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Ulrich Lüttge
Francisco M. Cánovas
Rainer Matyssek *Editors*

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Ulrich Lüttge

Part I
Review

Transport Processes: The Key Integrators in Plant Biology

Ulrich Lüttge

Contents

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Abstract In the 57 years of research reviewed in this essay, transport functions were studied in a variety of plant systems. Processes of membrane transport are essential in the operation of various glands, such as nectary glands, the glands of carnivorous plants, and the salt glands of halophytes. In the photosynthetic mode of crassulacean acid metabolism (CAM), a central feature is nocturnal accumulation of organic acids in the vacuoles. Thus, CAM poses a transport problem, which was resolved by the identification of the complement of an H^+-transporting ATPase, a malate channel, and a passive diffusion of non-dissociated malic acid at the tonoplast. The free running endogenous rhythm of CAM is operated by a biochemical-biophysical oscillator where the tonoplast acts as a master switch.

The paths of transport with apoplastic and symplastic transport and diffusion in the gas phase of aerenchymas couple and integrate cells within tissues. The energization of membrane transport is linked to the multicomponent network of energy metabolism. Transport in roots and leaves was investigated to show this.

All these features of plant biology indirectly or directly bear relations to physiological ecology of field performance. CAM is an ecophysiological adaptation to limited water supply which was studied intensively in the field in various tropical environments with respect to physiological autecology and ecosystem-relevant synecology.

Whole-plant physiology shows that transport is the basis of the functioning of entire plants. Transport is the pathway for interaction and integration creating plant's individuality as unitary organisms. The integration of modules via transport leads to the emergence of holistic systems across a large range of scalar levels from compartments within cells to cells and eventually the whole biosphere. Comprehending emergence of holism leads to understanding life beyond mechanistic modularity.

1 The Scene of Transport in Plants in the 1950s

Transport in plants: How did the landscape of research look in the 1950s? First, there were the membranes, barriers to, and paths of *short-distance transport*. Two alternative views were dominated by the lipid-permeability theory for permeation

of lipid membranes by Overton (1899) and by the ultrafilter theory for permeation of porous membranes by Ruhland (1912; Ruhland and Hoffmann 1925), respectively. Both theories were combined within the lipid-filter theory (Wartiovaara and Collander 1960). Second, there were connections for *medium-distance transport* especially in the symplast of tissues. The symplastic transport was intensely studied by Arisz (1956, 1960). Third, there were the avenues of *long-distance transport* in the xylem and phloem. Two researchers covered this in the Institute of Forstbotanik (Forest Botany) of the Ludwig Maximilian University of Munich. Bruno Huber was advancing the cohesion–tension theory of xylem sap flow, applying the heat pulse method for measurements of velocity and derivation of flow. This theory was challenged time and again (Ziegler et al. 2009). Hubert Ziegler was dedicated to transport in the phloem (Ziegler 1956) based on the pressure flow theory of Münch (1930).

I started my experimental work in 1957 in this Institute of Forstbotanik. At that time laboratories studying transport in plants were not very numerous. Even in the 1960s, it proved easy to assemble all relevant books on the topic on one's personal small book shelve, namely, Briggs, Hope, and Robertson (1961), Sutcliffe (1962), Jennings (1963), and Robertson (1968). It is hard to imagine today that at the end of the 1970s, it appeared still possible to publish a book covering the entire scope of transport in plants (Lüttge and Higinbotham 1979). Since then the field exploded, currently identifying a vast number of families of membrane transporters at the molecular level, such as ATPases, carriers, channels, and porins. The last decades of the twentieth century and the first decades of the twenty-first century might be named an era of transport physiology.

In this essay, I shall try to develop the progress of my interests in transport physiology within context of knowledge and not stringently chronologically. Reviewing the various topics in such a way, I shall try to provide the links to the current states of research. However, space does not allow developing this in depth. Rather, the essay will essentially remain a historical treatise. The integrating power of transport overcoming borders and barriers allowed advancing from the platform of transport studies to physiological ecology of tropical plants and consideration of emergence at higher scalar levels. Much chance, serendipity, guidance by excellent teachers, stimulation by admirable peers and friends, and achievements of wonderful dedicated coworkers and students were involved. All of these were exceptional presents that now allow looking back with gratitude on what could be completed.

2 Glands, Salt Hairs, and Epidermal Bladders

2.1 Nectaries

The great question in Hubert Ziegler's reflections on phloem transport was the function of the companion cells. They were supposed to secrete sugars into the

sieve tubes for their long-distance transport. However, inside the phloem tissue, the companion cells were not accessible for direct experimental studies (Ziegler 1956). As an analogy, Hubert Ziegler had made acquaintance with nectar glands, another example of sugar-secreting cells, during a stay in the laboratory of Albert Frey-Wyssling in Zürich in 1956/1957. Hence, when I joined him in 1957 as his first Ph. D. student, he asked me to work on nectaries.

I used all the available analytical techniques for analyzing the chemical composition of nectar from various species, with the profile of sugars mainly of sucrose, and glucose and fructose mostly in stoichiometrically equal amounts, and some oligosaccharides, carbonic acids, amino acids, mineral ions, protein, and vitamins (Lüttge 1961, 1962a; Ziegler et al. 1964). At that time our major interest in these analyses was to assess the array of solutes that are transported. Subsequently, others continued the chemical analyses revealing the rich chemical composition of nectar including secondary metabolites such as alkaloids (Kessler and Baldwin 2007; Manson et al. 2010). The sugar and amino acid spectra of nectars are of ecological relevance particularly with respect to mutualism of plants and the preferences of specific pollinators (Baker and Baker 1977; Corbet et al. 1979; Alm et al. 1990; Erhardt 1992; Schmidt-Lebhuhn et al. 2007; Nepi et al. 2012). Proteins and enzymology of nectars are studied to understand the metabolic activity of the secretion. A redox cycle has been shown to be active with redox compounds and reactive oxygen species such as H_2O_2 and ascorbate for the control of microbial contamination (Carter and Thornburg 2000, 2004a, b; Carter et al. 2007; Horner et al. 2007; González-Teuber et al. 2010; Hillwig et al. 2010, 2011; Escalante-Pérez and Heil 2012). Extrusion of vesicles [“granulocrinous” secretion *sensu* Fahn (1979) and Schnepf (Schnepf and Christ 1980)] might be the mechanism of secretion of macromolecules such as proteins.

For me the major challenge of studying gland functions remained that of transport physiology. The established task of the companion cells was to control the composition of the transported sieve tube sap. This would involve both secretion and reabsorption of compounds. Just like the phloem-sap, the chemical composition of nectar is rich in qualitative terms considering the diversity of compounds. However, quantitatively, the sugars are absolutely dominant. Thus, there is specificity in the secretion. I could show that the degree of specificity regarding the dominance of sugars and the low concentrations of noncarbohydrate solutes in the nectar was related to the degree of the anatomic specialization of the glandular tissue (Lüttge 1961). Transport processes could create the specificity by specific secretion or reabsorption. Active transport and specific transporters, explicitly carrier proteins, were thought to be involved, but at the time not any transporter had been characterized in plants, and the molecular basis remained pure speculation. Respiratory activity of nectaries is usually high (Lüttge and Schnepf 1976) and can drive energy-demanding transport processes. Many nectaries are green, and their photosynthetic capacity can provide energy as well as part of the carbohydrates secreted (Lüttge 2013a).

A central question was if nectaries do not only secrete but also reabsorb solutes and if different transport processes can operate simultaneously in opposite

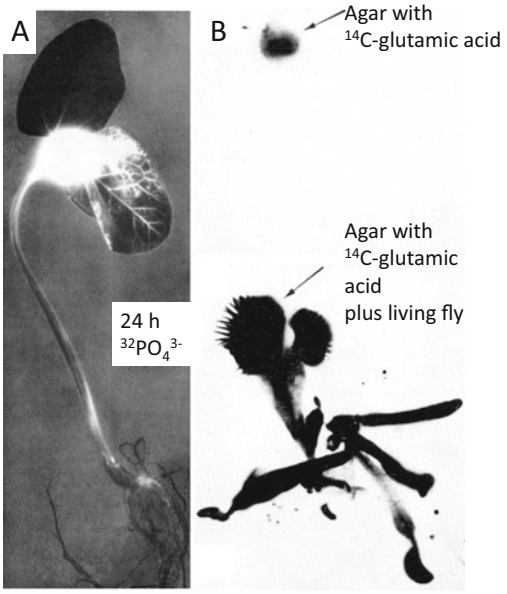

Fig. 1 Autoradiographs demonstrating resorption by glands. (**a**) A droplet of $^{32}PO_4^{3-}$ solution was placed on the extrafloral nectary of the left cotyledon of this seedling of *Ricinus communis* L. (Lüttge 1961). (**b**) A trap of *Dionaea muscipula* Ellis was fed (*arrows*) with ^{14}C-glutamic acid in dry agar (*top*) and with a living fly in addition to the labeled agar (*bottom*) (Lüttge 1963). Note that (**a**) is reproduced as a negative and (**b**) as a positive of the X-ray film, so that radioactivity appears *white* and *black*, respectively

directions. It proved useful to develop imaging techniques. The labeling technique of the time was that with radioactive isotopes. I applied radioactively labeled solutes to the nectaries and after some time of incubation pressed and freeze-dried the whole plants and covered them with X-ray film. In this way uptake of solutes by the nectaries and translocation in the whole plant could be demonstrated (Fig. 1a; Ziegler and Lüttge 1959; Lüttge 1962b). This contributed to explaining the specific chemical composition of nectar. At the end of the pollination period, entire surplus nectar may also be reabsorbed for economy of the plant's resources (Búrquez and Corbet 1991; Nepi and Stpiczyńska 2007). Thus, the studies on nectar secretion exerted some feedback on the reflections about companion cell functions in the phloem.

The refinement of autoradiography to make the anatomical level accessible then became a major occupation. It was essential to develop procedures preventing the artificial redistribution of water-soluble compounds during preparation for microscopic inspection. The task was tedious but highly rewarding for many further studies. The developed silver grains in the film of the micro-autoradiographs allowed localization at the cellular and subcellular level. Quantification was possible by densitometry or measurement of the reflection of incident illumination by the silver grains. The most precise way although extremely wearisome proved to be grid-based counting of silver grains under the microscope. It was proven that absorption indeed is by the nectary gland tissue (Lüttge 1962b). A book on methodology emerged from this work (Lüttge 1972). I met André Läuchli who had similar aims of cellular solute localizations and was engaged in X-ray microanalysis. A long friendship emerged, and André came in 1972 for 6 years as professor to our department in Darmstadt.

2.2 *Carnivorous Plants*

The glands of carnivorous plants fulfill a number of different functions. They secrete digestive enzymes and serve absorption of low molecular compounds from the digested prey. They secrete mucilage where this is involved in the capture of prey. In the pitchers of *Nepenthes*, they secrete chloride for the acidification of the pitcher fluid with hydrochloric acid (Fig. 2b; Lüttge 1966a, b). Hence, in

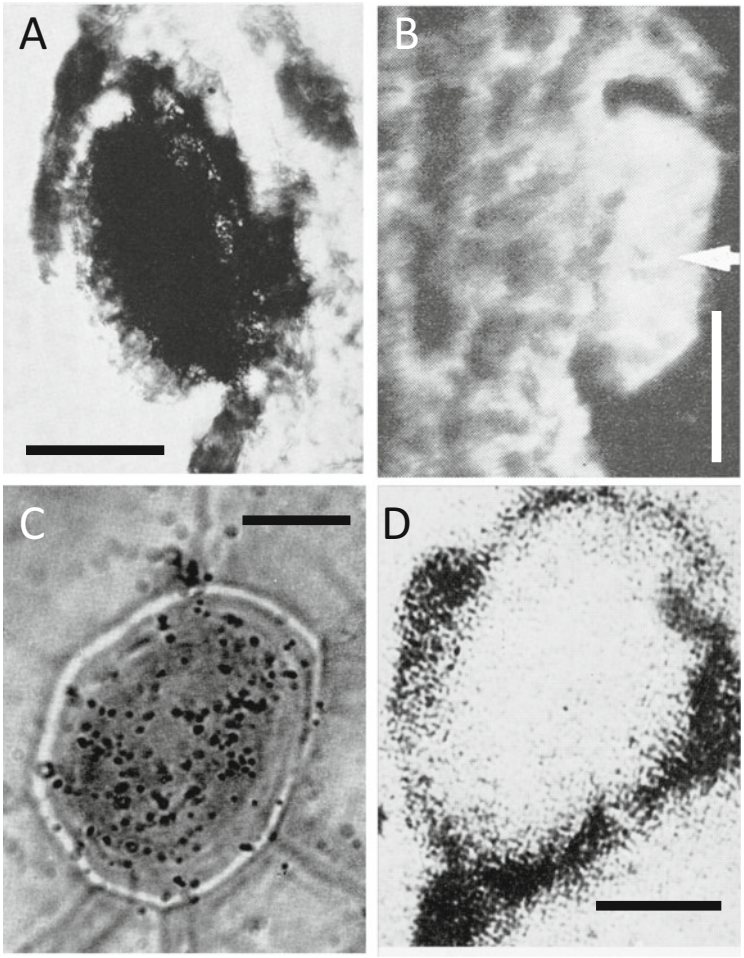

Fig. 2 Secretion and absorption by glands shown by micro-autoradiography. (**a**) Gland of *Nepenthes* where the pitcher was fed with $^{35}SO_4^{2-}$. Scale bar: 50 µm (Lüttge 1965). (**b**) Gland of *Nepenthes* secreting $^{36}Cl^-$ (*arrow*) fed to the pitcher wall tissue. *Scale bar*: 50 µm (Lüttge 1966b). (**c**) Uptake of $^{35}SO_4^{2-}$ by a hydropote gland of *Nymphaea*. *Scale bar*: 10 µm (Lüttge 1964b). (**d**) Stalk and bladder cells of *Atriplex spongiosa* James whose leaves were supplied with $^{35}SO_4^{2-}$. *Scale bar*: 25 µm (Osmond et al. 1969, copyright http://www.publish.csiro.au/nid/280/paper/BI9690797.htm). Note that in transmitted light, the silver grains of the micro-autoradiographs are *black* (**a**, **c**, **d**) and in incident light they are *bright white* (**b**)

carnivory, it appeared clear at the outset that glands would perform transport in two opposite directions.

The two lobes of the leaves of Venus' fly trap (*Dionaea muscipula* Ellis) close by a thigmonastic turgor movement when one of the six trigger hairs (three on each lobe) is touched twice or two different hairs are touched once. With each touching, an action potential is triggered and the two action potentials in series elicit trap closure. However, a chemical signal is required for a firm closure of the trap by a chemonastic growth movement (Fig. 37.7 in Lüttge et al. 2010) and for the induction of gland activity. Placing a dead fly, some dry cheese, or agar into the trap, this latter movement does not occur, and upon mechanic stimulus only the trap soon reopens. However, a living fly excreting fluid while struggling for life, a piece of moist meat, or a piece of moist cheese will elicit the chemonastic movement and also gland activity for absorption, e.g., of amino acids, as I could show by autoradiography (Fig. 1b; Lüttge 1963, 1965). Micro-autoradiography demonstrated the role of the pitcher glands of *Nepenthes* for absorption (Fig. 2a) which is specific. Proportional rates of absorption from 1 mM solutions in the pitcher of L-alanine/phosphate/sulfate were 1 : 0.4 : 0.1 (Lüttge 1965). Both specific transporters, such as channels and carriers, and vesicle endocytosis can be involved in absorption (Adlassnig et al. 2012).

The pitcher glands secrete digestive fluid before the lid of the pitcher opens. The fluid in the closed pitchers is microbiologically sterile, i.e., not contaminated. It contains proteinase activity which is of genuine origin of secretion by the plants (Lüttge 1964a). The proteinase processed to electrophoretic homogeneity has a pH optimum close to pH 2.2 (Steckelberg et al. 1967). The pH of open pitchers with prey was recorded at around pH 3.5 (Lüttge 1964a). The plasma membrane H^+-ATPase appears to be responsible for such acidification (An et al. 2001). The pitcher glands secrete chloride as shown by micro-autoradiography (Fig. 2b; Lüttge 1966b). Chloride secretion is inhibited by cyanide and arsenate. The chloride concentration of the fluid of still closed pitchers was about 30 mM on average, ranging up to about 65 mM (Lüttge 1966a). Hence, it is HCl that acidifies the pitcher fluid. The proteinase has biochemical properties akin to the pepsin of our own stomach. This was subsequently detailed by determination of amino acid sequences of the enzyme protein now named nepenthesin (Jentsch 1972; Athauda et al. 2004; Takahashi and Tanji 2007). Hydrochloric acid also acidifies the digestive fluid of *D. muscipula* (Rea 1983; Rea et al. 1983). Unfortunately space does not allow this essay to further pursue the fascinating research on carnivorous plants, "the most wonderful plants in the world" (Król et al. 2012).

2.3 Salt Glands and Salt Hairs

2.3.1 Salt-Excreting and Salt-Absorbing Glands

In many halophytes, the adaptation to salinity is based on the transport function of very specific structures, namely, salt glands excreting sodium chloride (Lüttge

1975). With the micro-autoradiographic imaging technique, chloride excretion by the salt glands of *Limonium vulgare* Miller was depicted (Ziegler and Lüttge 1967). For the hydropote glands on the lower, i.e., submerged, surface of the floating leaves of water lily (*Nymphaea*), this imaging showed that their function is salt uptake (Fig. 2c; Lüttge 1964b).

2.3.2 Salt Bladder Hairs of *Atriplex spongiosa* (James)

My major occupation with gland-type salt transport, however, arose from some serendipity. In 1965/1966 in the laboratory of George G. Laties at the University of California, Los Angeles, I had shared benches with C. Barry Osmond as postdocs. Barry spent another year with Tom ap Rees in Cambridge, UK, while I had returned to Darmstadt in September 1966. Barry visited me there for a few weeks on his way back to Australia (September–October 1967). Barry had been irreversibly infected with the C_4-fever after this new mode of photosynthesis had been discovered by Kortschak et al. (1965) and Hatch and Slack (1966). With this he also had fallen in love with *Atriplex spongiosa* James, not so much because of its nature as a halophyte, but since it was the C_4 partner of a couple together with *Atriplex hastata* L. serving as the C_3 partner for interspecific comparisons of the two modes of photosynthesis. *A. spongiosa* has epidermal salt hairs composed of a stalk cell and a large bladder cell. My interests in salt transport given, during Barry's visit in Darmstadt, we performed some micro-autoradiographs. The stalk cells are densely filled with cytoplasm, and like salt gland cells they function in secretion of salt into the large vacuoles of the bladder cells (Fig. 2d; Osmond et al. 1969).

Together with Ralph O. Slatyer, Barry invited me to come for an entire year to the just founded Research School of Biological Sciences at the Australian National University in Canberra (August 1968–July 1969). Our interests in photosynthesis and salt transport were combined to fathom the energetics of salt concentrating in the bladder cells of *A. spongiosa* (Osmond et al. 1969; Lüttge and Osmond 1970). Chloride accumulation in the bladder vacuoles is strongly stimulated by light. However, the bladder, stalk, and epidermis cells are not photosynthetically active. In plants grown on 0.25 M NaCl, the accumulation of NaCl in bladders is about 4–5 times higher than in the green lamina (Table 1). Hence, energy captured by photosynthetic light absorption in the lamina is able to energize active salt export by the distant stalk cells and accumulation in the bladder vacuoles (Lüttge and

Table 1 Light dependence of NaCl accumulation in salt bladders of *A. spongiosa* plants grown with 0.25 M NaCl solution (Osmond et al. 1969)

	Bladders	Green lamina
Photosynthetic CO_2 fixation mol $g_{FW}^{-1}\,h^{-1}$	0.2	10.6
Light stimulation of Cl^- accumulation	4.7×	1.9×
Na^+ concentration (M)	1.02	0.28
Cl^- concentration (M)	0.72	0.13

Osmond 1970). In this way, the salt load on the metabolically active lamina tissue is highly reduced.

2.4 *Giant Epidermal Bladders:* Mesembryanthemum crystallinum *L. and* Capsicum annuum *L.*

The work on *A. spongiosa* led to a serendipity which marked the starting point of a significant revolution in plant stress physiology with now of a global dimension. In 1969 on the way back from Australia, I made a stopover in California, where I also was invited to visit the laboratory of Andy Benson in La Jolla. I assembled all my courage and all I knew about photosynthesis and lipids to meet the great man. However, at dinner in a Mexican restaurant, he asked me what I was really interested in, and I told him the *Atriplex* story. Andy immediately got excited, and in the darkness of this evening of 31 July 1969 he drove me to the beach and came up with collecting samples of *Mesembryanthemum crystallinum* L. There are really large huge epidermal bladder cells on the leaves and stems with a volume of up to 2 mm^3, and I should work with these. I shall continue telling the anecdote and its consequences below (Sect. 6.1.2) when it comes to talk about crassulacean acid metabolism.

Seeds of *M. crystallinum* were taken back to Darmstadt. Plants were grown, and a student was asked to check if the bladders accumulated salt. Although other authors later reported and believed that they did, we did not find NaCl concentrations in bladder cell sap higher than in the leaf mesophyll when plants were grown with watering by NaCl solutions of up to 500 mM (Lüttge et al. 1978). The bladder cells of *M. crystallinum* were already described by Haberlandt (1904) as peripheral water storage cells. In the leaves, they may sequester salt due to their sheer size, but they do not concentrate it. By contrast to the salt hairs of *Atriplex*, they are just inflated epidermal cells and there is no gland like cell underneath. The water storage capacity of the bladder cells of *M. crystallinum* is large. Biophysical studies showed how their cellular water transport dynamically integrates and stabilizes water relations of the leaves (Steudle et al. 1975, 1977; Lüttge et al. 1978; Rygol et al. 1986, 1989).

Giant cells with large cell sap vacuoles are an attribute of plant succulence. In a subepidermal cell layer of the inner pericarp wall of *Capsicum annuum* L., the cells have a similarly large volume as the bladder cells of *M. crystallinum*, namely, 0.5 mm^3 on average and up to 1.7 mm^3. The water relation parameters of the large *C. annuum* cells, i.e., higher hydraulic conductivity, L_p, and volumetric cell wall elasticity modulus, ε, and shorter half-life of water exchange as compared to the much smaller mesocarp parenchyma cells, led us to an ecophysiological interpretation of the biophysics of succulence in relation to water storage and remobilization (Rygol and Lüttge 1983).

3 Paths of Transport: Coupling and Integration Within Tissues

For the transport within tissues, there are three different types of pathways. Solutes in aqueous media can be transported in the apoplastic space of the cell walls and in the symplasm, i.e., outside the plasma membranes and within the cytoplasm, respectively. The third way is diffusion of gaseous substances in the gas phase within intercellular spaces, in particular, of leaf aerenchyma.

3.1 Apoplastic and Symplastic Transport

With respect to the entry of solutes into plant tissues, the apoplastic space was termed "apparent free space" (Briggs et al. 1961) because it appears to be freely accessible without metabolic control over the uptake into and the transport within this space. Conversely uptake across the plasma membrane is required before transport in the symplasm can occur. For his studies of symplastic transport, the authoritative investigator Arisz (1956, 1960) used the long band-like submerged leaves of *Vallisneria*.

Our micro-autoradiographic imaging contributed to visualizing the transport pathways. Frequently one sees that the cell borders are particularly labeled when radioactive-labeled solutes are transported. In some cases, the resolution was not high enough to distinguish apoplast versus cytoplasm as it was the case in the nectar glands of *Heracleum sphondylium* L. (Fig. 3a) and the root cortex of *Zea mays* L. (Fig. 3d). Transport in nectary tissues potentially occurs both in the apoplastic and the symplastic space (Sawidis 1991; Vassilyev 2010). In the bladder hairs of *A. spongiosa*, distinctively the dense cytoplasm of the stalk cells and the bladder cytoplasm were preferentially labeled (Fig. 2d). In the mesophyll and gland cells of *Nepenthes*, labeling of the cytoplasm and, hence, the symplastic pathway also became evident (Fig. 3b, c).

The transport from the external medium across the root can take place both apoplastically and symplastically in the cortex up to the endodermis which constitutes a barrier for apoplastic transport. Using micro-autoradiography, we found that entry of labeled sulfate into the stele was blocked at the endodermis by the metabolic inhibitor azide (Weigl and Lüttge 1962). This is the final point where transported solutes must undergo metabolically controlled transport from the apoplast into the symplast across the plasma membrane to move further towards the xylem vessels of the stele. In the tertiary endodermis, only the living passage cells allow transport between the cortex and stele of roots (Fig. 3e). Later Ernst Steudle and his collaborators devoted a large and impressive body of work to the elucidation of these pathways for the transport of ions and water, and they developed the "composite model" of transport across the root (Steudle 2011).

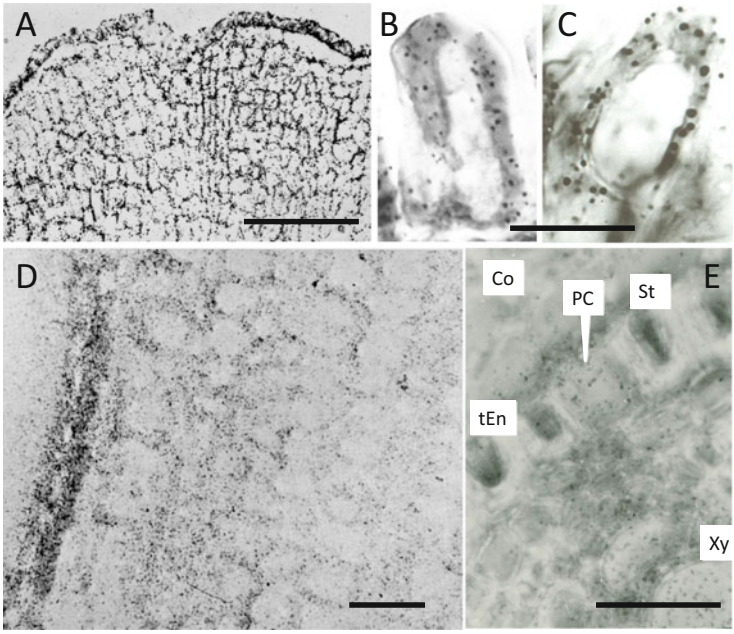

Fig. 3 Micro-autoradiographic imaging of transport in tissues. (**a**) Nectary of *Heracleum sphondylium* L. after resorption of $^{35}SO_4^{2-}$ (Lüttge 1962a). (**b**) Gland cell and (**c**) mesophyll cell of the pitcher wall of *Nepenthes* secreting $^{36}Cl^-$ (Lüttge 1966b). (**d**) Maize root cortex after uptake of $^{35}SO_4^{2-}$ (Weigl and Lüttge 1962; Lüttge and Weigl 1965). (**e**) Root of *Iris pumila* L. to which $^{35}SO_4^{2-}$ was administered via the transpiration stream in the stele. Co cortex, PC passage cell, tEn tertiary endodermis, St stele, Xy xylem (Ziegler et al. 1963). *Scale bars*: (**a**, **d**) 100 µm, (**b**, **c**), 25 µm, (**e**) 50 µm

3.2 Cell Coupling

Integration of cells in tissues requires their coupling. The essential structural basis of such coupling is the plasmodesmata, i.e., tubing-like bridges of endoplasmic-reticulum membranes, between the cytoplasm of adjacent cells (Spanswick 1976), resulting in a symplastic network of connected cells, i.e., the symplasm. By precipitating Cl^- ions with Ag^+ ions to obtain electron-dense AgCl particles, we visualized ion transport through plasmodesmata (Fig. 4).

The continuity of plasma membranes across the plasmodesmata allows electrical coupling. This was studied by Spanswick and collaborators (Spanswick 1976). Our own contributions made use of the phenomenon of transient changes of membrane electric potential in green cells when light is switched on or off (see below Sect. 4.3.2). This signal depends on photosynthesis and is not produced in nongreen cells. However, we could pick up such signals with microelectrodes in the nongreen bladder cells of *A. spongiosa* and *Chenopodium album* L. (Lüttge and Pallaghy 1969; Osmond et al. 1969; Pallaghy and Lüttge 1970) and in the nongreen cells of variegated leaves of *Oenothera* (Brinckmann and Lüttge 1974) demonstrating their electrotonic coupling to adjacent green mesophyll cells.

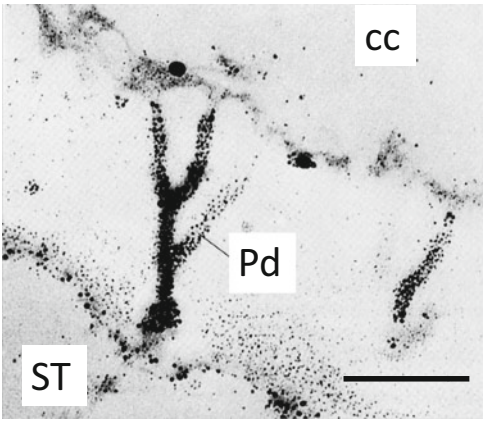

Fig. 4 Plasmodesmata (Pd) between a companion cell (CC) and a sieve tube (ST) in the phloem of a leaf of *Limonium vulgare* Miller transporting Cl^-. *Scale bar*: 250 nm. Cl^- was precipitated by addition of Ag-acetate to the electron-microscopic fixation medium; the *black dots* are precipitates of AgCl (Ziegler and Lüttge 1967)

3.3 The Gas Phase: Integrating Cells in Leaves with CAM

Our interests in the third pathway, i.e., lateral diffusion in the gas phase of leaves, arose later when we were involved in studies of crassulacean acid metabolism (CAM) (Sect. 6). The discussion of diffusion in the gas phase of leaves is determined by the heterobaric/homobaric leaf concept, i.e., whether the gas partial pressures are heterogeneous or homogeneous within the air space of whole leaves (Neger 1912, 1918; Terashima 1992; Pieruschka et al. 2005). Anatomical constraints leading to compartmentalization of the leaf air space are responsible for heterobaric conditions.

The friendship with Barry Osmond initiated a new methodological development in our laboratory. He had received the Alexander-von-Humboldt-Forschungspreis and arrived in our laboratory with a camera and a strong interest in chlorophyll fluorescence imaging. We studied nonuniform patchiness of photosynthetic activity in virus-infected leaves of *Abutilon* (Osmond et al. 1998). Heterobaric conditions in leaves are responsible for stomatal patchiness (Beyschlag and Eckstein 1997) because CO_2 is a signal molecule for stomatal movements (Lüttge and Hütt 2006). Nonuniform, i.e., patchy, photosynthetic activity in leaves is associated with this. Photosynthetic patchiness, however, can also arise independent of stomata as we found with Barry when studying wilting and recovery of leaves. Under such conditions, patchiness in chlorophyll fluorescence proved to arise from metabolic interference of drought stress (Osmond et al. 1999). The technique of imaging was then advanced in our laboratory by Uwe Rascher and later Heitor M. Duarte when they were Ph.D. students and the creative electronics expert Karl Schuller in the workshop of the Institute of Botany at Darmstadt. This enabled us to measure gas exchange, photorespiration, and spatially resolved photosynthetic electron transport synchronously and online.

The CAM plant *Kalanchoë daigremontiana* Hamet et Perrier has a rather uniform mesophyll of large succulent spherical cells, and therefore structurally

the leaves would be homobaric. However, the cells are densely packed impeding lateral gas diffusion, and therefore the leaves become functionally heterobaric (Rascher et al. 2001; Maddess et al. 2002; Duarte et al. 2005b; Lüttge and Hütt 2006). Imaging shows that heterogeneity or patchiness of relative quantum use efficiency of photosystem II (Φ_{PSII}) is rather low in phase III of CAM during the light period. This is the phase when stomata are closed and CO_2 concentration in the gas phase of the leaves (p_i^{CO2}) is very high due to the CO_2 remobilization from organic acid, mainly malic acid, accumulated nocturnally in phase I of CAM (Lüttge 2002). High CO_2 concentration drives lateral diffusion and reduces patchiness. Conversely, patchiness of Φ_{PSII} is high in phases II and IV when stomata are open at the beginning and towards the end of the light period, respectively, at fairly low p_i^{CO2}. Patchiness is particularly high in the transition between phases III and IV, when stomata open after nocturnally accumulated organic acid is consumed. At that stage, p_i^{CO2} equilibria within the leaves are rearranged (Rascher et al. 2001; Maddess et al. 2002; Rascher and Lüttge 2002; Duarte et al. 2005b; Lüttge and Hütt 2006; Duarte and Lüttge 2007a). The transition is a highly dynamic spatiotemporal event. In phase III due to high p_i^{CO2}, Φ_{PSII} is high. During the transition between phases III and IV, low Φ_{PSII} patches develop from which waves of high Φ_{PSII} may run towards each other and extinguish each other when they meet (Fig. 5).

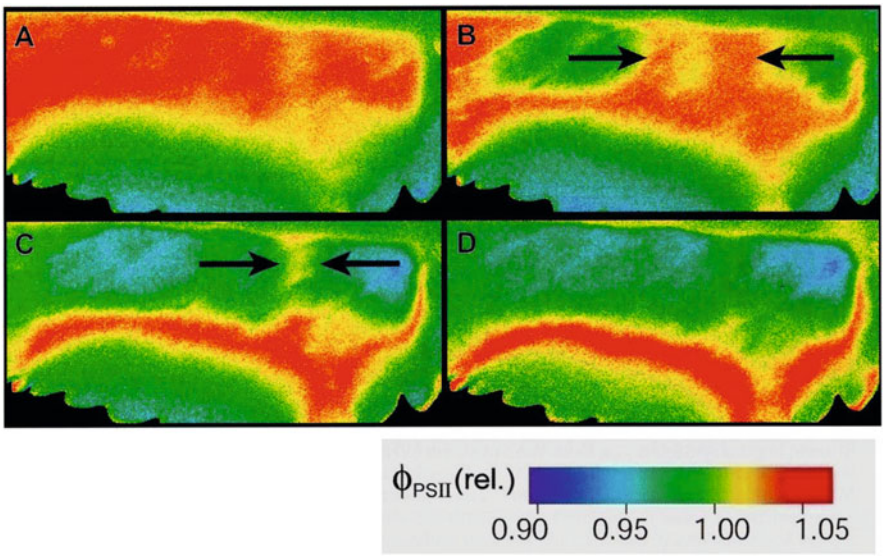

Fig. 5 Relative quantum use efficiency of photosystem II (Φ_{PSII}) in a leaf of *Kalanchoë daigremontiana* Hamet et Perrier during the transition between phases III and IV of CAM. Frames (**a**) to (**d**) were taken 20 min apart. Wavefronts of high Φ_{PSII} (*red*, see color code), which were initiated at different spots on the leaf, ran in opposite directions (*arrows*) and extinguished each other when they met (Rascher and Lüttge 2002, Copyright John Wiley and Sons)

4 Compartmentation: Transport at the Cellular Level

In assessing transport dynamics that govern cellular compartmentation, our work for some while focused on studies of transport kinetics (Lüttge 1968), namely, uptake kinetics (Sect. 4.1) and efflux kinetics (Sect. 4.2). In addition we used electrophysiology (Sect. 4.3). With this we were looking at the major cell compartments apoplast, cytoplasm, and vacuole.

4.1 Uptake Kinetics of Mineral Ions: Michaelis-Menten Hyperbolae

In the early 1950s, Emanuel Epstein had discovered that the concentration dependence of ion uptake by root tissues followed hyperbolic kinetics similar to the Michaelis-Menten kinetics of enzymes. With his group he elaborated this in a remarkable series of studies (Epstein and Hagen 1952; Epstein et al. 1963; Epstein and Rains 1965). They concluded that the enzyme kinetics of transport demonstrated the involvement of carrier molecules, which bind ions as substrates during the transport process across membranes. Moreover, they found that the kinetics were complex showing a dual hyperbolic isotherm, one saturating at low concentrations, i.e., with high affinity, and the other one saturating at high concentrations, i.e., with low affinity.

Epstein and his group had assumed that both the high and the low affinity systems were located at the same membrane, i.e., the plasma membrane, operating in parallel. When I came to George Laties' laboratory in October 1965, they had just tested an alternative view, namely, that the high affinity system was at the plasma membrane and the low affinity system was at the tonoplast (Torii and Laties 1966). They compared root tips, where the cells were not yet vacuolated having no tonoplasts, with vacuolated root tissue. In the non-vacuolated cells, they found the high affinity isotherm at low external ion concentrations and a linear concentration dependence of ion uptake at high concentrations. Their interpretation was that the high affinity system operated and limited transport into the cells at low concentration, whereas the linear kinetics indicated dominance of passive diffusion at high concentrations. In the vacuolated cells, having both plasma membrane and tonoplast, they found the dual isotherms indicating activity of both systems.

As the symplastic route of transport of ions from the medium across the root towards loading the xylem conduits does not involve vacuoles and tonoplasts (Sect. 3.1), George had postulated that it should follow the same kinetics as uptake by non-vacuolated cells, i.e., with the high affinity isotherm at low and a linear relationship at high concentrations. I got to check this, and indeed in a number of studies (Lüttge and Laties 1966, 1967a, b), the kinetic evidence corroborated these expectations (Fig. 6).

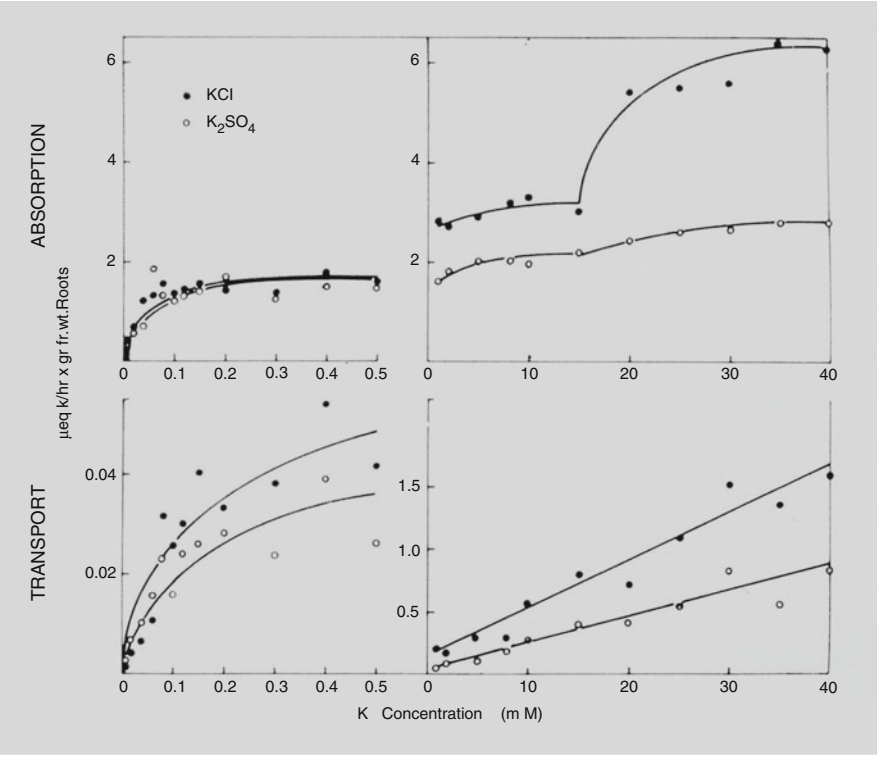

Fig. 6 Potassium absorption by the root tissue (*above*) and transport into xylem exudates (*below*) by maize seedlings from KCl (*closed circles*) and K_2SO_4 (*open circles*) solutions in a low (*left*) and high (*right*) concentration range (Lüttge and Laties 1966, www.plantphysiol.org, Copyright American Society of Plant Biologists)

For visualizing the uptake of ions into the cytoplasm and checking its isotherm, we returned to micro-autoradiography and used a trick. When one gently centrifuges tissues, one can precipitate the cytoplasm within cells without damaging the cells. The procedure enhances the resolution of imaging the cytoplasm. After uptake of labeled ions, the high affinity isotherm can be derived from quantifying the autoradiograms (Fig. 7), and similarly X-ray microanalysis can be used with the centrifuged material (Läuchli and Lüttge 1968).

Epstein's discovery of enzyme kinetics of ion uptake proved to be extraordinarily fruitful. From molecular characterizations in several laboratories, we now know that there are a plethora of transporters for various ions both with high and low affinity at both the plasma membrane and the tonoplast. On such grounds, the cellular localization of mechanisms which bring about the various Michaelis-Menten isotherms and compartmentation of the high and low affinity transport processes need new interpretation.

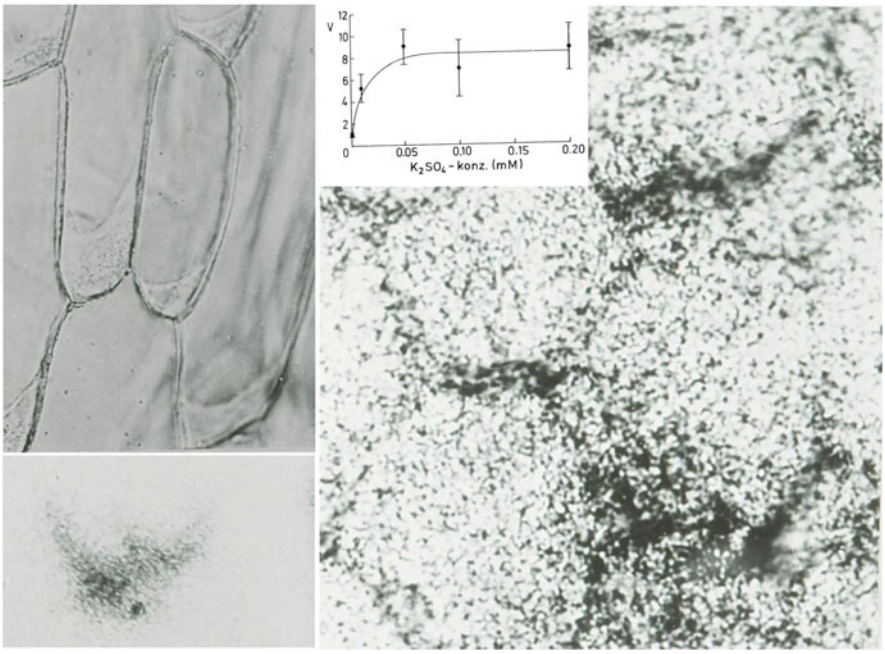

Fig. 7 *Left*: Cells of a centrifuged adaxial epidermis of onion bulb (*Allium cepa* L.) (*above*) with micro-autoradiograph (*below*) after uptake of $^{35}SO_4^{2-}$ for 3 h from 7.5 mM K_2SO_4. *Right*: Micro-autoradiograph of cells from a centrifuged leaflet of the moss *Plagiomnium cuspidatum* (Hedw.) T.J. Kop after uptake of $^{35}SO_4^{2-}$ for 7 h from 5 mM K_2SO_4, where the insert shows the relative rate of $^{35}SO_4^{2-}$ uptake derived from the density of the silver grains in micro-autoradiographs after uptake from increasing concentrations of K_2SO_4 in a low concentration range (Läuchli and Lüttge 1968)

4.2 Efflux Kinetics

While the uptake kinetics discussed in Sect. 4.1 are based on influx of labeled ions into the cells, for measuring efflux kinetics, the tissue is first labeled by extended incubation in the radioactive ion solution up to establishment of kinetic equilibrium of influx and efflux. The tissue is then transferred to a non-labeled solution with the same ion concentration as that of the labeling solution, and the rate of label efflux is measured. This essentially means that rates of isotope exchange are assessed. Basically efflux shows three linear phases: (1) a rather rapid one, (2) a slower one, and (3) a quite slow one extending over many hours. The phases arise from exchanges at the apoplast (1), the cytoplasm (2), and the vacuoles (3), represented by efflux of labeled and influx of non-labeled ions at efflux–influx equilibrium. A set of equations is developed relating fluxes and pools, i.e., for assessing the individual fluxes at the plasma membrane and tonoplast and the ion contents of cytoplasm and vacuole (Pitman 1963; Lüttge 1968). A shoulder of efflux in phase (2) indicated further sub-compartmentation of the cytoplasm (Pallaghy et al. 1970;