955). No calibrations were attempted to eliminate F_d, but F_d was constant for all treatments in any one experiment.

In some experiments, amounts of radioactivity were estimated by counting developed silver grains in the film under the microscope. In this case, radioactivity is directly proportional to the number of grains detected. A relative estimate of uptake is then:

$$\text{Uptake} = z \cdot \frac{1}{S} [F_c \cdot \mu\text{mole}] \tag{3}$$

where z is the number of grains per unit area and S is again the specific activity of the external solution [$\mu c \cdot \mu mole^{-1}$]. As in Eq. (2), the relative units of uptake are proportional to μmoles of ion taken up and F_c(c stands for "count") is a factor of proportionalty depending on experimental details. F_c, of course, is different from F_d in Eq. (2), but it is also a constant for any given experiment. For each experimental treatment, usually three slides holding three sections each were prepared. Statistical treatment of the results is indicated in the text and in the subscripts of tables and graphs.

Results

Fig. 2 shows a time course of $^{35}SO_4^{--}$ uptake into hydropotes and epidermal cells from 0.05 mM K_2SO_4 at room temperature (25 °C). A similar time course is found for an external concentration of 0.1 mM at 3 °C after preloading of the tissue with non-radioactive SO_4^{--} under the same conditions (*see* Fig. 3).

A number of experiments were performed to analyze the effect of external concentration between 0.01 and 0.5 mM K_2SO_4 on SO_4^{--} uptake by the two types of cells. The uptake was 3 hr at 25 °C. Relative rates of uptake have been measured either by grain counts or by densitometry. Since the

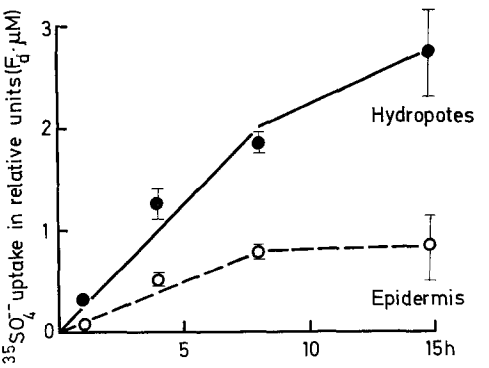

Fig. 2. Time course of $^{35}SO_4^{--}$ uptake into hydropotes and epidermal cells from 0.05 mM K_2SO_4 at 25 °C. The leaf was harvested in March. Errors shown are standard deviations of the mean, where n was taken as the number of sections on each of which numerous densitometer readings were taken

proportionality factors F_d and F_c vary from experiment to experiment data were normalized by setting the radioactivity of the hydropotes for al experiments on a given point at 0.1 mM K_2SO_4. The rest of the data wa: then recalculated so that the ratios of the relative rates remained the same as in the original measurements.

The Michaelis-Menten equation

$$v = V_M \frac{[S]}{[S] + K_m} \tag{4}$$

seems applicable to most ion uptake processes in higher plants (Epstein, 1966). v is the rate of uptake at a given concentration, V_M is the maximal rate of uptake, $[S]$ is the external concentration, and K_m the Michaelis constant. In order to test if our uptake data fit this relationship, we used the following linear transformation of Eq. (4):

$$[S]/v = K_m/V_M + 1/V_M \cdot [S] \tag{5}$$

(*see* Dowd & Riggs, 1965). The linear regression lines for $y = [S]/v$ and $x = [S]$ were calculated. The results are shown in Table 1. The correlation coefficients (r) show that the correlation of our results with the Michaelis-Menten kinetics is quite good. Notwithstanding the differences between grain counts and densitometer readings respectively, K_m values obtained are on the order of magnitude of Michaelis constants usually found for metabolically dependent ion uptake by plant tissues in this concentration range.

It was shown by Lüttge (1964) that uptake of labelled SO_4^{--} into hydropotes is sensitive to metabolic inhibitors. Table 2 confirms these earlier findings.

Table 1. *Regression lines, V_M and K_m values as calculated from the data using Eq.* (5)

Cells	Data	$[S]/v = K_m/V_M + 1/V_M \cdot [S]$	V_M	K_m	r[a]
			(mmoles/liter)		
Hydropotes	Densitometer readings	$y = 0.0021 + 0.0220 \cdot x$	45.5	0.096	0.90
	Grain counts	$y = 0.0009 + 0.0298 \cdot x$	33.5	0.030	0.94
	Densitometer readings plus grain counts	$y = 0.0016 + 0.0266 \cdot x$	37.6	0.060	0.93
Epidermal	Densitometer readings	$y = 0.0043 + 0.0487 \cdot x$	20.5	0.088	0.92
	Grain counts	$y = 0.0015 + 0.0363 \cdot x$	27.5	0.041	0.86
	Densitometer readings plus grain counts	$y = 0.0032 + 0.0396 \cdot x$	25.3	0.081	0.84

[a] Correlation coefficient.

Table 2. *The effect of inhibitors on SO_4^{--} uptake into hydropotes and epidermal cells at 25 °C for two different SO_4^{--} concentrations in the external solution*[a]

Inhibitor	$K_2\,^{35}SO_4$			
	0.05 mM		3 mM	
	Hydropotes	Epidermis	Hydropotes	Epidermis
Control	1.25 ± 0.07	0.69 ± 0.03	5.52 ± 0.43	2.15 ± 0.17
2×10^{-4} M KCN	1.16 ± 0.07	0.58 ± 0.01	3.96 ± 0.38	1.59 ± 0.17
10^{-5} M Cl–CCP[b]	0.73 ± 0.04	0.35 ± 0.02	3.31 ± 0.31	1.53 ± 0.18

[a] The uptake period was 3 hr. The figures shown are relative rates, i.e., $F_d \cdot \mu M \cdot hr^{-1}$. Errors given are standard deviations of the mean, where n was taken as the number of sections on each of which *numerous* densitometer readings were taken. After treatment with 1 mM NaN_3 and at 0 °C (no inhibitor, respectively), radioactivity in the tissue was too small for autoradiographic detection. The leaf was harvested in July.

[b] *m*-chloro-carbonylcyanide-phenyl-hydrazone.

Table 3. *Amount of radioactivity incorporated from ^{35}S-labelled solutions into the TCA-insoluble fraction of Nymphaea leaf epidermis shown as % of the total radioactivity taken up. The leaf used was harvested in September*

Temp.	% activity incorporated			
°C	3-hr uptake		16-hr uptake	
	ext. concn.		ext. concn.	
	0.2 mM	5.0 mM	0.2 mM	5.0 mM
3	6	11	–	–
25	7	8	12	10

Table 3 shows the amount of ^{35}S incorporated under various treatments into the trichloroacetic acid (TCA)-insoluble fraction of *Nymphaea* leaf epidermis. Epidermal sections were labelled as for the autoradiographic experiments; free space was exchanged, and the sections were then extracted with boiling TCA. The figures shown in Table 2 give the radioactivity found in the TCA-insoluble fraction as % of the total activity taken up. It can be seen that this fraction is never more than 12%.

The effects of poly-L-lysine are shown in Tables 4 and 5 and in Fig. 3. Results from individual densitometer readings were calculated according to Eq. (1) and analyzed statistically. The mean *amounts* of SO_4^{--} taken up into hydropotes and epidermal cells in relative units are listed for each

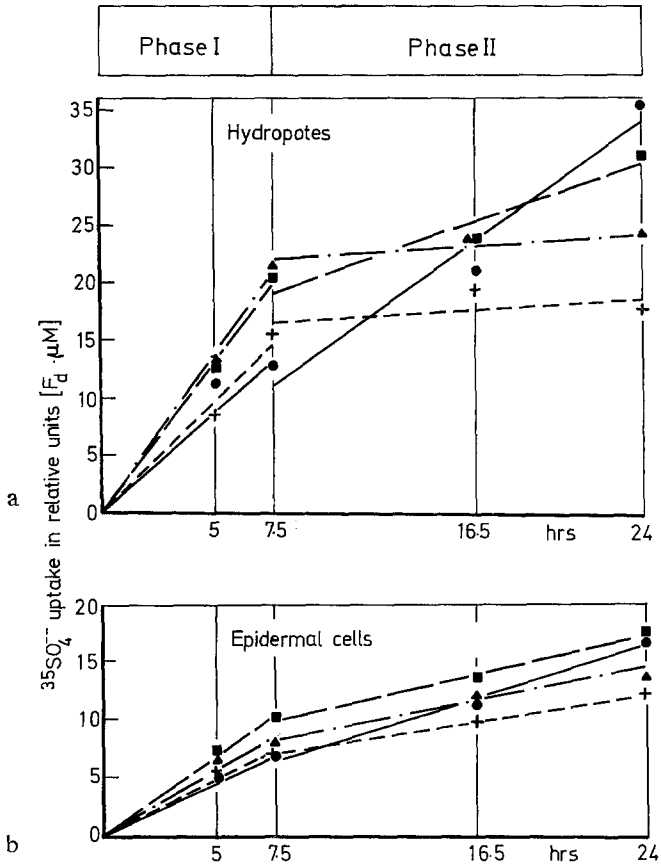

Fig. 3. Time course of $^{35}SO_4^{--}$ uptake from 0.1 mM K_2SO_4 into hydropotes (a) and epidermal cells (b) at 3 °C for various concentrations of poly-L-lysine. The leaf was harvested in September. The symbols indicate the means shown in Table 4.
•——• controls; ▪— — —▪ 10^{-8} M, ▲—·—·▲ 10^{-7} M, +------+ 5×10^{-7} M poly-L-lysine

treatment in Table 4. For the purpose of comparing the effect of "short-term" and "long-term" treatment with poly-L-lysine on the relative rates of SO_4^{--} uptake at various poly-L-lysine concentrations, the data were treated in the following manner. The rates of SO_4^{--} uptake were approximated to be linear between 0 and 7.5 hr and between 7.5 and 24 hr. The regression lines shown in Fig. 3 are based on this assumption. Table 5 shows a comparison of the slopes (i.e., relative rates of uptake in the presence of various concentrations of poly-L-lysine) with the appropriate controls.

It can be seen that in the first part of the time course (0 to 7.5 hr, phase I) low concentrations of poly-L-lysine (10^{-8} to 10^{-7} M) significantly increase SO_4^{--} uptake into both hydropotes and epidermal cells. Higher poly-L-lysine concentrations reduce this increment in such a way that treatments

Table 4. *The effect of poly-L-lysine on SO_4^{--} uptake into the hydropotes and epidermal cells. Statistical analysis of individual densitometer readings. The leaf was harvested in September*

Cells	Poly-L-lysine concn. (M)	Time (hr)	$^{35}SO_4^{--}$ uptake			
			mean	S.D.	S.D.M.	No. of readings
			($F_d \cdot \mu M$)			
Hydropotes	Control	5.0	11.41	3.52	0.65	29
		7.5	12.64	2.80	0.40	49
		16.5	21.12	4.73	0.59	66
		24.0	35.54	7.97	1.10	59
	10^{-8}	5.0	12.59	3.00	0.60	26
		7.5	20.18	4.23	0.55	56
		16.5	23.75	5.61	0.60	88
		24.0	30.91	5.93	0.65	92
	10^{-7}	5.0	12.82	6.09	0.92	43
		7.5	21.59	7.90	1.20	43
		16.5	23.95	4.20	0.60	50
		24.0	24.05	3.71	0.45	74
	5×10^{-7}	5.0	8.02	1.53	0.32	23
		7.5	15.14	2.20	0.32	48
		16.5	19.56	9.57	1.10	68
		24.0	17.59	3.67	0.49	77
Epidermal	Control	5.0	5.05	1.58	0.17	27
		7.5	6.91	1.67	0.24	49
		16.5	11.25	2.43	0.30	65
		24.0	16.98	2.88	0.37	59
	10^{-8}	5.0	7.04	1.81	0.35	27
		7.5	10.28	1.93	0.25	56
		16.5	13.55	2.47	0.27	81
		24.0	17.55	3.63	0.36	108
	10^{-7}	5.0	6.53	2.33	0.38	42
		7.5	8.06	1.76	0.27	42
		16.5	12.29	2.55	0.36	50
		24.0	14.29	2.07	0.23	77
	5×10^{-7}	5.0	5.30	1.14	0.24	23
		7.5	7.06	1.25	0.18	48
		16.5	9.78	1.98	0.25	66
		24.0	12.33	2.39	0.28	71

with 5×10^{-7} M poly-L-lysine are not significantly different from the controls. In the second part of the time course (7.5 to 24 hr, phase II), raised poly-L-lysine concentrations (10^{-8} to 5×10^{-7} M) increasingly inhibit SO_4^{--} uptake.

Table 5. *Slopes of the lines shown in Fig. 2 and statistical comparison of slopes in th presence of poly-L-lysine with the appropriate controls*

Cell	Poly-L-lysine concn. (M)	Time (hr)	Slope[a] ($F_d \cdot \mu M \cdot hr^{-1}$)	t[b]	n[c]	p[b]
Hydropotes	0	0–7.5	1.809	–	78	–
	10^{-8}	Phase I	2.660	10.53	82	<0.001
	10^{-7}		2.780	7.56	86	<0.001
	$5 \cdot 10^{-7}$		1.940	2.20	71	0.05
	0	7.5–24	1.388	–	174	–
	10^{-8}	Phase II	0.667	8.05	236	<0.001
	10^{-7}		0.142	13.56	167	<0.001
	$5 \cdot 10^{-7}$		0.129	12.71	193	<0.001
Epidermal	0	0–7.5	0.938	–	76	–
	10^{-8}	Phase I	1.377	10.74	83	<0.001
	10^{-7}		1.146	4.67	84	<0.001
	$5 \cdot 10^{-7}$		0.962	0.68	71	0.5
	0	7.5–24	0.612	–	173	–
	10^{-8}	Phase II	0.445	0.94	245	0.4
	10^{-7}		0.376	2.95	169	0.01
	$5 \cdot 10^{-7}$		0.324	3.61	185	0.001

[a] The slopes reflect relative rates of uptake.

[b] t and probability values (p) from t-tests. $p < 0.001$ is considered to be highly significant.

[c] Total number of densitometer readings, i.e. number of readings taken at 5 and 7.5 hr (phase I) and at 7.5, 16.5 and 24 hr (phase II), respectively.

Discussion

The results show that at external SO_4^{--} concentrations between 0 and 0.5 mM SO_4^{--} uptake into hydropotes and epidermal cells behaves typically like cation and anion uptake in many other plant tissues: (1) it follows a hyperbolic isotherm which in this concentration range would be expected for both vacuolated and non-vacuolated cells (Torii & Laties, 1966); (2) it is sensitive to temperature and depends on metabolic activity, both uncouplers and inhibitors of electron flow being inhibitory; and (3) the accumulated ^{35}S is ionic in form and is not assimilated into organic macromolecules, since an incorporation of approximately 10% of the total radioactivity into the TCA-insoluble fraction lies within the margin of error of most of the autoradiographic measurements.

The time course of SO_4^{--} uptake into the hydropotes and epidermal cells shows an apparent leveling off after about 8 hr for most experiments

(Figs. 2 and 3). In the poly-L-lysine experiments, the rates of $^{35}SO_4^{--}$ uptake by the controls (i.e. in the absence of poly-L-lysine) are slightly smaller in phase II of the time course (7.5 to 24 hr) than in phase I (0 to 7.5 hr). This can be readily explained by tracer efflux. Initially the rate of uptake of labelled $^{35}SO_4^{--}$ will reflect largely influx alone, but as the specific activity continues to build up in the various compartments of the cells, the efflux component will become increasingly important.

The apparent opposite effects of poly-L-lysine on SO_4^{--} uptake in phases I and II are more difficult to explain. Using the simple model of a plant cell where an inward anion pump is the only driving force for the accumulation of ions (Briggs, 1963), it can be shown that an increase in the membrane permeability to anions also reduces the ion content of the cell. Thus the marked reduction in $^{35}SO_4^{--}$ uptake by poly-L-lysine applied for longer periods (phase II) would be consistent with a high-permeability low-salt status of the tissue in the presence of poly-L-lysine, which has similarly been suggested for K^+ distribution in red beet cells (Osmond & Laties, 1970). At the low temperature (3 °C) used in these experiments, the increase of $^{35}SO_4^{--}$ uptake in phase I by low poly-L-lysine concentrations would also be consistent with an effect of poly-L-lysine on membrane permeability. If the passive component of SO_4^{--} influx is sufficiently large in comparison to the active component of SO_4^{--} influx at this temperature, an increased membrane permeability will increase the initial rate of apparent tracer uptake.

However, the observation that 5×10^{-7} M poly-L-lysine shows no enhancement of $^{35}SO_4^{--}$ uptake during phase I is unexpected if the action of poly-L-lysine were to simply increase the ease of diffusion of ions across the outer membranes. This suggests that there is also an effect of poly-L-lysine on cell metabolism driving SO_4^{--} uptake. This may be an effect of poly-L-lysine on mitochondria. The electron micrograph of a hydropote cell from a leaf sampled in July (Fig. 4b) shows mitochondria occupying almost all the space left by the cell wall protuberances, the white areas being caused by shrinkage during tissue preparation. This obervation together with the fact that poly-L-lysine has been shown to affect characteristics of mitochondrial membranes (Johnson *et al.*, 1967; Schwartz, 1965) makes it likely that the poly-L-lysine effects observed are also somehow related to mitochondrial activities.

The data have shown that SO_4^{--} influx into both hydropotes and epidermal cells is metabolic. Although the rate of SO_4^{--} uptake in the hydropotes is about twice as high as that found for epidermal cells, the characteristics of SO_4^{--} uptake are essentially identical for both cell types.

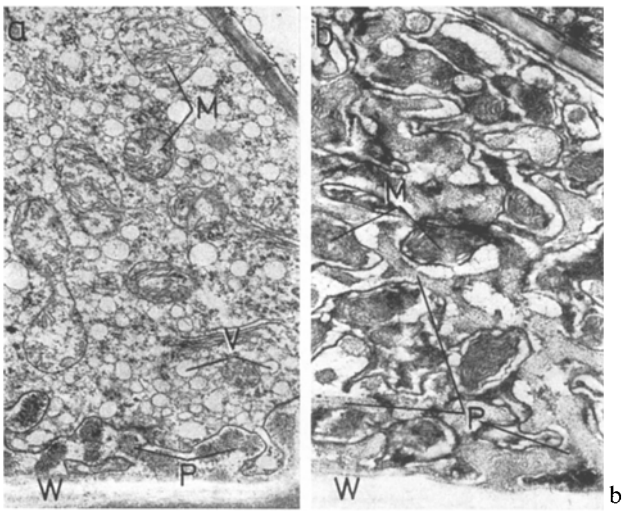

Fig. 4a and b. Electron micrographs of the hydropote gland cytoplasm. (a) Fixation in April; (b) fixation in July. It can be seen that cell wall protuberances develop during the summer to form an extensive labyrinth. Mitochondria are extremely numerous in the July specimen. For further details, *see* Lüttge and Krapf (1969). Fixation with 1% OsO_4 (Palade); embedding in vestopal; staining with lead citrate (Reynolds, 1963); sectioning with a LKB-ultrotome; microscopy with a Zeiss EM 9. ×13,000. *M* mitochondria, *V* small cytoplasmic vesicles, *W* cell wall, *P* cell wall protuberances. Preparations and electron microscopy by Gisela Krapf

The results point to an effect of poly-L-lysine on cell metabolism as well as on membrane permeability. Our findings have demonstrated that poly-L-lysine affects the anionic as well as the cationic (Osmond & Laties, 1970) distribution in plant tissues.

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