Compartmentation of Solutes and the Role of Tonoplast ATPase in *Hevea* Latex

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The latex of *Hevea brasiliensis* could be regarded as issued from a tissue specialized in the biosynthesis of rubber. From all the biochemical investigations conducted on this material, the idea emerges that the lutoids, a single membrane-bound organelle, form a dispersed vacuolar compartment where the properties are analogous to those of the central vacuome of higher plants (D'Auzac et al, 1982). In addition, those vacuoles form a cell compartment where the relationship with the cytoplasm plays an extremely important biological role, especially in all events involved in the biosynthesis of rubber. However, as pointed out by Boudet et al (1984), like the other vacuolar compartment isolated from fungi and higher plants, the lutoids, initially described as a rather inert compartment mainly devoted to maintain the osmotic pressure, must now be also considered as a multifunctional compartment involved in different areas of plant functioning (Table 1). The aim of this contribution is to expose the current issues about the compartimentation of solutes, the energization of their transport and the processes of their accumulation at the tonoplast in *Hevea* latex.

Hevea Latex as a Source of Vacuoles Having preserved Their Native Properties

Hevea brasiliensis is a tropical tree characterized by a complex laticiferous system consisting of anastomosed cells arranged in monocellular layers around the cambium (Dickenson, 1964; Hebant and DeFay, 1980). In addition to the typical organelles found in all tissues of higher plants, namely, nuclei, mitochondria and ribosomes, these cells contain a great number of rubber particles, some Frey-Wyssling particles, structurally equivalent to degenerated plastid type organelles and numerous lutoids, which form a polydispersed vacuo-lysosomal system (cf. Pujarniscle, 1968; Ribaillier et al, 1971). Lutoids must be regarded as a type of specialized vacuome, fundamentally comparable with the central vacuole of higher plants. By tapping, it is possible to obtain easily the content of the laticiferous vessels devoid of any nuclei and mitochondria, which have a parietal intracellular location (Southorn, 1969; D'Auzac et al, 1982). Consequently, the collected latex must be identified to a true cytoplasm, from where the isolation of vacuoles is easy and allows the obtention of organelles having preserved their native properties, especially without rupture of the tonoplast membrane (Marin, 1982).

Solute Content of *Hevea* Vacuoles

When resuspended in an adequate medium, which respect their osmosensitivity (in the presence of 0.3 M mannitol, usually), it is possible with several differential centrifugations to separate and purify large quantities of vacuoles, sufficiently to analyze their solute content. Thus, by quantitative comparison of the solute composition of

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Officially changed name: Chrestin Hervé, from Crétin Hervé

Table 1. Lutoids as a multifunctional compartment involved in different areas of plant function (adapted from Wagner, 1982, and Boudet et al, 1984)

- maintenance of the osmotic pressure in concert with the cytoplasm and cell wall
  - mechanical support
  - tissue movement
  - motive force for cell expansion
  - stress reaction (by bursting) in the stopping of latex flow
- ion balance and storage (H^+, Ca^{2+}, Mg^{2+})
- metabolite storage (reversible)
  - at long term (major protein reserves)
  - at short term (inorganic phosphate, citrate, basic amino-acids)
- metabolite sequestration (irreversible)
- lytic functions related with development

  (destruction of the transversal wall of the latex tube cells upon the formation of the latex tubes), with any stress modifying the steady-state (phenomenon of coagulation, microbial invasion of the latex tubes,...) and with the senescence (peroxidative degradation of the tonoplast membrane)
- intracellular and intercellular mixing via transvacuolar strands and plasmodesmata
- minimization of the volume of the cytoplasm, maximization of the cytosol-tonoplast interface and provision for efficient distribution of the photosynthetic apparatus

vacuoles and latex, and after calculation that of cytoplasm, it is possible unambiguously to estimate the distribution of solutes between the cytoplasm and the vacuolar compartment, as shown in Table 2.

Thus, the accumulation ratio is almost 1 for K^+, 8 for Mg^{2+} and 6 for Ca^{2+}. It ranges between 8 and 15 for inorganic phosphate. Consequently, divalent cations and inorganic phosphate are accumulated in the vacuolar compartment. Among the organic molecules, only citric acid is significantly accumulated. Amino-acids are distributed differently between the cytoplasm and the vacuolar compartment according to their nature (Brzozowska et al, 1974). It is noted that the basic amino-acids (arginine, ornithine, lysine, δ-aminobutyric acid) are accumulated from 5 to 20-fold in the vacuoles while acid and neutral acids are 3-fold more concentrated in the cytoplasm. In addition, to be complete, from 30 to 50 phenolic aglycones are present in the latex of *Hevea brasiliensis* but they seem to be present in both fractions at the same concentrations (Hanower et al, 1979).

In parallel, it has been observed that, in the cytoplasm, certain ions or molecules inhibit very effectively important steps in the isoprenoid metabolism when their contents cross (exceed) some threshold. This is the case of Mg^{2+} as regards invertase (Tupy, 1973; Jacob et al, 1982), of citrate as regards pyruvate kinase (Jacob et al, 1981), of phospho-fructokinase (Jacob, 1970), of Ca^{2+} which inhibits phosphoenol-carboxylase (Jacob et al, 1980) and copper which inhibits pyruvate-kinase and phosphoenol-pyruvate-carboxylase (Jacob et al, 1980 and 1981) (Fig. 1). Consequently, the dynamic nature of the exchange of these ions and molecules through the tonoplast is very important to regulate the functioning of the biochemical syntheses involved in the latex regeneration process (D'Auzac et al, 1982). As evidenced in Table 2, their vacuolar storage avoids the development of too high a concentration in the cytoplasm, which could cause a specific feedback inhibition of the synthetic enzymes involved in the rubber biosynthesis. The knowledge of their transport and the processus of their accumulation in the vacuolar compartment in *Hevea* latex is fundamental.

Characteristics of Solute Uptake at Tonoplast Level in *Hevea* Latex

All the data about the characterization of the mechanisms of solute uptake and accumulation and their energization either on the undamaged lutoids (vacuoles) or the vesicles formed from the tonoplast membranes are self-consistent (Marin et al, 1982; Crêtin, 1984).

In the absence of any metabolic energy supply, such as MgATP, *Hevea* vacuoles *in vitro* are capable of taking up such varied solutes as Ca^{2+}, phosphate, arginine, lysine or citrate against a transmembrane concentration gradient (Ribaillier, 1972; D'Auzac and Lioret, 1974; Montardy and Lambert, 1977; D'Auzac et al, 1977; Hanower et al, 1977; Crêtin 1984). Consequently, such process involves an active transport system where the kinetic parameters are clearly defined (Tables 3,4 and 5). Each temperature-dependent uptake is linear for at least 30 min. Its initial rates as a function of the substrate concentration displays often simple Michaelis-Menten kinetics, and Lineweaver-Burk plots yield straight lines for all solutes tested over a 20-fold concentration range. Moreover, it shows a strict pH-dependence with an optimum value around 7.0, the value reported for the cytoplasm of *Hevea* latex (Hanower et al, 1977). In addition, this uptake corresponds to a true increase of the accumulation ratio (Crêtin, 1984). Such data is confirmed with tonoplast vesicles (Marin, 1982).

The presence of MgATP with the vacuoles in the incubation medium or their preincubation with this complex, described as the substrate of the tonoplast-bound ATPase (D'Auzac, 1977; Gidrol, 1984; Marin, unpublished data) leads to an increase of the solute uptake (D'Auzac

Table 2. Solute accumulation in vacuoles from Hevea latex (from D'Auzac et al, 1982)

solute	concentration (mM) $C_{vacuole}$	concentration (mM) $C_{cytoplasm}$	ratio $\frac{C_{vacuole}}{C_{cytoplasm}}$
K^+	31.2	30.1	1.0
Mg^{2+}	64.2	8.3	8.0
Ca^{2+}	1.51	0.25	6.0
Cu^{2+}	0.046	0.021	2.0
inorganic phosphate (acid-soluble P_i)	76	9.1	8.7
sucrose	5.8	40.5	0.1
citrate	53.0	5.7	9.3
malate	17.3	14.6	1.2
amino-acids (a):			
acidic	22.9	56.9	0.4
neutral	21.1	36.4	0.6
basic	56.9	6.6	8.6

(a) percent of total amino-acids

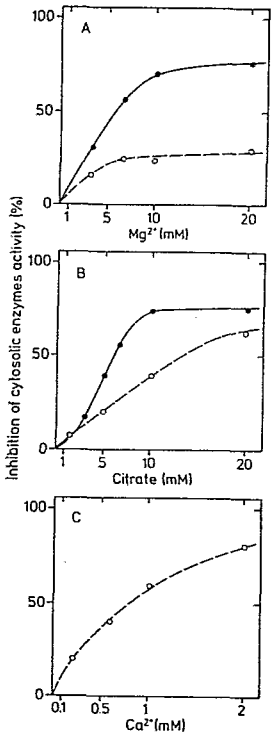

Fig. 1. Inhibition of some cytosolic enzymes in Hevea latex by magnesium, calcium or citrate at physiological concentrations: effect of Mg^{2+} on invertase at pH 6.5 (●——●) or 7.2 (o——o) (A); effect of citrate on phospho-fructo-kinase (●——●) and PEP-carboxylase (o——o) (B); effect of Ca^{2+} on PEP-carboxylase (o——o) (C) (from Jacob and d'Auzac, 1967, 1969, 1972; d'Auzac and Jacob, 1969; Tupy, 1973; Jacob et al, 1979)
The activities are measured in artificial buffers

Table 3. Characteristics of the uptake of organic acids by vacuoles and tonoplast vesicles from Hevea latex. o = ineffective; - = inhibition; + = stimulation; nd = not determined (from Ribaillier, 1972; D'Auzac and Lioret, 1974; Montardy and Lambert, 1977; D'Auzac et al, 1977; Marin, 1982; Marin et al, 1981; Crétin, 1984)

	citric acid	malic acid	succinic acid
Properties:			
time-course (during 30 min)	linear	linear	linear
pH optimum	6 - 7	7	7
dependence of uptake on solute concentration			
K_m (mM)	5 - 7	7.5	10
V_m (nmol · min^{-1} · mg^{-1} protein)	1.5 - 5.8	1.0	1.7
specificity	no effect of structural analogs	nd	nd
stimulation by MgATP	++++	++++	++++
nucleotide specificity of this stimulation	ATP>>>GTP>CTP>UTP>ADP	nd	nd
exsorption	function of the material tested:		
-vacuoles	o	nd	nd
-tonoplast vesicles	++++	nd	nd
sensitivities:			
proton conductors			
2,4-DNP	++	+	+
NH_4Cl	o	o	o
FCCP	++	nd	nd
CCCP	++	nd	nd
S-13	++	nd	nd
ATPase inhibitors			
DCCD	++	nd	nd
EEDQ	++	nd	nd
TMT	++	nd	nd
SH-group reagents			
pCMB	++	nd	nd
mersalyl	++	nd	nd
NEM	++	nd	nd
iodoacetamide	++	nd	nd

Table 4. Characteristics of the uptake of amino-acids by vacuoles and tonoplast vesicles from *Hevea* latex. o = ineffective; - = inhibition; + = stimulation; nd = not determined (from Brzozowska et al, 1977; Niamien N'Goran and Crétin, unpublished data; Marin, unpublished data)

	L-lysine	arginine
Properties:		
time-course (during 30 min)	linear	linear
pH optimum	7.0 - 8.0	7.0 - 8.0
dependence of uptake on solute concentration		
K_m (mM)	10 - 12	6
V_m (nmol · min^{-1} · mg^{-1} protein)	0.1	nd
specificity	competitive inhibition with L-arginine (K_i = 6 mM)	nd
stimulation by MgATP	++++	++++
nucleotide specificity of this stimulation	ATP>>>ADP	ATP>>>ADP
exsorption	o	o
sensitivities:		
proton conductors		
2,4-DNP	+	+
NH_4Cl	+	+
FCCP	++	nd
CCCP	++	nd
S-13	++	nd
ATPase inhibitors		
DCCD	++	++
EEDQ	++	nd
TMT	++	nd
SH-group reagents		
pCMB	+	+
mersalyl	+	nd
NEM	+	nd
iodoacetamide	+	nd

Table 5. Characteristics of the uptake of calcium by vacuoles and tonoplast vesicles from *Hevea* latex. o = ineffective; - = inhibition; + = stimulation; nd = not determined (from Crétin, 1984; Marin, unpublished data)

	Ca^{2+}
Properties:	
time-course (during 30 min)	linear
pH optimum	6.8 - 7.2
dependence of uptake on solute concentration	
K_m (mM)	10 - 20
V_m (nmol · min^{-1} · mg^{-1} protein)	5 - 20
specificity	nd
stimulation by MgATP	+++
nucleotide specificity of this stimulation	ATP>>>GTP>CTP>UTP>>ADP
exsorption	++
sensitivities:	
proton conductors	
2,4-DNP	++
NH_4Cl	++
FCCP	++
CCCP	++
S-13	++
ATPase inhibitors	
DCCD	++
EEDQ	++
TMT	++
SH-group reagents	
pCMB	++
NEM	++
iodoacetamide	++

and Lioret, 1974; Montardy and Lambert, 1977; D'Auzac et al, 1977; Hanower et al, 1977; Crétin, 1984). In the best cases, the steady state level of solute uptake and accumulation is 4 - 5 fold higher than the level obtained in the absence of any energy source. In parallel, an increase of transtonoplast ΔpH is observed (Lambert, 1975). A close relationship between the magnitude of this ΔpH and its increase and the uptake of citric acid and lysine has been established with vacuoles and tonoplast vesicles (Hanower et al, 1977; Marin et al, 1981; Marin, 1982). In addition, agents usually described as proton conductors (2,4-dinitrophenol, NH_4Cl, FCCP, CCCP and S-13) cause a considerable reduction in the uptake of citrate and lysine (D'Auzac and Lioret, 1974; Hanower et al, 1977; Marin, 1982; Crétin, 1984). Similar inhibition patterns are observed in response to the different tonoplast-bound ATPase inhibitors known to block the H^+-channel, namely DCCD, EEDQ and TMT (Marin, 1983a). SH-group reagents (pCMB, mersalyl, NEM, iodoacetamide) are reported to be effective. Consequently, the energy indispensable to each uptake, which is developed against a concentration gradient, seems to be originated from the electrochemical proton gradient at tonoplast level (Marin et al, 1981a; Marin, 1982; Crétin, 1984). This $\Delta\tilde{\mu}_{H^+}$ is under the dependence of two enzymes located at tonoplast level: the ATPase which catalyzes a proton influx and the NADH-cytochrome c-reductase which catalyzes a proton efflux (Marin et al, 1981a and b; Marin, 1982 and 1983; Crétin, 1982 and 1984; Gidrol, 1984).

Relationships Between the Accumulation of Solutes and the Protonmotive Force at Tonoplast Level

The main results obtained with *Hevea* vacuoles are listed in Table 6. They concern only Ca^{2+} and citrate (Crétin, 1984).

Thus, for Ca^{2+}, whatever the method used, any increase of the transtonoplastic ΔpH is immediately followed by an increase in the uptake of Ca^{2+}. In contrast, any dissipation of the initial ΔpH causes the opposite effect. The intravacuolar accumulation of Ca^{2+} is strictly closed to the magnitude of the transtonoplast proton gradient. In addition, in spite of the way used, any positivation of the tonoplast is accompanied by a large efflux of Ca^{2+}. As discussed below, this effect is due to a modification of the transtonoplast Donnan potential, which induces a change of the large intravacuolar Ca^{2+} pool, resulting from the interaction of Ca^{2+} with negatively charged intralutoidic structures.

For citric acid, no effect is observed with intact vacuoles. Whatever the method used to change one of the two components of the protonmotive force (or both) no efflux of citrate could be shown. These results contrast entirely with those reported with tonoplast vesicles, where any increase of ΔpH or depolarization is accompanied by an increase of uptake of citric acid. As discussed below, such differences are in favour of a sequestration of citric acid.

Reversibility of the Solute Uptake

The data reported by the different authors in the literature about the reversibility of solute uptake process at tonoplast level in *Hevea* latex are entirely different according to the molecule species studied and the nature of material used (whole vacuoles or tonoplast vesicles).

Thus, for citric acid and lysine, when vacuoles are incubated in the presence of radioactive tracers for 30 - 60 min and then placed in a non-radioactive medium, no sizeable efflux of the incorporated molecules is observed (D'Auzac and Lioret, 1974; Montardy and Lambert, 1977; Hanower et al, 1977). After 45 - 60 min, at most 13 - 15% of the organic acids or of the basic amino-acids taken up are released

Table 6. Relationships between the accumulation of solutes and the protonmotive force a tonoplast level in *Hevea* vacuoles (Crétin, 1984)

	Effects observed on *Hevea* vacuoles	Ca^{2+} flux	citrate flux
changes of ΔpH			
- HCl or MES	ΔpH ↘	----	o
- NaOH or Tris-base	ΔpH ↗	++++	o
- 10 - 50 µM nigericin + 160 mM KCL	ΔpH ↘	---	o
changes of ΔΨ			
- 100 - 130 mM KCl + valinomycin (10 µg · ml^{-1})	$\Delta\Psi$ ↗$^+$	----	o
changes of ΔΨ and ΔpH			
- 10 mM NH_4Cl	ΔpH ↘ $\Delta\Psi$ ↗$^+$	n.d.	o
- TPP^+ or $TPMP^+$ (until 6 mM)	ΔpH ↘ $\Delta\Psi$ ↗$^+$	----	o
- 2.5 mM CaATP in presence of 5 mM $MgCl_2$	ΔpH ↗ $\Delta\Psi$ ↗$^+$	+++	+++
- 3 - 5 mM MgATP	ΔpH ↗ $\Delta\Psi$ ↗$^+$	+++	o
- 20 - 100 µM FCCP or 200 µM CCCP	ΔpH ↘ $\Delta\Psi$ ↘	---	o
- 20 µM FCCP + 5 mM MgATP	ΔpH ↘ $\Delta\Psi$ ↘	+	o
- 20 µM A-23187	dissipation of any concentration gradient of native free Mg^{2+} or Ca^{2+}	n.d.	-
- 20 µM A-23187 + 0.5 mM $CaCl_2$	ΔpH ↘	---	n.d.
- 20 µM A-23187 + 5 mM $MgCl_2$	ΔpH ↘	n.d.	++

(Montardy and Lambert, 1977; Hanower et al, 1977). This efflux is attributed to the breakage of a certain proportion of tightly-sealed vacuoles unvoidable during such experimentation. In addition, any modification of the parameters capable of having some effect upon the efflux, such as the collapse of one of the two components of the protonmotive force (or both) and the change of the internal concentration of free Mg^{2+} or Ca^{2+}, fails to induce a sizeable efflux, limited at most to 5% of the internal pool (Crêtin, 1984). In contrast, the results obtained with tonoplast vesicles, at least for citric acid, show an important efflux of uptaken solute (Marin, 1982). This temperature-dependent efflux increases with the external citrate concentration. In addition, the isotopic enrichment of the internal space does not exceed 8% of the external specific activity. Consequently, isotopic equilibrium is never reached. All these data could be considered as concordant only if the differences observed between the whole vacuoles and the tonoplast vesicles are explained by the loss of some endogenous factors, during the vesiculation process in an artificial medium (Marin, 1982; Crêtin, 1984). Consequently, *in vivo*, if an isotopic exchange occurs, it is practically not measureable and, no net efflux could be shown even by enzymatic analysis (Crêtin, 1984). All the citrate incorporated by vacuoles from *Hevea* latex is sequestrated in the interior of the vacuole space.

In contrast, for Ca^{2+}, sizeable efflux of the incorporated ions is observed when the transtonoplast proton gradient decreases (cf. Table 6). Thus, *in vivo*, when the NADH-cytochrome c-oxidoreductase functions, when it reduces the ΔpH, an important efflux of Ca^{2+} is observed (Crêtin, 1984), assuming the close relationship between the exchangeable Ca^{2+} level inside the vacuoles and the magnitude of ΔpH. An increase of the efflux is also obtained when the Donnan potential is affected with high concentrations of permeant molecules. But, in this case, Ca^{2+} originates from an other pool, as outlined below.

The Functioning of the Citrate Translocator as a Model for the Transport and the Accumulation of Vacuolar Solutes

All the data obtained with vacuoles and tonoplast vesicles suggest the existence of an internal compartmentation of the vacuolar citrate in two clearly distinct pools, only one of them being directly accessible to isotopic exchange (Marin, 1982). This can only be accounted for by a kinetic pool since no morphological compartmentation is visible.

Nevertheless, as Mg^{2+} is present in stoichiometric concentrations with citrate in *Hevea* latex (Coupé, 1977; Jacob, unpublished data), it could be suggested that a part of vacuolar citrate is able to chelate this cation. Acid-base equilibrium calculations and associations with Mg^{2+} predict an accumulation of the complex $(citrate)^{3-}$-Mg^{2+}, considered as the unpermeant form, as also the formation of a pool of $(citrate)^{2-}$, regarded as the permeant form as judged from the shape of the curves of citrate uptake *vs.* pH but also the variation of K_m *vs.* pH (Marin, 1982; Marin, unpublished data). In addition, the acidification of the medium and the alkalinization of the vesicles which follow citrate addition to the medium in absence of MgATP, suggest that there is a $n\,H^+ - m$ citrate antiporter (Marin, 1982). The electrogenic character of citrate translocation observed in absence of ATP infers that a net negative charge is transported. The tentative model which accounts for the whole set of data is as follows: citrate (as $citrate^{2-}$) is transported antiportly with a proton, one Mg^{2+} being simultaneously transferred from the cytoplasm to the internal vacuolar space, as shown in Fig. 2 (Marin, 1982; Marin et al, 1982). Kinetic simulations based on this model are quite reconcilable with the experimental data. They account for the electric

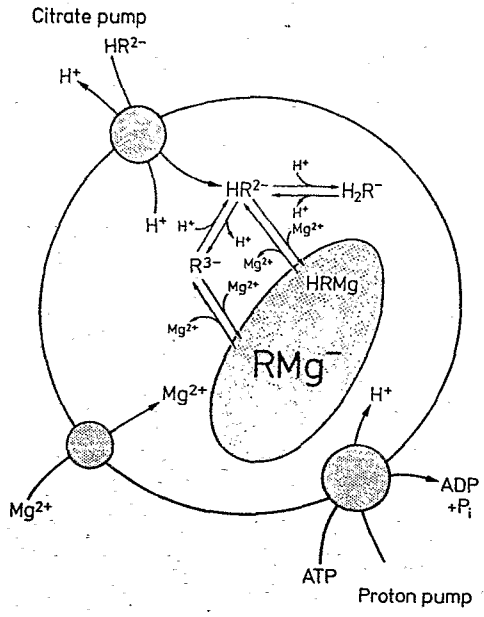

Fig. 2. Mechanism of incorporation and trapping of citrate in the vacuoles from Hevea latex (from Marin, 1982)

This figure summarizes the present status of the knowledge on citrate transport in Hevea vacuole. The mechanism of citrate translocation is a citrate-proton antiporter associated with a magnesium influx. Under the conditions of internal vacuolar space, the permeant form of citrate (HR^{2-}) is sequestrated under a non-permeant form (RMg^-). The proton pump assumes the tonoplast energization. By a side effect due to the acidification of intravacuolar medium, it assumes also the formation of a pool of non-permeant solute.

character, the effect of the pH of the medium, the effect of the ΔpH and the stoichiometry with Mg^{2+}. In addition, in these conditions, all the vacuolar citrate is present as the unpermeant complex $(citrate)^{3-}Mg^{2+}$ inside the vacuolar space. Such model explains clearly how the citrate transport is energized and how the two components of the proton-motive force are used by the translocator.

Recent data on Ca^{2+} transport suggest also the occurrence of two pools inside the vacuoles (Crétin, 1984). The first pool corresponds to a large exchangeable Ca^{2+} pool sensitive to any changes of the protonmotive force at tonoplast level, especially the ΔpH component. The second pool corresponds to all the Ca^{2+} bound to intravacuolar anionic structures. This Ca^{2+} could be released from vacuoles when the vacuoles are incubated in the presence of high concentrations of permeant cations.

In each case, the tonoplast-bound ATPase supplies the energy sufficient to control the accumulation of citrate and Ca^{2+}. However, their coupling is indirect and involves essentially the protonmotive force, and especially the ΔpH component.

CONCLUDING REMARKS

The compartmentation of solutes in *Hevea* latex corresponds to a better functioning of the biochemical synthesis involved in the latex regeneration process. Thus, Ca^{2+} and citrate are two potential effectors of the metabolism of rubber synthesis. These molecules are removed from the cytoplasm when their concentrations exceed a value above which they largely inhibit some enzymes involved in the synthesis of rubber (Jacob et al, 1979, 1980 and 1981). These values correspond to the optimum conditions of their uptake and their accumulation in the vacuoles. The mechanisms of storage involved seem to be different and complex but, in each case, they emphasize the importance of pH gradients between the cytoplasm and the vacuoles in these processes. In addition, they suggest the occurrence of two compartments inside the vacuoles. The first compartment is exchangeable and its size is function of the tonoplast energization, the protonmotive force being derived from the functioning of the tonoplast-bound ATPase (Marin, 1982; Crétin, 1982 and 1984). The second compartment corresponds to a storage pool where the solute is accumulated either reversiblely or not, according to its nature. The main differences between Ca^{2+} and citrate are the relative size of these two compartments: the exchangeable compartment is important for Ca^{2+} whereas it is reduced or non-existent for citrate. Consequently, the role of these two molecules should be very different.

It appears clearly that the protonmotive force induced by the operation of the tonoplast-bound ATPase of lutoids not only maintains a pH gradient between the cytoplasm and the internal vacuolar space, but also drives the active transport of Ca^{2+} and citrate. However, its extent could be reduced by the tonoplast-bound NADH-cytochrome c-oxidoreductase which catalyzes an electrogenic proton efflux (Crétin, 1984). Consequently, these two activities control the compartmentation of solutes between the cytoplasm and the vacuoles, their transport and their accumulation. In addition, when an efflux is possible, they control also their release from the vacuoles. Thus, any variation of ΔpH modulates the flux of exchangeable vacuolar Ca^{2+} and its consequence on the metabolism of rubber synthesis is very important.

Hevea latex corresponds typically to the model described by Boudet et al (1984): "the tonoplast-located transport systems could function as transmembrane signal transducers implicating the cytoplasm pH as a potential messenger, small shifts in cytoplasmic pH being able to modify diverse pH-sensitive processes".

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FOOTNOTES

Abbreviations used:

CCCP, carbonyl-cyanide-m-chlorophenyl-hydrazone; DCCD, N,N'-dicyclohexyl-carbodiimide; $\Delta\mu_{H^+}$, electrochemical proton gradient; $\Delta\Psi$, membrane potential; ΔpH, transmembrane pH gradient; EEDQ, N-ethoxy-carbonyl-2-ethoxy-1,2-dihydroquinoline; FCCP, carbonyl-cyanide-p-trifluoromethoxy-phenyl-hydrazone; NEM, N-ethylmaleimide; pCMB, p-chloromercuribenzoate; S-13, 5-chloro-3-tert-butyl-2'-chloro-4-nitrosalicylanilide; TMT, trimethyltin chloride.

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