$\Delta\bar{\mu}H^+$-CONTROLLED REVERSIBLE FLUXES OF H^+ AND CALCIUM AT THE TONOPLAST BUT QUASI-TOTAL CITRATE SEQUESTRATION WITHIN THE INTACT VACUOLES FROM THE LATEX CELLS OF HEVEA BRASILIENSIS. IMPLICATIONS IN THE PRODUCTION OF NATURAL RUBBER

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The latex of Hevea brasiliensis is a fluid cytoplasm which is expelled from wounded latex vessels (articulated, anastomosed cells) (Archer et al., 1963). It contains a vacuolar compartment - the so-called "lutoids" - consisting of micro-vacuoles which can be easily isolated and purified by simple differential centrifugation (Pujarniscle, 1968; Ribaillier et al., 1971; D'Auzac et al., 1982).

Like all plant vacuoles, lutoids exhibit a lower internal pH (about 5.5) than that of their cytosolic environment (about 7.0). They accumulate, in vitro and in vivo, numerous mineral and organic cations such as Mg^{2+}, Ca^{2+}, Cu^{2+}, etc ., and basic amino-acids, as well as anions such as inorganic phosphate and citrate (Ribaillier et al., 1971; D'Auzac and Lioret, 1974; Brzozowska et al., 1974).

Production of latex reflects the intensity of the metabolism within these specialized laticiferous cells. Indeed this regenerative metabolism must be sufficiently active to compensate for the loss of latex (50 to 300 ml or more with a mean value of 35% dry rubber content) at each tapping (generally twice a week).

Rubber production has been shown to be correlated positively with the pH of the cytosol of the latex cells (Table 1), and negatively with the intravacuolar pH (Coupé and Lambert, 1977; Brzozowska-Hanover et al., 1979; Chréstin, 1985). Furthermore, a highly significant inverse relationship was demonstrated between the pH of the cytosolic compartment and the changes in intravacuolar pH (lutoidic pH), strongly suggesting the existence of vectorial H^+ fluxes at the level of the lutoidic tonoplast (Table 1) (Brzozowska-Hanower et al., 1979; Chréstin, 1985).

Furthermore, multivariate analysis showed that the latex from high yielding rubber-trees was characterized not only by a slightly alkaline cytosolic pH and a high transtonoplastic H^+ gradient, but also by a pronounced accumulation of citrate in the vacuoles, resulting in a high transtonoplastic gradient of citrate, i.e. a low citrate concentration in the cytosol (Table 1).

These relationships were satisfactorily explained by the extreme pH sensitivity (in the physiological pH range) of numerous key enzymes of the cytosolic metabolism

Table 1

Correlation Coefficients
Linking the Latex Production (g dry rubber/tapping/tree),
Cytosolic pH, Transtonoplastic pH Gradient,
Cytosol and Vacuole Citrate Concentrations (mM) in Latex
and the Resulting Transtonoplastic Citrate Gradient

	Latex Production	Vacuolar (Citrate)	Cytosolic (citrate)	(Citrate) Gradient	Cytosolic pH	Trans-tonoplastic pH Gradient
Latex Production	1	+ 0.562 ***	- 0.768 ***	+ 0.755 ***	+ 0.894 ***	+ 0.822 ***
Vacuolar (Citrate)		1	- 0.369 **	+ 0.778 ***	+ 0.675 ***	+ 0.640 ***
Cytosolic (Citrate)			1	- 0.678 ***	- 0.705 ***	- 0.752 ***
(Citrate) Gradient				1	+ 0.765 ***	+ 0.800 ***
Cytosolic pH					1	+ 0.935 ***
Trans-tonoplastic pH Gradient						1

Data were obtained from freshly collected latex from 56 rubber trees, (***): very high significance; (**): high significance.

and their inhibition by certain ions such as Mg^{2+}, Ca^{2+}, citrate, etc., at physiological concentrations (Jacob and D'Auzac, 1967, 1969 and 1972; D'Auzac and Jacob, 1969; Tupy, 1969; Jacob et al., 1979; Chrestin et al., 1984).

All these data led to seeking a mechanism able to control transtonoplastic fluxes of protons and citrate, and therefore wondering about the role of the vacuolar compartment in the control of the cytosolic metabolism within the latex cells of Hevea.

TWO OPPOSING H^+-TRANSLOCATING SYSTEMS AT THE TONOPLAST

H^+ accumulation within intact latex vacuoles was shown to originate from two complementary processes :

- a large, nearly constant pool of protons (accounting for up to 1 pH unit of the transtonoplastic pH gradient) remains sequestered at the thermodynamic equilibrium within the vacuolar compartment, owing to the existence of a transtonoplastic Donnan potential (Crétin, 1982),

- transtonoplastic H^+ fluxes, which determine the cytosolic as well as the vacuolar pH changes (accounting for 0.1 to 1 pH unit of the total transtonoplastic pH gradient), are shown to be under the control of two opposing H^+ translocating systems both located at the level of the tonoplast.

The first is the tonoplastic ATPase dependent on Mg^{2+}, revealed and partially described by D'Auzac (1975 and 1977) and more carefully described by Marin and his group (Marin, 1985; Marin et al., 1985 and 1986 a and b). This constitutive membrane ATPase works as a proton pump, catalysing H^+ influx into the vacuole (vacuolar acidification shown by the accumulation of the ΔpH probe ^{14}C-methylamine in the vacuolar compartment), causing the alkalinization of the cytosol and an increase in the transtonoplastic pH gradient. As expected, the ATP-dependent transtonoplastic H^+ fluxes were shown to be inhibitable by protonophores such as FCCP (Fig. 1-A and B) (Marin et al., 1981; Crétin, 1982; Crétin et al., 1982; Marin, 1982; Chréstin, 1985; Marin, 1985).

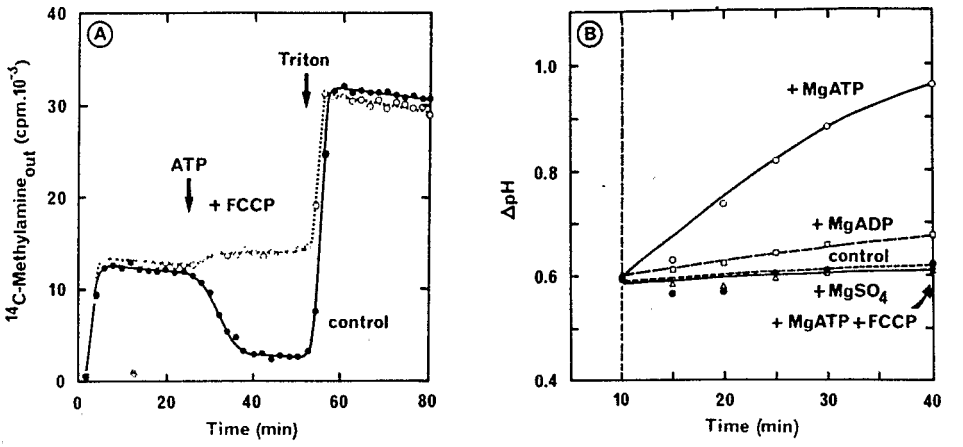

Figure 1

Change in time of ΔpH across the tonoplast (inside acid) in the presence of ATP and effects of FCCP

ΔpH changes were monitored by following the accumulation of the ΔpH probe ^{14}C-methylamine, using either the flow dialysis technique with intact lutoids (Fig. 1-A) or centrifugation with reconstituted tonoplast vesicles (Fig. 1-B).

Furthermore, in the absence of any energy supply, intact lutoids (Crétin, 1982; Chréstin, 1985) as reconstituted tightly-sealed tonoplast vesicles (Marin, 1982 and 1985 a and b; Marin et al., 1981 and 1985) accumulated $^{86}Rb^+$ (in the presence of valinomycin) and the cationic phosphonium probes, indicating a transmembrane electrical potential difference which was a more negative inside. The addition of MgATP to both materials results in a rapid change in the distribution of the potential probes, corresponding to a transmembrane depolarization (Fig. 2-A and B).

All these results confirmed by the use of fluorescent probes (Marin , unpublished data) led to the conclusion that the Mg-dependent ATPase located on the lutoidic tonoplast works as an electrogenic proton-pump, setting up a high (inside more positive) electrochemical gradient ($\Delta\overline{\mu}_H+$) across the lutoidic tonoplast, then against the thermodynamic equilibrium.

Finally, the proton-pumping ATPase of the lutoidic tonoplast has more recently been fully described and solubilized (Gidrol et al., 1985; Marin et al., 1985 and 1986).

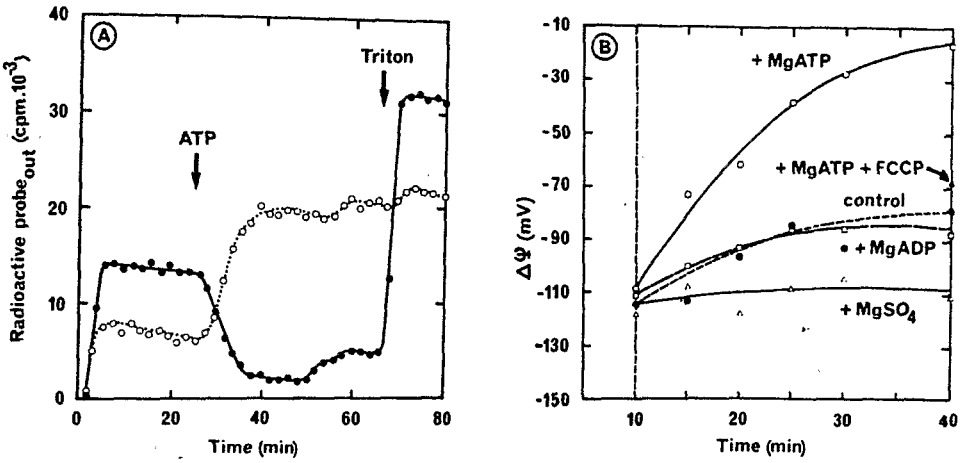

Figure 2

Change in time of the transtonoplastic electrical potential ($\Delta\Psi$) in the presence of Mg^{2+}-ATP

$\Delta\Psi$ was monitored either following the accumulation of ^{86}Rb in the presence of valinomycin using flow dialysis technique with intact lutoids (open circles, Fig. 2-A) or lipophilic cation probe ^{14}C-TPP^+ with reconstituted tonoplast vesicles using centrifugation (Fig. 2-B).

The Outward H^+-Translocating Activity of a Tonoplastic Redox System

A second H^+-translocating moiety has been revealed on the lutoidic tonoplast. It consists of a NADH-cytochrome c (artificial acceptor)-oxido-reductase (Crétin, 1983), perharps the same as the one, including cytochromes b-type, revealed by Moreau et al. (1975). Isopycnic centrifugation experiments confirmed the tonoplastic location of this H^+-pumping redox system, the activity of which closely followed the distribution of the typical lutoidic acid phosphatase and tonoplastic ATPase activities throughout the density gradient profile (Fig. 3) (Chréstin, 1985). The functioning of this redox chain induces H^+ efflux from freshly isolated intact latex vacuoles resulting in acidification of the cytosol and a collapse of transtonoplastic pH gradient (Fig. 4-A) (Crétin, 1983).

The H^+-translocating redox system was shown to work electrogenically and to cause membrane hyperpolarization (inside more negative : Influx of Rb^+) (Fig. 4-B), leading to the collapse of the transtonoplastic electrochemical gradient of protons (Chréstin, 1985).

The partial characterization of this redox H^+-pump showed that it is insensitive towards the classic inhibitors of the cytochromic respiratory chains (KCN, Antimycin A, etc.) and also those of the mitochondrial alternate pathway (Chréstin, 1983 and 1985).

ENERGIZATION OF SOLUTE TRANSPORT AT LATEX CELL TONOPLAST BY THE TWO ELECTROGENIC H^+-PUMPS

The differential functioning of these two opposing H^+-pumps at the latex cells tonoplast is then able to modulate the transtonoplastic electrochemical proton gradient. The latter was shown to energize numerous solute transports through the lutoidic tonoplast (Marin et al., 1982; Marin and Chréstin, 1985). As models, this paper focuses in particular on the processes involved in Ca^{2+} and citrate transport and accumulation within latex cell vacuoles.

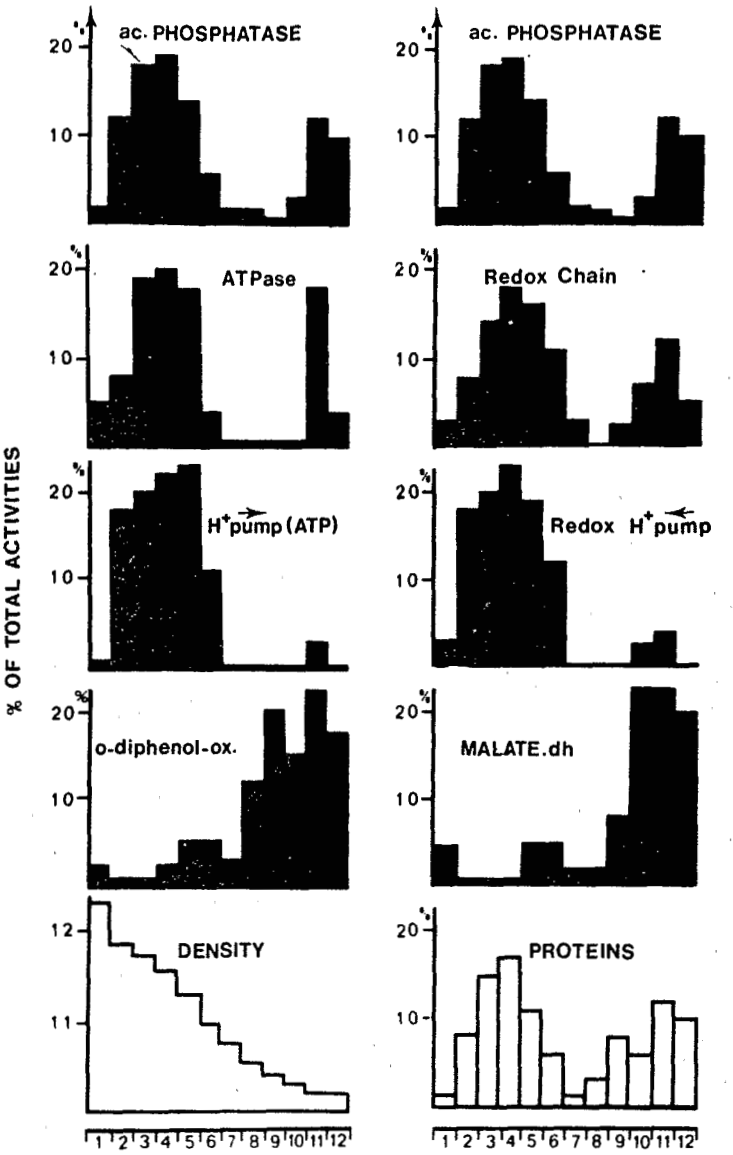

Figure 3

Localization of the proton-pumping activities
by density gradient centrifugation
of the latex organelles

An isotonic suspension of the bottom fraction from freshly centrifuged latex was layered on the top of the continuous sucrose gradient (0.6 to 1.8 M sucrose) containing 0.3 M mannitol, 50 mM Hepes-Tris at pH 7.0, and centrifuged 75 min. at 95,000 x g at 8°C. The different fractions were analyzed for their enzymatic and H^+-pumping activities according to Crétin (1982 and 1983).

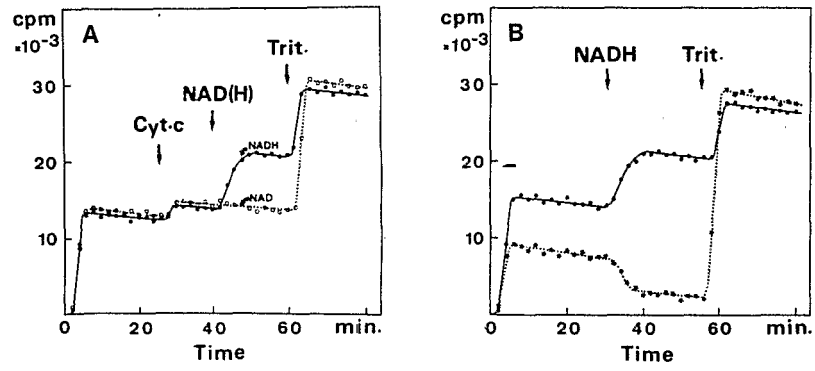

Figure 4

Evolution in time of the transtonoplastic ΔpH and $\Delta\Psi$ changes during the working of the lutoidic NADH-cytochrome c reductase

ΔpH changes were monitored by recording the transmembrane fluxes of methylamine using flow dialysis technique with intact lutoids (Fig. 4-A, with NADH: dots, with NAD: circles; and Fig. 4-B, dots and line).
$\Delta\Psi$ changes were monitored using $^{86}Rb^+$ in the presence of valinomycin (Fig. 4-B: stars and dotted line).

$\Delta\mu H^+$-controled Reversible Fluxes of "Free" vacuolar Ca^{2+} At the Lutoidic Tonoplast

Using flow dialysis method (Crétin, 1982), it was observed that even in the absence of any energy supply intact freshly isolated lutoids could intensively accumulate Ca^{2+} (freed by lutoid lysis). Since the addition of divalent cations (up to 2.5 mM Ca^{2+} or 12.5 mM Mg^{2+}) did not lead to release of the accumulated $^{45}Ca^{2+}$, we concluded that there were no isotopic exchanges and that Ca^{2+} retention by the lutoids could not be attributed to major adsorption on the external surface of the membrane (Chréstin, 1985; Marin et al., 1982).

Changes in the external pH induced rapid movement of Ca^{2+} across the tonoplast (Fig. 5-A) : Decrease in transtonoplastic ΔpH by acidification of the medium resulted in efflux of calcium while increase in ΔpH by alkalinization of the medium resulted in calcium influx in the vacuoles.

The supply of the suspension with Mg-ATP, which induces an intravacuolar acidification, and hence an supplementary increase in the transtonoplastic $\Delta\overline{\mu_H}^+$, led to simultaneous accumulation of $^{45}Ca^{2+}$ within the lutoids (Fig. 5-A and B) (Chréstin et al., 1984).

Ionophores such as nigericin or FCCP had no significant effect on Ca^{2+} fluxes when added to a vacuolar suspension kept in the resting state (no ATP-energized $\Delta\overline{\mu_H}^+$), but when added after the energization of the vacuolar Ca^{2+} uptake by MgATP, these protonophores were shown to induce a significant efflux of Ca^+ from the vacuoles, equivalent to the level of the ATP-stimulated uptake (Fig. 5-B) (Marin et al., 1982; Chréstin, 1985).

The addition of the non-electrogenic protonophore NH_4Cl to a non ATP-energized suspension, which at least in part decreases the non energized ΔpH

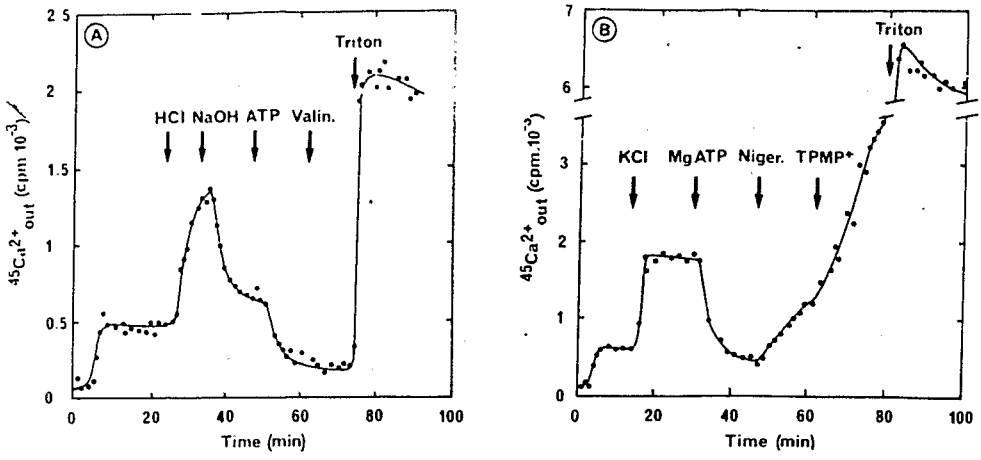

Figure 5

Effect of the changes in tonoplastic proton-motive force on transtonoplastic calcium fluxes in a suspension of intact lutoids

Fresh vacuoles were preincubated in the presence of 0.5 mM $CaCl_2$ and $^{45}Ca^{2+}$ at pH 7.5. Additions were as follows in the upper chamber of the flow-dialysis cell: A. HCl (to pH 6.0), NaOH (to pH 7.0), Ca-ATP (2.5 mM), valinomycin (0.8 μg/ml) and Triton (0.1% final concentration). B. Addition of KCl (130 mM plus valinomycin), Mg-ATP (5 mM), nigericin (16 μg/ml), $TPMP^+$ (2.5 mM) and Triton X-100 (0.1%).

(Donnan potential), did not induce significant efflux of Ca^{2+} (Marin et al., 1982). In contrast, the depolarization of the tonoplast by high external concentration of KCl (with valinomycin) or triphenylmethylphosphonium ($TPMP^+$) led to an important efflux of the intravacuolar calcium (Fig. 5-B).

Furthermore, the addition of the ionophore A-23187 (specific for the free divalent cations) was shown to induce only a small efflux of Ca^{2+} when added to resting vacuoles (Chrétin, 1985). In contrast, when added to ATP-energized vacuoles, A-23187 induced a significant efflux of Ca^{2+}, equivalent to the size of the Ca^{2+} pool accumulated in the presence of ATP (like FCCP) (Chrétin et al., 1984). This suggests that the ATP-energized Ca^{2+} transport through the tonoplast leads to the vacuolar accumulation of a pool of free Ca^{2+} against a thermodynamic equilibrium.

Furthermore, the functioning of the tonoplastic H^+-pumping system by addition of NADH plus cytochrome c to a suspension of lutoids, preloaded with $^{45}Ca^{2+}$ in the presence of MgATP, leads to an efflux of Ca^{2+} equivalent to the size of the ATP-energized Ca^{2+} pool (Fig. 6), simultaneously with the redox-pump dependent discharge of transmembrane $\Delta\bar{\mu}_{H^+}$ (Chrétin, 1985).

All these data seen as a whole led us to postulate the existence of two kinetic pools of Ca^{2+} within the latex cells vacuoles :

- a major pool sequestrated within the vacuolar compartment, owing probably to adsorption on intravacuolar structures by a Donnan type effect, and which could only be dissipated by high concentrations of KCl (+ valinomycin) or $TPMP^+$;

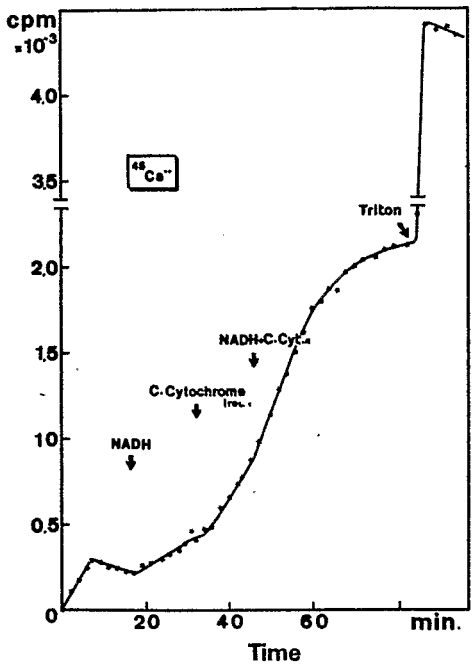

Figure 6

Efflux of calcium from the vacuolar compartment
during the operation of the tonoplastic NADH-cytochrome c-reductase

Intact fresh lutoids were preloaded with $^{45}Ca^{2+}$ (as 0.5 mM $CaCl_2$) in the presence of Mg-ATP (2.5 mM) and then transferred to the flow-dialysis cell. NADH (0.5 mM) and cytochrome c (0.2 mM) were then added first individually and then together. Finally the lutoids were lysed by addition of Triton X-100 (0.1%).

- an exchangeable pool of "free" Ca^{2+}, accumulated inside the vacuolar compartment against a thermodynamic equilibrium which could be dissipated by ionophores such as FCCP, nigericin + K^+ and ionophore A-23187 and the specific free divalent cations. This pool of free Ca^{2+} accumulated in the vacuolar compartment through ATP-energized $\Delta\overline{\mu_H}^+$ could be released into the external medium, both in vivo and in vitro, by the action of the tonoplastic redox protonpump which has been shown to dissipate the transtonoplastic $\Delta\overline{\mu_H}^+$.

Finally, as it could be shown that successive additions of small amounts of Ca^{2+} to an ATP-energized vacuolar suspension led to progressive collapse of ΔpH and even $\Delta\mu_H^+$ (Chréstin, 1985), we propose the existence of transport processes corresponding to a Ca^{2+}/H^+ exchange at tonoplast level, and that the adverse transtonoplastic fluxes of free Ca^{2+} remain under the energy-dependent control of both opposing H^+-pumps at the tonoplast.

Sequestration of citrate within the vacuoles of the Latex Cells

As the latex cell vacuoles accumulate citrate against a steep concentration gradient in vivo (Ribaillier et al., 1971), a lot of work was carried out to characterize the citrate transport processes and energization at latex cell tonoplast in vitro, using either freshly isolated intact lutoids (D'Auzac and Lioret, 1974; Montardy

and Lambert, 1977; Chrestin, 1985) or tonoplast vesicles reconstituted from lyophilized lutoids (Marin et al., 1981; Marin, 1982).

The native lutoids, and the tonoplast vesicles as well, were shown to accumulate, in vitro, exogeneous citrate against a steep concentration gradient. The kinetic parameters were clearly defined : Citrate uptake was temperature-dependent and linear for at least 30 min, even in the absence of any metabolic energy supply. Its initial rate as a function of citrate concentration in the medium was shown to display simple Michaelis-Menten kinetics with an apparent K_m value of 7 mM (then in the physiological range), where the three dissociated form predominates (Marin, 1982). The addition of MgATP to a suspension of intact freshly isolated lutoids as well as tonoplast vesicles was shown to generate a large increase in the magnitude of citrate uptake. In the presence of MgATP, the steady state level of citrate uptake and accumulation was shown to be 2 to 5 times higher than the level obtained in the presence of any energy source. Furthermore, the addition of protonophores such as NH_4Cl, FCCP and S-13 caused considerable reduction of citrate uptake in the absence fo energy supply, and completely stopped its activation by Mg-ATP. Finally, it was shown that all the known inhibitors of the lutoidic tonoplast ATPase did inhibit the activation of citrate uptake in the presence of MgATP (Marin et al., 1981; Marin et al., 1982; Marin, 1983 a and b; Chrestin, 1985).

It was then concluded that the energy indispensable for citrate uptake by the lutoids originated from the transtonoplastic gradient of proton, resulting from the functioning of the H^+-pumping ATPase located on the lutoidic tonoplast.

The direct, highly significant relationship linking the amplitude of the transtonoplastic gradient of citrate to the gradient of proton as determined in vivo (Table 1) agrees well with a force derived from the transtonoplastic ΔpH as the energy source for citrate uptake and accumulation in the vacuoles, in vivo, as in vitro.

Moreover, as it was shown that any change in the magnitude of the transtonoplastic gradient of proton (ΔpH) as well as in the transtonoplastic electrical potential gradient ($\Delta\Psi$) induced parallel changes in the magnitude of citrate uptake (Fig. 7) it was definitely concluded that both components of the proton-motive force, then $\Delta\overline{\mu}_{H^+}$ itself, were involved in the energization of citrate uptake. Finally, as uptake of citrate was shown to induce internal alkalinization, Marin (1982) proposed the functioning of a tonoplastic H^+/citrate antiporter.

Since it has been wondered that role of citrate accumulation plays in lutoids as a storage or a detoxifying process, many attempts were made to characterize any mechanism able to control efflux of the citrate accumulated in the lutoids as well as in tonoplast vesicles.

Whatever the technic used : centrifugation and washing (Montardy and Lambert, 1977) or flow dialysis (Chrestin, 1985) and whatever the strategy adopted to try to induce citrate efflux (labile citrate accumulated in vitro, or cold citrate accumulated in vivo) from intact isolated lutoids, such as changes in the transtonoplastic $\overline{H^+}$ gradient by imposed external pH variations, the use of protonophores or diverse ionophores, the functioning of the outwards H^+-pumping tonoplastic redox chain, or changes in the transtonoplastic potential using either KCl + valinomycin or lipophilic cations (TPP^+ or $TPMP^+$), in the presence or absence of various concentrations of exogenous citrate in the medium, and at three temperatures (20, 30 and 40°C), neither of the authors was able to shown any significative efflux of the citrate that had been accumulated in vivo or in vitro by intact vacuoles (Fig. 8-A and B) (Montardy and Lambert, 1977; Chrestin, 1985). It was then concluded that the quasi totality of the citrate accumulated remained definitively entrapped within the native lutoids, constituting in a true detoxification process.

On the contrary, working with tonoplastic vesicles reconstituted from lyophilized lutoids, Marin (1982) did show evidence for the occurrence of massive efflux of citrate : up to 80% of the previously accumulated citrate. This efflux was shown to be temperature-dependent, and to increase with the concentration

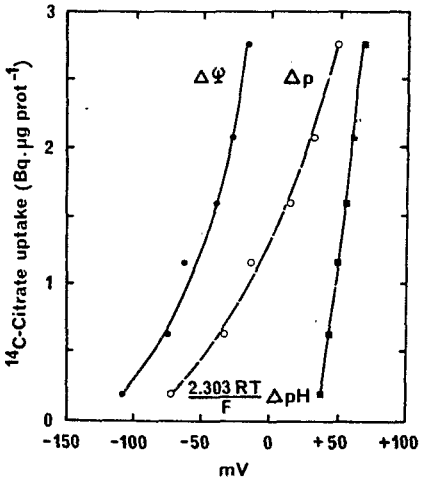

Figure 7

Relation between the components of the proton-motive force (Δp) and citrate incorporation by tonoplast vesicles

of citrate in the medium. Yet, upon the uptake of labelled citrate by these vesicles, the isotopic enrichment of the internal compartment did not exceed 8% of the external specific activity. As a result, isotopic equilibrium was never reached.

From these data obtained on native vacuoles and tonoplastic vesicles, it was finally concluded that there might be exist an "internal compartmentation" of the vacuolar citrate into two distinct kinetic pools: a minor, directly exchangeable pool, and a major one (more than 97% of the total vacuolar citrate) assumed to remain sequestrated within the native vacuoles. Insofar as it was shown that citrate and Mg^{2+} are present in quasi stoichiometric concentrations in latex, and in particular in the lutoids (Coupé, 1977; Jacob, unpublished data), it was proposed that the vacuolar citrate might be sequestrated in the complex form citrate^{3-}-Mg^{2+} in intact native lutoids (Marin, 1982; Marin et al., 1982). It was then suggested that the massive efflux of citrate observed in tonoplast vesicles was somewhat artifactual (i.e. non physiological), and might be due to the loss of intralutoidic sequestrating factors, including Mg^{2+}, during tonoplast vesiculation from lyophilized lutoidic membrane in vitro. It was then assumed that in vivo the triacid is accumulated and sequestrated inside the vacuolar compartment in the complex form citrate^{3-}-Mg^{2+}, considered as the impermeant form which represents by far the largest pool of the vacuolar citrate (more than 97%). A very minor pool (less than 3%) of free citrate^{3-}, might exist as a possible exchangeable pool. Computer kinetic simulations based on this model were shown to be quite reconciliable with these experimental data (see Marin, 1982). Such models involved the working of citrate translocator antiporterly with a proton, one molecule of Mg^{2+} being simultaneously transferred from the cytosol to the intra-lutoidic space, then being sequestrated in this complex non-permeant form (Marin, 1982). Fig. 8-A agrees

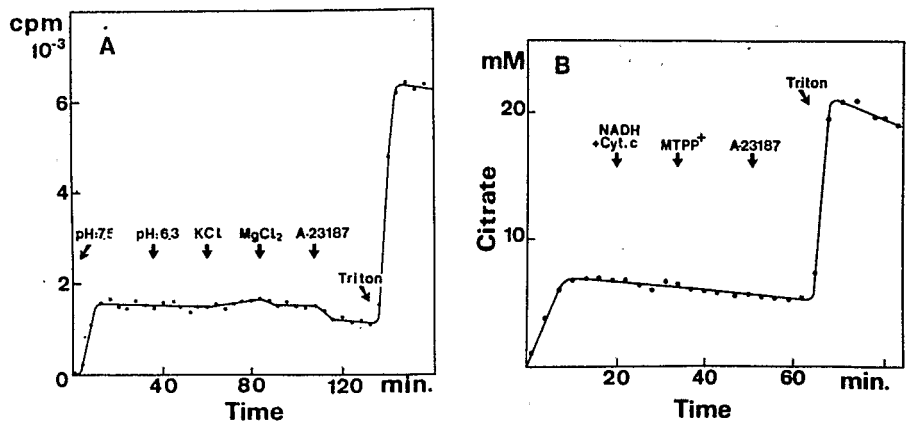

Figure 8

Unfruitful attempts to provoque efflux of the intravacuolar citrate with intact lutoids

A. Intact fresh lutoids were preloaded with ^{14}C-citrate at pH 7.5 (external citrate: 5.5 mM), and transferred to the flow dialysis cell. The medium was then acidified by addition of HCl. Further additions were as follows : KCl (130 mM + valinomycin), $MgCl_2$ (2.5 mM), A-23187 (0.2 µg/ml) and then Triton X-100 0.1%.
B. Intact lutoids were incubated in unlabelled citrate (7 mM) for 30 min and then transferred to the flow-dialysis cell. Additions were as follows: NADH (1 mM) plus cytochrome c (0.5 mM), then $TPMP^+$ (2 mM) and then A-23187 (0.2 µg/ml) without Mg^{2+}. Finally, the lutoids were lysed with Triton X-100 (0.1%). Citrate was determined enzymatically in the flow-dialysis effluent.

with the suspected important role of Mg^{2+} in citrate uptake and accumulation, as far as in the presence of the divalent cation ionophore A-23187 and the addition of excess $MgCl_2$ to the suspension induces a significant uptake of citrate by intact lutoids.

Taking into consideration the fact that citrate (a potent inhibitor of some cytosolic key enzymes) remains mainly entrapped within the native lutoids, it is proposed that this vacuolar compartment essentially plays a role of detoxifying trap, ensuring detoxification of the cytosolic metabolism against any excessive accumulation of this triacid in the cytosol. This proposal fully explains the direct highly significant relationships linking the production of latex (rubber) with high transtonoplastic gradient of citrate in the latex, i.e. low concentration of citrate in the cytosol but high accumulation in the vacuolar compartment in vivo (Table 1).

CONCLUSION

Control of metabolism within the latex cells by intracellular pH and by ionic composition of the cytosol has attracted more and more interest during the past few years, because of their major impact on natural biosynthesis of rubber, and hence on production. When present in excess, H^+, Ca^{2+} and citrate which are potent inhibitors of some key enzymes of the cytosolic metabolism, are removed from the latex cell cytosol and accumulate in the vacuoles.

As far as protons are concerned, all the data reported here demonstrate the existence of two H^+-pumping systems, definitely located on the lutoidic tonoplast, able to control opposite transtonoplastic fluxes of protons. Their respective sensitivity towards the pH of the medium (see Chrestin et al., in this issue) is in good agreement with their functioning as a true biophysical pH-stat, controlling adverse fluxes of H^+ across the lutoidic tonoplast using an energy-consuming system, thus regulating the cytoplasmic pH. It was shown that there are two kinetic pools of accumulated protons inside intact vacuoles. A more or less constant one, equivalent to 1 pH unit, accumulated at the thermodynamic equilibrium in the vacuolar compartment owing to some Donnan potential. This immobilized pool could only be released by the artificial neutralization of the Donnan potential through additions of permeant cations. A second pool of H^+ with highly variable size (accounting for 0.1 to 1 pH unit of the total transtonoplastic ΔpH gradient), forms an exchangeable pool of free protons accumulated in the vacuolar space against the thermodynamic equilibrium through the functioning of the tonoplastic H^+-pumping ATPase. This exchangeable pool of H^+ can be reinjected in the cytosolic compartment through the working of the outwards tonoplastic H^+-pumping redox system.

The two opposing H^+-pumps were shown to work electrogenically, and then to modulate the amplitude of the transtonoplastic electrochemical proton gradient ($\Delta\overline{\mu}_{H^+}$), which has been shown to energize transports of various solutes across the membrane (Marin, 1982; Marin and Chrestin, 1985), and in particular citrate and calcium.

Each of the citrate and the calcium pools accumulated in the vacuolar space could be subdivided into two distinct kinetic pools. The first and major pool corresponds to a non exchangeable pool where solutes remain entrapped within the vacuolar compartment according to some Donnan potential (this is the case of H^+ and probably Ca^{2+}) or other trapping processes such as immobilization of citrate in the impermeant complex form $citrate^{3-}$-Mg^{2+}. The second, minor pool, whose size varies considerably, reversibly accumulated in the vacuolar compartment. These exchangeable pools can be placed again at the disposal of the cytosolic metabolism through the functioning of outward H^+-pumping redox system. The exchangeable pool can be relatively important as far as Ca^{2+} is concerned (up to 25% of the total vacuolar calcium), whereas it is reduced (3 to 5% to non-existent) for citrate.

Consistently with the functioning of the two opposing H^+ pumps at the lutoidic tonoplast, we propose that the lutoids, the vacuolar compartment of the latex cells, play a triple role as a "biophysical pH-stat", a detoxifying trap (citrate, etc.) and a storage compartment (Ca^{2+}, H^+, ..), thus controlling homeostasis in the cytosol and favouring active metabolism within the cells, thus resulting in high latex (natural rubber) production.

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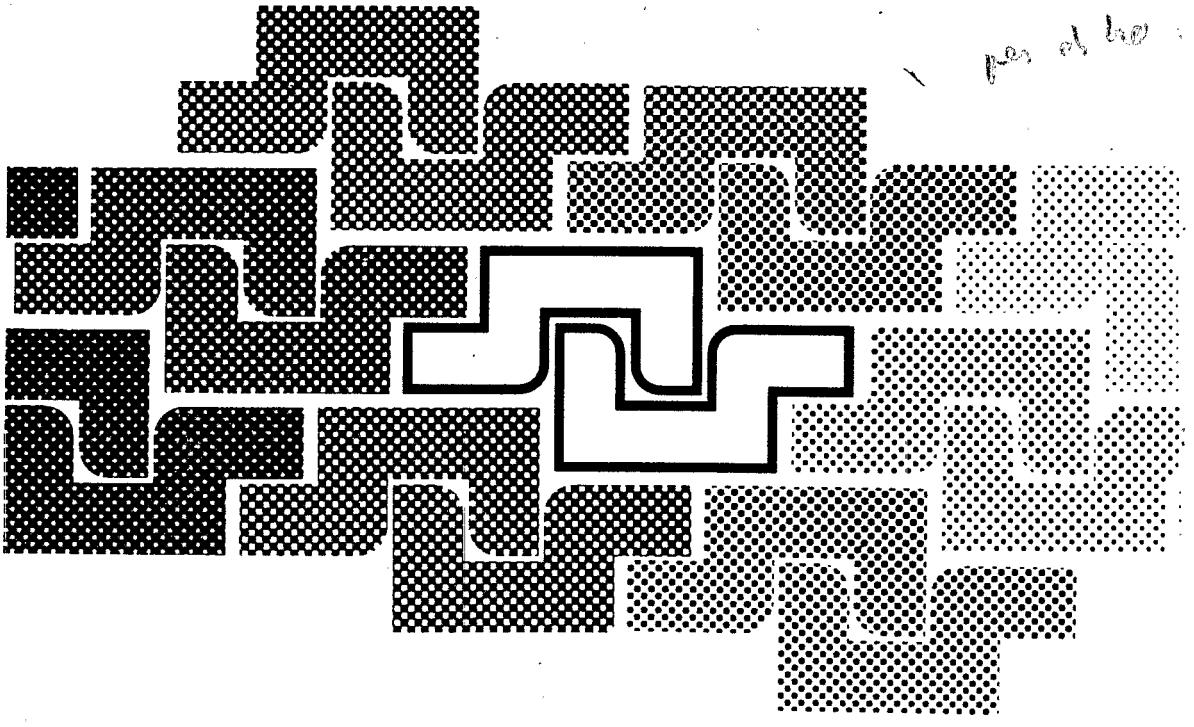

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Edited by

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