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Review

A plant vacuolar system:
the lutoids from *Hevea brasiliensis* latex

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Abstract. Lutoids, which comprise nearly 20% of the latex volume, are unit-membrane organelles from 1 to 5 µm in diameter. They constitute a dispersed lysosomal vacuome in a specialized cytoplasm, that is the latex. Lutoids accumulate certain compounds from the cytoplasmic medium. Pi, citrate and Mg^{2+} are about 10 times more concentrated and basic amino acids are also accumulated. Acid hydrolases, characteristic of animal lysosomes, are present in the lutoids which also contain peroxidase, lysozyme and α-mannosidase. The lutoid membrane has an Mg-dependent ATPase which ensures an influx of protons and, therefore, vacuolar acidification. A membranous NADH-cytochrome *c*-reductase may ensure a proton efflux from the lutoids; it could evolve into a $NADH-O_2$ reductase, generator of superoxide ions. The physiological roles of lutoids in the latex is analysed both for the situation where they remain intact and where they liberate their content into the laticiferous cytoplasm.

Key words: latex, lutoids, vacuoles, lysosomes, Hevea brasiliensis.

Résumé. *Les lutoïdes, constituant près de 20% du volume du latex, sont des organites monomembranaires de 1 à 5 µm de diamètre. Ils constituent au sein du cytoplasme spécialisé qu'est le latex un vacuome lysosomal polydispersé. Les lutoïdes accumulent certains constituants du milieu cytoplasmique. Pi, citrate et Mg^{2+} y sont concentrés environ 10 fois; les acides aminés basiques y sont également accumulés. Les hydrolases acides, caractéristiques des lysosomes animaux, sont présentes dans les lutoïdes, qui contiennent aussi peroxydase, lysozyme et α-mannosidase. La membrane lutoïdique possède une ATPase Mg-dépendante, assurant un influx de protons et donc l'acidification vacuolaire. Une NADH-cytochrome c réductase membranaire assurerait un efflux de protons à partir des lutoïdes; elle pourrait évoluer en une $NADH-O_2$ réductase génératrice d'ions superoxydes. Les rôles physiologiques des lutoïdes dans le latex sont présentés selon que ces organites conservent leur intégrité ou qu'ils libèrent leur contenu dans le cytoplasme laticifère.*

Mots clés : latex, lutoïdes, vacuoles, lysosomes, *Hevea brasiliensis*.

INTRODUCTION

The first work on latex was done about 1930 by FREY-WYSSLING and a Dutch team in Bogor. The rise of biochemistry led LYNEN during the 1950 s to use latex to discover the way in which isoprenoi are synthesized. Then, with latex likened to a specialized, easily accessible cyto   , it served as material in diverse laboratories. So, as early as 1966, PUJARNISCL suggested that the latex particles called lutoids, which represented nearly 20% of   latex volume, had all the characteristics of animal lysosomes. The study of th e lutoids has largely contributed to the definition of the vacuo-lysosomes of the plant kingdom.

Laticiferous system, latex and lutoids

Hevea brasiliensis latex is harvested by tapping. This most often entails the periodic removal of a thin bark layer on a tapping cut of the *Hevea* trunk. This cuts the latex tubes of the bark, where they are arranged in concentric layers around the cambium which engenders them periodically. Each coat consists of anastomosed latex tubes (BOBILLIOFF, 1923). Such an arrangement favors the flow of a relatively large quantity of latex (100 to 500 ml in the course of a tapping).

The *Hevea* latex flows for several hours after tapping and consists of the fluid part of the latex tube cell cytoplasm (MILANEZ, 1946; DICKENSON, 1964, 1969). A thicker parietal cytoplasm remains attached to the latex tube walls and electron microscopy shows that this viscous latex contains the few nuclei and mitochondria remaining in the adult latex tube (DICKENSON, 1969). The latex which flows during tapping contains an average of 35% rubber, that is *cis*-polyisoprene, present in the form of globules of a few tenths of a micrometer in diameter surrounded by a phospholipoprotein membrane (HO *et al.*, 1975).

Ultracentrifugation of latex (50,000 g; 60 min), first done by COOK and SECKHAR (1955), reveals at least 3 fractions. The rubber particles rise above an almost clear supernatant and a voluminous, more or less yellow pellet representing up to 20% of the latex volume. The centrifugation of latex on a sucrose gradient (PUJARNISCLE, 1968; RIBAILLIER, 1972; MARIN and TROUSLOT, 1975) has permitted a finer resolution, in that the sediment is separated into slightly coloured lutoids comprising the larger part and into a lesser particulate fraction of a vivid yellow colour, discovered as early as 1929 by FREY-WYSSLING. Today these latter particles are considered to be a special type of plastid (GOMEZ and MOIR, 1979; HÉBANT, 1981).

Lutoids appear in electron microscopy as spherical particles from 0.5 to 3 μm in diameter circumscribed by a simple membrane about 80 Å thick (DICKENSON, 1969). They are extremely osmosensitive (PUJARNISCLE, 1968). In the interior of the latex tubes, these lutoids can present two different forms. Most often they are optically empty. Sometimes, particularly in the youngest latex tubes, they contain fibrillar proteins regrouped in strands (DICKENSON, 1960, 1969; HÉBANT, 1981).

Sequential differential centrifugation has revealed the presence of ribosomes and functional polysomes (COUPÉ and D'AUZAC, 1972, 1974). In addition, the presence of peroxisomes, characterized by a malic dehydrogenase and a non-cytoplasmic catalase, has been predicted (COUPÉ *et al.*, 1972; CRÉTIN and HANOWER, unpublished results).

Chemical composition of lutoids

The content of lutoids

Respecting the osmosensitivity of lutoids, it is possible with several differential centrifugations to separate and purify large quantities of lutoids, before analyzing their content in relation to the cytoplasm in which they are immersed. As early as 1957, WIERSUM noted the facility with which lutoids absorbed neutral red and this led him to hypothesize that the lutoids constituted a dispersed vacuome in the interior of the latex.

In order to verify the vacuolar character of lutoids, the distribution of ions between the lutoidic medium and the cytoplasm was determined (COOK and SEKHAR, 1955; RIBAILLIER *et al.*, 1971, COUPÉ, 1977). The ratio between intralutoidic and cytoplasmic concentrations is almost 1 for K^+ (1.0-1.2), between 5 and 10 for Ca^{2+} and Mg^{2+}. It is between 1.5 and 3 for Cu^{2+}. It ranges between 8 and 15 for

TABLE I

Solute accumulation in lutoidic compartment

Solute	Concentration (mM)		Lutoids
	lutoids	cytoplasm	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
Pi.	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.

acid soluble phosphate (Pi) (*tab.* I). Divalent cations and Pi are therefore accumulated in the lutoidic compartment while K^+ is not.

The principal latex sugar is sucrose at a concentration of about 10 mM (TUPY and RESING, 1968). It is at a lower concentration in the lutoidic medium (about 10% latex sucrose) (RIBAILLIER *et al.*, 1971; JACOB, unpublished results).

Citric and malic acid are the main latex organic acids (D'AUZAC, 1965). The ratio of citrate accumulation in lutoids is near 10, but is only 1 for malate (RIBAILLIER *et al.*, 1971).

Amino acids are distributed differently between the cytoplasm and the lutoidic medium according to their nature (BRZOZOWSKA *et al.*, 1974). So it is that the basic amino acids (Arg, Orn, Lys, γ-aminobutyric acid, etc) are accumulated from 5 to 20 times in the interior of the lutoids, while acid and neutral amino acids are 3 times more concentrated in the cytoplasm (*tab.* I). Such accumulation of basic amino acids, and particularly of arginine, has been observed in *Saccharomyces cerevisiae* vacuoles (WIEMKEN and DURR, 1974; BOLLER *et al.*, 1975). The same holds for diverse basic amino acids in *Candida utilis* vacuoles (WIEMKEN and NURSE, 1973).

In addition, from 30 to 50 phenolic aglycones are present in latex. They seem to be present in both fractions (HANOWER *et al.*, 1979). Studying the composition of the intralutoidic serum, ARCHER *et al.* (1969) showed the absence of low molecular weight nucleotides, while confirming the presence of alkaloids such as ergothioneine and trigonelline, the former having been detected there at the same time as hercynine by TAN CHEE HONG and AUDLEY (1968). These authors, analyzing latex from different sources, came to the conclusion that ergothioneine is of fungal origin, and that it accumulates in the latex lutoids to the extent that the soil contains the fungi which secrete it.

Lutoids contain about 20% protein in relation to dry weight (ARCHER and SEKHAR, 1955 *a*; ARCHER and COCKBAIN, 1955; ARCHER *et al.*, 1969). Heveine, with a molecular weight of 10,000, represents about 70% of these proteins. It is characterized by a sulphur content of about 5% (ARCHER, 1960). Moreover, as early

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as 1942, Roe and Ewart showed the presence in latex of high isoelectric point proteins, which later proved to come from the lutoids. Thus, two basic proteins were isolated by Archer (1976): hevamine A and B. In addition, fibrillar proteins, coming mainly from the lutoids of young tissues, were demonstrated (Dickenson, 1964, 1969; Hébant, 1981). They present a tightly bound helix structure (Dickenson, 1969; Gomez and Yip, 1975).

The lutoids contain not only 80S ribosomes but also RNA ribosomes which can be more or less broken down. Unbroken, they have a molecular weight of 1.3 and 0.7×10^6 and a composition identical to that of cytoplasmic RNA ribosomes (Marin *et al.*, 1974; Marin, 1978; Marin and Trouslot, 1975).

Lutoidic membrane

The biochemical composition of the lutoidic membrane has been studied by Dupont *et al.* (1976). It is characterized by an unusual content of phosphatidic acid (80% lipid phosphorus) and the absence of nitrogen phospholipids (phosphatidyl-choline and phosphatidylethanolamine). These last two compounds are, in contrast, present in the membrane which surrounds the rubber particles. Precaution taken by the authors eliminates a possible artefact due to the action of a phospholipase.

Furthermore, the fatty acid composition of the lutoidic membrane is original. The ratio between saturated and unsaturated fatty acids ($C_{16:0}$; $C_{18:0}$; $C_{18:1}$; $C_{18:2}$) is close to 1. Linolenic acid ($C_{18:3}$) is undetectable (*fig.* 1). The relative abundance of saturated fatty acids suggests to Dupont *et al.* (1976) resemblance between the lutoidic and other biological membranes, such as the outer membrane of mitochondria or chloroplasts: The membrane's lack of fluidity due to such a composition could be responsible for the fragility of the lutoidic membrane when confronted with osmotic shocks and physical constraints (Pujarniscle, 1968; Southorn, 1969). The only other work on the biochemical composition of a plant tonoplast seems to be that of Marty and Branton (1980) on sugar beet vacuoles. That tonoplast contains only 12% phosphatidic acid and nitrogen bases are present. It therefore differs appreciably from the lutoidic membrane. It could be that the large quantity of negative charges carried by the phosphatidic acid favours the colloidal stability of the principal elements which figure in latex, that is, the rubber particles and the lutoids, both of which are negatively charged (Jacob *et al.*, 1975; Dupont *et al.*, 1976).
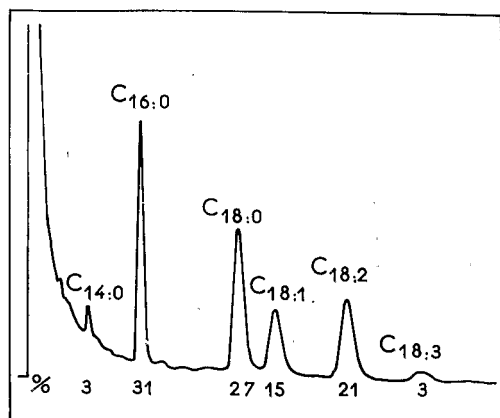

FIG. 1. — Fatty acid composition of lutoidic membrane (from Dupont *et al.*, 1976).

pH and transmembrane potential difference

The value of the intralutoidic pH greatly depends on the low molecular weight soluble compounds but also undoubtedly on the macromolecular compounds which have just been cited. Lutoids, like all vacuolar systems, have an acid internal pH, from 5.5 to 6.0 (LAMBERT, 1975; BRZOZOZOWKA-HANOWER et al., 1979). These values result from pH measurements made directly on the medium of burst lutoids. Estimates were also made from the transmembrane distribution of lipophilic probes (such as ^{14}C-methylamine) accross the intact organelle tonoplast (CRÉTIN, 1982 a; CRÉTIN et al., 1982).

Estimates of the transmembrane potential value have also been made on suspended fresh lutoids from the transmembrane distribution either of ^{86}Rb in the presence of valinomycin or of tetraphenyl-phosphonium. For the lutoids immersed in an isotonic medium (mannitol), in the presence of 10 mM KCl, the estimated $\Delta\Psi$ ranges from -70 to -80 mV, the interior being negative (CRÉTIN, 1982 a; CRÉTIN et al., 1982).

It is generally found that the vacuoles have a positive membrane potential relative to the cytoplasm (RONA, 1973; DUNLOP, 1976; RONA et al., 1980 a and b). The negative value found here may be explained, on the one hand, by the fact that the lutoid suspension medium is not the laticiferous cytoplasm and, on the other hand, by the fact that the lutoids are not functionning. It is indeed known that, in the presence of ATP, there is a strong depolarization of lutoids, which tends to cancel the negative value of $\Delta\Psi$ (CRÉTIN, 1982 a; CRÉTIN et al., 1982).

Lutoid enzymatic activities

Soluble lutoidic enzymes

It is obvious that an understanding of a cellular organelle requires description of its enzymatic activities. With the exception of the lutoidic phosphohydrolasic activities signalled by SMITH (1954) and ARCHER et al. (1963), the principal points have been elucidated by PUJARNISCLE (1965, 1966, 1968, 1969). In the lutoid compartment, he was able to show the presence of hydrolase equipment identical to that regularly found in animal lysosomes (phosphatase, phosphodiesterase, β-glucosidase, β-galactosidase, β-N-acetyl-glucosaminidase, cathepsine, ribonuclease, deoxyribonuclease). All the enzymes have an acidic activity optimum pH ranging from 4 to 6, and they show latency, that is, they are dosable only after the bursting of the lutoidic membrane (osmotic shock, detergent...).

It subsequently became apparent that 60 to 80% of the latex peroxidases were localized in the vacuolar compartment (COUPÉ et al., 1972). This would represent, along with the demonstration by GROB and MATILE (1980) of the presenve of 70% root peroxidase is horseradish vacuoles, the only biochemical localization of vacuolar peroxidase, while its cytochemical localizations are more classic (POUX, 1969; CZANINSKI and CATESSON, 1969; HALL and SEXTON, 1972). An o-diphenol oxidase is, in contrast, localized in FREY-WYSSLING particles (COUPÉ et al., 1972; BRZOZOWSKA-HANOWER et al., 1979). TATA et al. (1976), after MEYER (1948), showed that the two most abundant basic proteins (hevamines) of the lutoidic fraction presented a lysozymic activity which ensures the hydrolysis of certain bacterial cell wall peptido-glucanes. The amino acid composition, identical between the two hevamines, is remarkably similar to that of the fig latex lysozyme. In a general way, the two latex lysozymes, both cationic proteins, demonstrate some analogies with plant and animal lysozymes (TATA et al., 1976).

An α-mannosidase is present in the intralutoidic medium, while a smaller fraction appears to be absorbed on the membrane (D'AUZAC, 1981). This enzyme was first considered as a vacuolar enzyme by VAN DER WILDEN *et al.* (1973); they situated it on the yeast tonoplast, while BOLLER and KENDE (1979), MARTINOIA *et al.* (1981) and BOUDET *et al.* (1981) present it as intravacuolar in some higher plants.

As early as 1968, PUJARNISCLE compared latex lutoids to animal lysosomes as they had been defined by the DE DUVE group (DE DUVE, 1959; DE DUVE, 1966). Subsequent to the work done on yeast vacuoles and on microvacuoles of meristematic cells, MATILE was able to confirm the existence of a vacuo-lysosomal compartment in the plant kingdom (MATILE, 1975, 1978). The latex lutoids fit logically into this classification, of which they are one of the most studied elements, a fact often ignored by many.

Lutoidic membrane enzymes

Because of their enzymatic content, lutoids have contributed to the definition of plant vacuo-lysosomes. The study of the lutoidic tonoplast has, for its part, elucidated the function of this membrane. The stimulation by ATP of the absorption of certain solutes by a suspension of lutoids (D'AUZAC and LIORET, 1974; HANOWER *et al.*, 1977) led to the search for an ATPase on the membrane. After an acid phosphatase activity, adsorbed on the membrane, was selectively inhibited by molybdate or phosphate or removed subsequent to detergent action, the presence of a membrane ATPase was clearly demonstrated (D'AUZAC, 1975, 1977).

LAMBERT'S assertion of an ATP action on the acidification of the intralutoidic medium (1975) led to the hypothesis of a lutoidic membrane ATPase functioning as a proton pump (D'AUZAC *et al.*, 1977 *a* and *b*). This hypothesis was fully demonstrated on fresh or aged lutoids and on the reconstituted vesicles from the lutoidic tonoplast (CRÉTIN, 1982 *a*; CRÉTIN *et al.*, 1982; MARIN *et al.*, 1981; MARIN, 1981). Thus, the lutoidic ATPase proves to be an electrogenic proton pump, intervening in the energization of active solute uptake by lutoids (MARIN, 1981; MARIN *et al.*, 1982; CRÉTIN, 1982 *b*; CRÉTIN, unpublished results).

In addition to an ATPase system, the lutoidic membrane also has a redox system. MOREAU *et al.* (1975) were able to demonstrate the presence, on this membrane, of a NADH-cytochrome *c* oxidoreductase insensitive to antimycin and therefore different from the mitochondrial enzyme but comparable to the endoplasmic reticulum enzyme. It can function with ferricyanide but not with NADPH, yet the physiological receptor of this enzyme remains unknown. A NADH-cytochrome *c* reductase, differing from the mitochondrial enzyme by its insensitivity to antimycin, is sometimes found on vacuolar or microsomal membranes (MATILE, 1975, 1978). Its role has not been clearly defined.

Recent findings have shown that, in a suspension of lutoids incubated in the presence of exogenous cytochrome *c*, the enzyme was apparently able to function as a proton pump. It would thus ensure, at the expense of the cytoplasmic NADH, a proton efflux from the lutoidic compartment (CRÉTIN, 1982 *b*). In addition, in the latex lutoids from overexploited trees, a lutoidic NADH oxidase, which could be the same enzyme, is likely to function as NADH-O_2 oxido-reductase in producing the superoxide ion O_2^- (CRÉTIN, 1982 *b*).

MOREAU *et al.* (1975) also demonstrated the existence of 2 type-*b* cytochromes on the lutoidic membrane: b_{563} and b_{561}. The former is reduced by NADH and ascorbate and not by NADPH; the latter, only by hydrosulfite.

Physiological roles related to lutoid bursting

In a latex or a lutoid suspension the intactness of these particles can readily be determined by measuring their bursting index (BI). This measure, defined by RIBAILLIER (1972), uses the ratio of free acid phosphatase to total phosphatase activity achieved in the presence of a detergent (generally 0.1% Triton X-100). Furthermore, the mere observation of the lutoids, after centrifugation, gives a clear picture of their condition.

Lutoid action in the stopping of latex flow

The principal cause of the stopping of latex flow after tapping is a progressive closing of the end of the latex tubes. This was shown by SOUTHORN (1968, 1969) who observed, under a microscope, bark taken from the area of the tapping cut. The latex tubes contain, at their open end, plugs consisting of coagulated rubber and damaged lutoids.

Lutoids are directly implicated in this coagulation process which enables the stopping of the flow. On the one hand, the fall in turgor pressure related to opening the laticifer results in water influx capable of breaking lutoids, which are particularly osmosensitive (PAKIANATHAN, *et al.*, 1966; PUJARNISCLE, 1968). On the other hand, the mechanical fragility of the lutoidic membrane has been demonstrated: when the latex is introduced under pressure into capillary tubes (YIP and SOUTHORN, 1968), microflocs are formed only when the latex used contains its lutoids. Such a shearing effect actually occurs at the point where the latex flows out of the latex tubes due to the strong pressure gradient at this point (SOUTHORN, 1969). The coagulation of latex by lutoids may be explained by the liberation of protons, divalent cations, and positively charged proteins, all of which contribute to the destabilization of the negative colloidal suspension, that is, the latex (YIP and SOUTHORN, 1968).

The lutoids' coagulating role has been demonstrated in several ways. MILFORD *et al.* (1969) showed that in clones whose flow stops quickly after tapping, re-tappings every 15 min was sufficient to obtain, time after time, a considerable latex flow increase. Elimination of a thin bark pellicle and the obturated part of the latex tube contained in this pellicle suffices to restart the flow. A latex tube plugging index was thus able to be established (MILFORD *et al.*, 1969). RIBAILLIER (1972) demonstrated the inverse correlation between the lutoid bursting index (mentioned above) and latex production. This correlation explains others, such as that between the production and plugging index (inverse correlation), and between the lutoid bursting index and the latex tube plugging index (direct correlation) (RIBAILLIER, 1972; ESCHBACH *et al.*, unpublished results).

The FREY-WYSSLING particles, which are just as fragile as lutoids, also contribute, upon bursting, to the external coagulation of latex upon contact with the oxygen in the air because of the freed *o*-diphenoloxidase (HANOWER *et al.*, 1976; BRZOZOWSKA-HANOWER *et al.*, 1978).

Hormonal stimulation of latex production

Injections of trace elements (Cu, B) at the tapping cut level (COMPAGNON and TIXIER, 1950) or treatment of the tapping panel with synthetic auxins (2,4-dichlorophenoxyacetic acid, naphtalene acetic acid; etc.) (CHAPMAN, 1951) have been used for a long time to increase the production of *Hevea* latex. These products are now replaced by 2-chloroethylphosphonic acid (Ethrel) (ABRAHAM *et al.*, 1968; D'AUZAC and RIBAILLIER, 1969). It soon became apparent that the action of all the stimulants (which have in common a capacity to produce ethylene *in situ*) involved an augmentation in the duration of latex flow. This last finding would reasonably
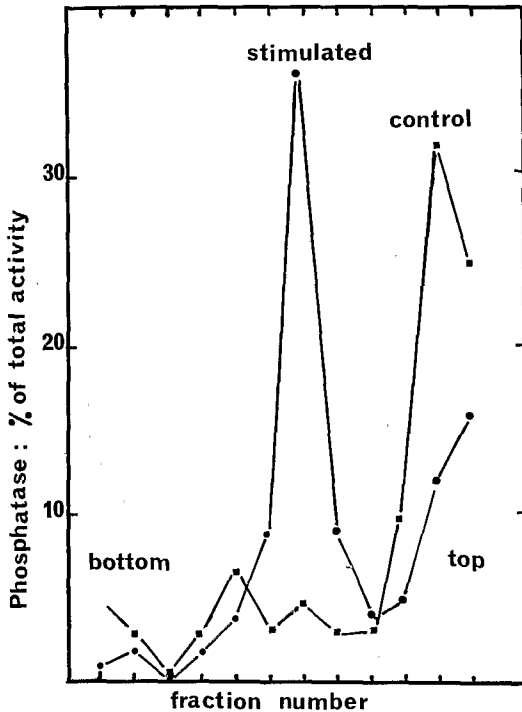
FIG. 2. — Ethrel stimulation of latex production: effect on lutoid stability as evidenced by their localization on a sucrose gradient after equilibrium centrifugation.

Lutoids in good condition are located in the middle of the gradient (refer figure 3), burst lutoids are on the top.

be explained by an increase of the lutoid's stability (*fig.* 2) and by a change of its membrane permeability (RIBAILLIER, 1972; COUPÉ and LAMBERT, 1977; HANOWER *et al.*, 1977; CRÉTIN, unpublished results). Nonetheless, biochemical analysis of the lutoidic membrane has not explained the apparent variation of the properties of this tonoplast (HANOWER *et al.*, unpublished results).

The physiological disease of dry bark

The overexploitation of *Hevea* can lead to a complete stopping of latex flow, consecutive to a drying up of the latex tubes. This phenomenon has been called "Encoche sèche" (Dry bark) in French and "Brown Bast" in English; it was characterized as early as 1921 (PETCH, 1921; RANDS, 1921; SANDERNSON and SUT-CLIFFE, 1921). A recent microscope study showed that the typical dry bark was characterized, in the early stages, by a coagulation of latex *in situ* and the invasion of the latex tubes by tylosoids from neighbouring parenchymatous cells (DE FAŸ, 1981).

Today the postulate of PUJARNISCLE and RIBAILLIER (1966) on the significant role played by the lutoids in dry bark is well founded. The examination of latex from trees with particularly dry bark reveals virtual absence of lutoids in the ultracentrifugation sediments, a particularly high bursting index and a considerable lightening of the residual lutoids (*fig.* 3) (CRÉTIN, unpublished results).

Recent work by CRÉTIN (unpublished) has led to a general hypothesis which, on the whole, explains the *in situ* coagulation of latex, observed by DE FAŸ (1981), at the onset of dry bark. In the latex from partially diseased trees, CRÉTIN showed, by polarography, marked absorption of oxygen by a suspension of lutoids in the presence of exogenous NADH, without the mitochondria being implicated. This absorption is limited, then suppressed, by the successive addition of superoxide
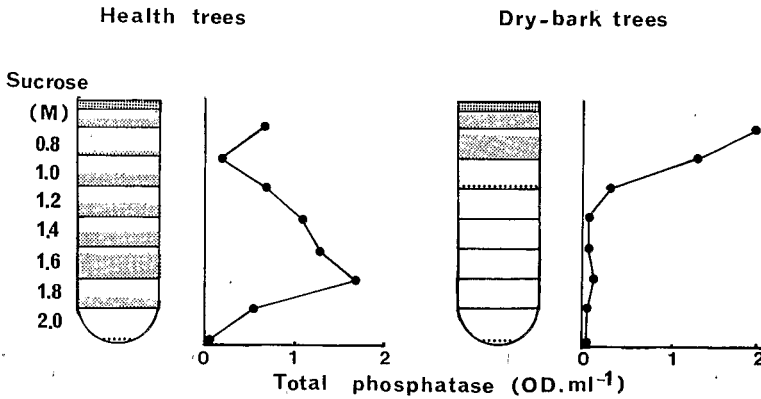
FIG. 3. — A physiological disease (dry bark) modifies lutoids localization on a sucrose gradient.

dismutase (SOD), then catalase. If NADH is added in the presence of SOD, O_2 consumption is very limited. It is here again suppressed by the addition of catalase (*fig.* 4).

Furthermore, on a suspension of lutoids from the same type of latex, the following is observed as a function of the time after the addition of NADH: an
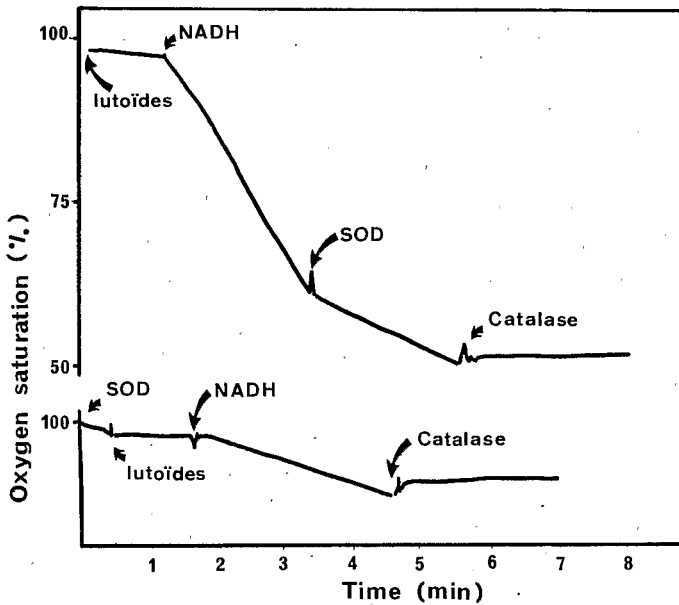

FIG. 4. — Polarographic determination of a NADH-O_2 reductase activity in lutoids after an ultrasonic treatment.

250 µl of lutoid suspension ws added to 0.1 M Tris-HCl (pH 7.4): 5 ml. Arrows indicate addition of 0.5 mM NADH; superoxide dismutase: 50 µl (1 mg. ml^{-1}); catalase: 50 µl (1 mg. ml^{-1}).

increase in the lutoid bursting index, a regular consumption of NADH related to that of oxygen, a regular liberation of malondialdehyde from exogenous linoleic acid (*fig.* 5).

All of these findings, together with the discoveries of superoxide dismutase in the latex and the action of complexed ion and copper, led CRÉTIN to propose that the breaking down of lutoids *in situ* is dependent on the production of toxic oxygen (H_2O_2, O_2^-, OH)susceptible to attack the unsaturated fatty acids present in the membranes. In the latex of healthy trees, the system would be nonfunctional as a result of the inactivity of $NADH - O_2$ reductase or the presence of protector systems, enzymatic (superoxide dismutase and catalase) or not (reduced thiols, ascorbic acid). It should be noted that direct correlation between the level of latex thiols (protectors) and yield has often been shown (D'AUZAC, 1965; CRÉTIN, unpublished results; VAN DE SYPE *et al.*, unpublished results). The scheme in figure 6 illustrates this hypothesis concerning the role of lutoids in coagulation *in situ* of the latex associated with dry bark.

It is clear that, in the case of dry bark, the lutoid action leads in the end to the autolysis of the tissue just as occurs upon senescence, for example, in *Ipomea purpurea* petals (MATILE and WINKENBACK, 1971) or wheat leaves (SHAW and MANOCHA, 1965). In all these phenomena, bursting of the vacuolar membrane is implicated (MATILE, 1975).

Physiological roles involving the intact lutoid

Intravacuolar storage involves reversible phenomena, while detoxification would be an irreversible absorption.

Storage and detoxification

Among the detoxifications occurring at the lutoid level, the most distinct concerns ergothioneine, produced by soil fungi, absorbed by *Hevea* and stored, at least in part, in the latex lutoids (ARCHER *et al.*, 1969). This seems to involve vacuolar detoxification specific to an exogenous alkaloid, which can be likened to that of such endogenous alkaloids as sanguinarine in *Chelidonium majus* latex vacuoles (MATILE *et al.*, 1970), or nicotine in *Nicotiana rustica* vacuoles (SAUNDERS, 1979) or morphine in *Papaver somniferum* latex vacuoles (FAIRBAIRN *et al.*, 1974). This is equally comparable with the accumulation of the cyanogenic glycoside dhurrin in *Sorghum bicolor* vacuoles (SAUNDERS and CONN, 1978) or that of betacyanin in *Beta vulgaris* vacuoles (LEIGH and BRANTON, 1976).

Various ions and molecules accumulate in the lutoids against a concentration gradient, but is not yet possible to ascertain whether this involves storage or detoxification. Inded, Mg^{2+} accumulating in the lutoids would destabilize the latex colloid (RIBAILLIER, 1968). Citrate may be trapped in the form of Mg^{2+} salt in the reconstituted lutoidic vesicles (MARIN, 1981), which would explain its complete retention in fresh lutoids (MONTARDY and LAMBERT, 1977). In contrast, it should be remembered that ^{14}C-citrate may be transformed by latex into *cis*-polyisoprene, which can confer upon it a possible role of reserve (D'AUZAC, 1965).

MARTY *et al.* (1980) propose that the amino acids, mainly basic, which accumulate in vacuoles yeast, probably constitute a reserve of soluble nitrogen capable of being mobilized in the case of a nitrogen deficiency. Likewise, DICKENSON (1969) considers that the protein microfibrils of young lutoids can be regarded as protein reserves, able to be utilized during latex tube development, thus making an analogy with the aleurone proteins of cereal grains.
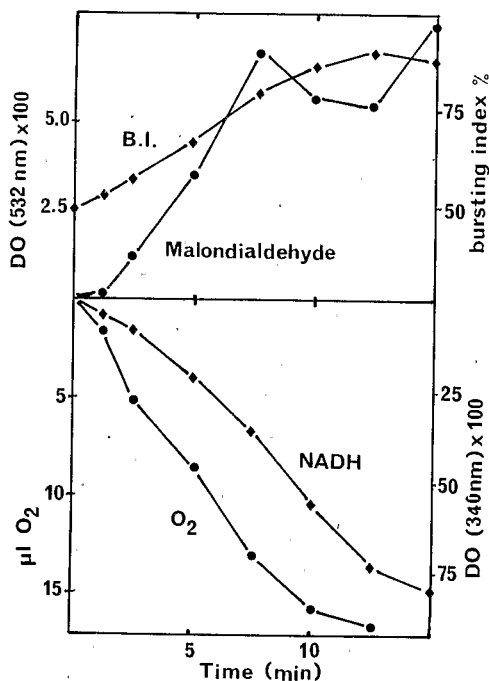
FIG. 5. — Evidence for degradation of lutoidic membrane in relation with NADH−O_2 reductase activity.

Conditions are the same as figure 4. After NADH addition, NADH utilization was monitored at 340 nm. O_2 consumption was followed by polagraphic determination. Bursting of lutoids was determined by the ratio of free and total phosphatase activities. By peroxidation, added linoleic acid (1 mM) generates malondialdehyde which was monitored at 532 nm.
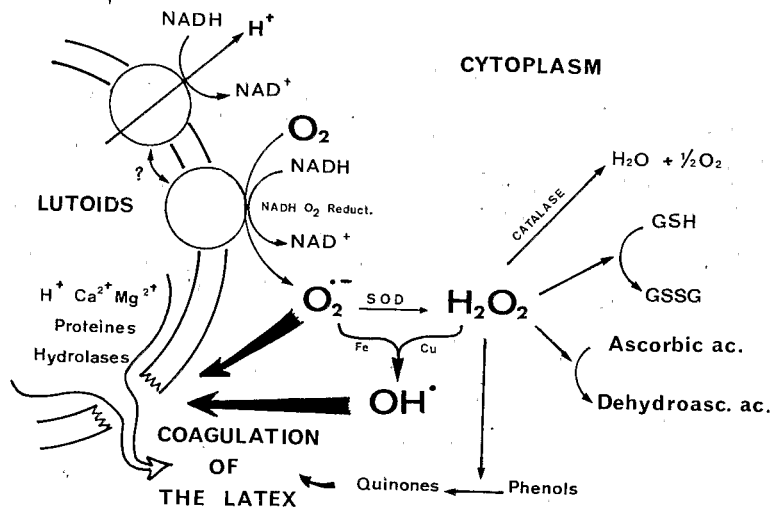

FIG. 6. — Interpretation for peroxidative degradation of lutoidic membrane and latex coagulation *in situ* related to dry-bark disease, upon disequilibrium of toxic oxygen producer and protector systems.

SOD, superoxide dismustase; GSH, reduced glutathione; GSSG, oxidized glutathione.

While sucrose is certainly cytoplasmic, it does not seem likely, that the lutoids constitute a storage site for glucides, as is the case for sugar beet root vacuoles (LEIGH *et al.*, 1979; DOLL *et al.*, 1979).

Lysosomal role

Lutoids contain a powerful arsenal of acid hydrolases whose utilization remains problematic. When lutoids burst *in situ*, it is known that this is followed by latex coagulation, and it is readily imagined that the hydrolases recycle utilizable metabolites to the availability of neighbouring cells. Note that this is an instance of an extreme case, almost pathological (dry bark). A more normal utilization of these hydrolases could occur by autophagy. The demonstration of degraded RNA in the lutoids favours the autophagy argument (MARIN *et al.*, 1974; MARIN, 1976).

A systematic investigation with electron microscopy of the latex tube cuts might reveal diverse phagocytoses. At present, relatively frequent presence of rubber particles in lutoids has been shown only in primary latex tubes. If such observations become more and more numerous, especially in secondary latex tubes, they would be in complete accordance with the argument that the lutoids play an autophagic role, traditionally attributed to plant vacuoles (MATILE, 1975; MARIN, 1978; BUVAT and ROBERT, 1979).

The presence of α-mannosidase in the lutoids (D'AUZAC, 1981) and particularly of β-N-acetylglucosaminidase (PUJARNISCLE, 1968) and of lysozyme (TATA *et al.*, 1976), three enzymes capable of attacking bacterial walls, intimates that the lutoids are capable by lysis or autophagy to deal with microbial invasions of the latex tubes.

The lutoidic hydrolases could intervene in the destruction of the transversal wall of the latex tube cells upon formation of the latex tubes (HÉBANT, 1981). However, it has been proven that a cellulase in the latex is localized in the cytoplasmic serum and not in the lutoids (SHELDRAKE and MOIR, 1970).

Regulation of cellular metabolism

The compartmentation at the interior of the lutoids of certain effectors of cytoplasmic enzymes can be considered, to a certain extent, as a regulation phenomenon. In effect, Pi, Mg, K, Ca, Cu, citrate and malate are recognized effectors of diverse cytoplasmic enzymes, such as NADP-phosphatase (JACOB *et al.*, 1970), also called 2'-nucleotidase (JACOB and SONTAG, 1973), and such as phosphoenol-pyruvate carboxylase (JACOB *et al.*, 1979), pyruvate kinase (JACOB *et al.*, 1980), invertase (JACOB *et al.*, 1981), glyceraldehyde-3-phosphate dehydrogenase (JACOB and D'AUZAC, 1972), and malic enzyme (JACOB and PRÉVOT, 1981).

An original aspect of the lutoid regulatory role, of lutoids metabolism in the laticifer is cytoplasmic pH regulation. Notably *via* invertase whose pH optimum is very narrow, slight latex pH variations lead to an activation of sugar metabolism and to an increase in latex yield (TUPY, 1969, 1973 *a* and *b*). Direct correlations between cytoplasmic pH and yield have been shown (PRIMOT *et al.*, 1978). In addition, direct correlations between ΔpH (between cytoplasmic and lutoidic media) and latex yield have been established (BRZOZOWSKA-HANOWER *et al.*, 1979; CRÉTIN *et al.*, 1980).

It appears that the lutoidic tonoplast ATPase functions to maintain a large ΔpH between the cytoplasmic and vacuolar compartments, while function in the opposite direction of a possible NADH-cytochrome *c* oxidoreductase would unfavorably influence the ΔpH (CRÉTIN, 1982 *b*). One can thus foresee the role of an ATP/NAD(P) H equilibrium in the pH regulation in the laticiferous cell.

Academically, the discussion remains open between the supporters of the internal acidification of lysosomes by DONNAN equilibrium (REIJNGOUD and TAGER, 1975; REIJNGOUD, 1978; HOLLEMANS, 1981) and the partisans of a lysosomal or vacuolar membrane ATPase involvement (SCHNEIDER, 1979). The results obtained with fresh lutoids (CRÉTIN, 1982 *a*) show that nearly 80% of the transmembrane pH gradient can be suppressed by the addition of K^+ in the presence of valinomycin. The conclusion was made that the functioning of the proton pump ATPase contributes to maintain the pH gradient, but also to energize the accumulations (especially of anionic substances) indispensable to the creation of DONNAN equilibrium (CRÉTIN *et al.*, 1982; MARIN *et al.*, 1982 *b*).

If one makes the very likely hypothesis that there exists a proton pump ATPase functioning in the sense of an efflux on the latex tube plasmalemma, the demonstrated presence of another ATPase on the tonoplast, which accumulates protons in the vacuole, contributes similarly to the maintenance of an essentially neutral cytoplasmic pH. In opposition to events contributing to cytoplasmic alkalinization, the active syntheses of malic and citric acid are essentially the results of the PEP carboxylase activity (JACOB *et al.*, 1979), while malic enzyme (JACOB and PRÉVOT, 1981) would constitute another element of the cytoplasmic pH regulator system: the pH-stat of DAVIES (1977) (JACOB *et al.*, 1978) (*fig.* 7).

Osmotic role of lutoids

In many adult plant tissues, the vacuoles represent a large percentage of the cellular volume; their high solute concentration confers on them, a very negative osmotic potential and a considerable role in cellular turgescence. However, demonstrations of such a role are quite rare. It is befitting to recall the case, cited by MATILE (1978), of *Candida utilis*, in which budding is related to a sudden increase of arginine absorption in the vacuole.
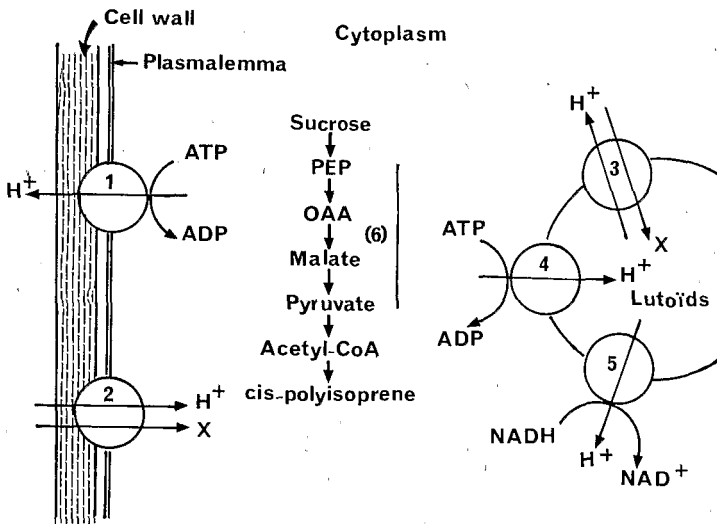

FIG. 7. — Interpretation for pH regulation in cytoplasm of laticiferous cells. Action of plasmalemma and tonoplast ATPases and of a pH-stat system.

1, plasmalemma ATPase; 2, proton symports; 3, antiports; 4, tonoplastic ATPase; 5, NADH-cytochrome *c* reductase; 6, pH-stat.

The fact that the volume of microvacuoles in *Hevea* latex does not represent any more than 20% of the harvested latex does not lead to according them a major role in the maintenance of osmotic pressure at the interior of the latex tubes. It is known, however, that latex osmolarity is about 350 to 450 milliosmol.1^{-1} (PAKIANATHAN *et al.*, 1966), while the turgescence pressure measured manometrically at the interior of soft bark tissues varies between 7.9 and 15 atm (350 to 700 milliosmol.1^{-1}) (BUTTERY and BOATMAN, 1966). However, PAKIANATHAN *et al.* (1966) accord a considerable role to the lutoids in order to explain latex coagulation. According to these authors the loss of turgor in the tissue upon tapping, due to opening the latex tubes, leads to a call for water and, by this, to an osmotic shock which promotes lutoid bursting and the appearance of microflocs which accumulate at the end of the latex tubes. It is ultimately followed by flow arrest (PAKIANATHAN *et al.*, 1966).

When considering which mineral ions might be the source of the osmotic potential of the lutoids, one notes a particularity in relation to the giant algae cells such as *Nitella translucens*. In these giant vacuoles K^+, Na^+ and Cl^- are the principal agents responsible for osmotic potential. They accumulate heavily in relation to the exterior medium (MAC ROBBIE, 1974), while Na^+ and Cl^- are more concentrated in the vacuoles than in the cytoplasm. These two ions are practically absent in the lutoids, while K^+ is divided equally between the cytoplasm and the vacuole. An equal distribution of K^+ between vacuole and cytoplasm has also been noted by LIN *et al.* (1977) in *Hippeastrum* and *Tulipa* petals.

CONCLUSION

Due to their ability to accumulate various ions and solutes, lutoids behave like classic vacuoles. However, the distinction between a detoxification and a reversible storage of absorbed solutes still remains elusive. The acidic hydrolase activities of the lutoids allows comparison with animal lysosomes, and the knowledge of this capacity has contributed greatly to the definition of plant vacuo-lysosomes. The way in which these hydrolases are utilized remains unclear, particularly when the intact compartment is conserved. With the exception of a few findings, an autophagic role for lutoids remains to be demonstrated.

Structures analogous to lutoids have been demonstrated in latex plants belonging to other families (SOUTHORN, 1964). One can reasonably surmise that these organelles do systematically intervene to stop the latex flow, as is clearly the case with *Hevea*.

Peroxidation of the lutoidic membrane *in situ*, following disequilibrium between toxic oxygen producer systems and protector systems, could be the primary mechanism of the physiological disease, dry bark, related to a drying up of the *Hevea* laticiferous system. Such a mechanism of peroxidative membrane breakdown, leading to loss of intracellular compartmentation, is known in the animal kingdom, notably in lysosomes (KUO LAN FONG *et al.*, 1973). It is beginning to be evoked in plants, in the phenomena of senescence (BRENNAN and FRENKEL, 1976; STEWART and BREWLEY, 1980), of degradation by an excess of light (RABINOWITCH and SKLAN, 1980) or of oxygen (FOSTER and HESS, 1980).

Whatever the case may be, the results presented here show a possible role for membranous NADH oxydoreductases, even if the main points undoubtedly remain to be discovered. The study of the lutoidic membrane has been particularly interesting owing to its biochemical composition, the originality of which has not yet been observed on similar structures, and due to the enzymes which are associated with it.

The evidence for an electrogenic ATPase proton pump on the lutoidic membrane constitutes the first unambiguous demonstration of the presence of a proton pump on a tonoplast. This pump is involved logically in the creation, at the vacuolar level, of a proton-motive force energizing the translocations which enable accumulation at the interior of this compartment. A more subtle role of the tonoplastic ATPase seems to be the regulation of cytoplasmic pH, in conjunction with a plasmalemma ATPase, both functioning to ensure proton efflux out of the cytoplasm. The lutoid ATPase is also involved in the maintenance of the proton gradient between the cytoplasm and the vacuole, either directly or by ensuring the active adsorption of anions likely to be involved in the Donnan equilibrium. Taking into account the correlations between cytoplasmic pH, or the pH gradient, and the *Hevea* latex yield, the importance of tonoplastic ATPase becomes evident.

The great facility to obtain from *Hevea* latex large quantities of pure, uninjured vacuoles contrasts with the difficulties encountered in the techniques, initiated by WAGNER and SIEGELMAN (1975), which entail the use of protoplasts.

The findings presented here clearly illustrate the typical vacuolar and lysosomal character of *Hevea* latex lutoids. Nevertheless it still remains true, as MATILE (1978) has remarked, that "Vacuoles differ biologically and functionally from one type of cell to another and perhaps even from the interior of a single cell." We would add, *a fortiori*, from one species to the next. Whatever the case may be a large part of the results obtained with lutoids contribute to knowledge of the plant vacuome.

REFERENCES

ABRAHAM P. D., WYCHERLEY P. R. and PAKIANATHAN S. W., 1968. — Stimulation of latex flow in *Hevea brasiliensis* by 4-amino-3,5,6, trichloropicolinic acid and 2-chloroethane-phosphonic acid. *J. Rubb. Res. Inst. Malaya*, **20**, 291-305.

ARCHER B. L., 1960. — The proteins of *Hevea brasiliensis* latex. 4. Isolation and characterization of crystalline heveine. *Biochem. J.*, **75**, 236-240.

ARCHER B. L., 1976. — Hevamine, a crystalline basic protein from *Hevea brasiliensis* latex. *Phytochemistry*, **15**, 297-300.

ARCHER B. L. and SEKHAR K. C., 1955. — The proteins of *Hevea brasiliensis* latex: I. Protein constituants of fresh latex serum. *Biochem. J.*, **61**, 503-508.

ARCHER B. L. and COCKBAIN E. G., 1955. — The proteins of *Hevea brasiliensis* latex. II. Isolation of the α-globulin of fresh latex serum. *Biochem. J.*, **61**, 508-512.

ARCHER B. L., AUDLEY B. G., COCKBAIN E. G. and MCSWEENEY G. P., 1963. — The biosynthesis of rubber: Incorporation of mevalonate and isopentenylpyrophosphate into rubber by *Hevea brasiliensis* latex fractions. *Biochem. J.*, **89**, 565-574.

ARCHER B. L., AUDLEY B. G., MCSWEENEY G. P. and TAN CHEE HONG, 1969. — Studies on the composition of latex serum and bottom fraction particles. *J. Rubb. Res. Inst. Malaya*, **21**, 506-569.

D'AUZAC J., 1965. — Étude de quelques réactions métaboliques liées, au sein du latex d'*Hevea brasiliensis*, à la biogenèse du caoutchouc. Sur quelques relations entre la composition, l'activité biochimique du latex et la productivité de l'*Hevea brasiliensis*. *Thèse Doct. Etat*, Université de Paris.

D'AUZAC J., 1975. — Caractérisation d'une ATPase membranaire en présence d'une phosphatase acide dans les lutoïdes du latex d'*Hevea brasiliensis*. *Phytochemistry*, **14**, 671-675.

D'AUZAC J., 1977. — ATPase membranaire de vacuoles lysosomales : les lutoïdes du latex d'*Hevea brasiliensis*. *Phytochemistry*, **16**, 1881-1885.

D'AUZAC J., 1981. — Une α-mannosidase dans un système vacuolaire végétal : les lutoïdes du latex d'*Hevea brasiliensis*. *C.R. Acad. Sc.*, **292**, série III, 1085-1087.

D'AUZAC J. and RIBAILLIER D., 1969. — L'éthylène nouvel agent stimulant de la production de latex chez l'*Hevea brasiliensis*. *C.R. Acad. Sc. Paris*, **268**, série D, 3046-3049.

D'AUZAC J., BRZOZOWSKA J., HANOWER P., LAMBERT C., LIORET C. and NIAMIEN N'GORAN., 1977 *a*. — Un modèle de structure vacuolaire isolée intacte : les lutoïdes du latex d'*Hevea brasiliensis*. I. Accumulation et pénétration du citrate et de la L-lysine dans les lutoïdes. In *Transmembrane ionic exchanges in plants*, M. THELLIER, A. MONNIER, M. DEMARTY and J. DAINTY, ed., Éditions du C.N.R.S., Paris, and Publications de l'Université de Rouen, Mont-Saint-Aignan, 391-398.

D'AUZAC J., DUPONT J., JACOB J. L., LANCE C., MARIN B. and MOREAU F., 1977 *b*. — Un modèle de structure isolée intacte : les lutoïdes du latex d'*Hevea brasiliensis*. II. Caractéristiques de la membrane lutoïdique. In *Transmembrane ionic exchanges in plants*, M. THELLIER, A. MONNIER, M. DEMARTY and J. DAINTY, ed., Editions du C.N.R.S., Paris, and Publications de l'Université de Rouen, Mont-Saint-Aignan, 399-406.

D'AUZAC J. and LIORET C., 1974. — Mise en évidence d'un mécanisme d'accumulation du citrate dans les lutoïdes du latex d'*Hevea brasiliensis*. *Physiol. Vég.*, **12**, 617-635.

BOBILIOFF D. W., 1923. — *Anatomy and physiology of* Hevea brasiliensis. *Part I: Anatomy of* Hevea brasiliensis. Art Institut Orell Fussli, Zurich.

BOLLER T. P., DÜRR M. and WIEMKEN A., 1975. — Characterization of a specific transport system for arginine in isolated yeast vacuoles. *Eur. J. Biochem.*, **54**, 81-91.

BOLLER T. and KENDE M., 1979. — Hydrolytic enzymes in the central vacuole of plant cells. *Plant Physiol.*, **63**, 1123-1132.

BRENNAN T. and FRENKEL C., 1976. — Involvement of hydrogen peroxide in the regulation of senescence in pear. *Plant Physiol.*, **59**, 411-416.

BRZOZOWSKA J., HANOWER P. and CHÉZEAU R., 1974. — Free aminoacids of *Hevea brasiliensis* latex. *Experientia*, **30**, 894-895.

BRZOZOWSKA-HANOWER J., HANOWER P. and LIORET C., 1978. — Étude du mécanisme de la coagulation du latex d'*Hevea brasiliensis*. II. Systèmes enzymatiques impliqués dans le processus. 1. Phénol-oxydases. *Physiol. Vég.*, **16**, 231-254.

BRZOZOWSKA-HANOWER J., CRÉTIN H., HANOWER P. and MICHEL P., 1979. — Variations de pH entre compartiments vacuolaires et cytoplasmique au sein du latex d'*Hevea brasiliensis*. Influence saisonnière et action du traitement par l'éthrel générateur d'éthylène. Répercussion sur la production et l'apparition d'Encoches sèches. *Physiol. Vég.*, **17**, 851-867.

BUTTERY B. R. and BOATMAN S. G., 1966. — Manometric measurement of turgor pressures in laticiferous vessels. *J. Exp. Bot.*, **17**, 283-296.

BUVAT R. and ROBERT G., 1979. — Vacuole formation in the actively growing root meristem of barley (*Hordeum sativum*). *Am. J. Bot.*, **66**, 1219-1237.

BOUDET A., CANUT H. and ALIBERT G., 1981. — Isolation and characterization of vacuoles from *Melilotus alba* mesophyll. *Plant Physiol.*, **68**, 1354-1358.

CHAPMAN G. W., 1951. — Plant hormones and yield in *Hevea brasiliensis*. *J. Rubb. Res. Inst. Malaya.*, **13**, 167- .

COMPAGNON P. and TIXIER R. P., 1950. — Sur une possibilité d'améliorer la production de l'*Hevea brasiliensis* par l'apport d'oligoéléments. *Rev. Gén. Caout.*, **27**, 525-526, 591-594, 663-665.

COOK A. S. and SEKHAR B. C., 1955. — Fractions from *Hevea brasiliensis* latex centrifuged at 59,000 *g*. *J. Rubb. Res. Inst. Malaya.*, **14**, 163-167.

COUPÉ M., 1977. — Études physiologiques sur le renouvellement du latex d'*Hevea brasiliensis*. Action de l'éthylène. *Thèse Doct. d'État*, Université des Sciences et Techniques du Languedoc, Montpellier.

COUPÉ M., PUJARNISCLE S. and D'AUZAC J., 1972. — Compartimentation de diverses oxydo-réductases dans le latex d'*Hevea brasiliensis*. *Physiol. Vég.*, **10**, 459-480.

COUPÉ M. and D'AUZAC J., 1972. — Mise en évidence de polysomes fonctionnels dans le latex d'*Hevea brasiliensis*. *C.R. Acad. Sc.*, **274**, série D, 1031-1034.

COUPÉ M. and D'AUZAC J., 1974. — Caractéristiques de l'incorporation d'acides aminés par les polysomes isolés du latex d'*Hevea brasiliensis*. *Phytochemistry*, **13**, 85-88.

COUPÉ M. and LAMBERT C., 1977. — Absorption of citrate by the lutoids of latex and rubber production by *Hevea*. *Phytochemistry*, **16**, 455-458.

CRÉTIN H., 1982 *a*. — The proton gradient across the vacuo-lysosomal membrane of lutoids from the latex of *Hevea brasiliensis*. I. Further evidence for a proton translocating ATPase on the vacuo-lysosomal membrane of intact lutoids. *J. Membr. Biol.* (sous presse).

CRÉTIN H., 1982 *b*. — The proton gradient across the vacuo-lysosomal membrane of lutoids from the latex *of Hevea brasiliensis*. II. The electrogenic proton efflux coupled to the vacuo-lysosomal membrane — bound NADH-cytochrome c-oxidoreductase activity. *J. Memb. Biol.* (sous presse).

CRÉTIN H., JACOB J. L., PRÉVOT J. C. and D'AUZAC J., 1980. — pH du latex d'*Hevea* : son influence sur la production et les éléments de sa régulation. *Rev. Gén. Caout. Plast.*, **603**, 111-115.

CRÉTIN H., MARIN B. and D'AUZAC J., 1982. — Characterization of a magnesium-dependent proton translocating ATPase on *Hevea* latex tonoplast. In *Plasmalemma and Tonoplast: Their Functions in the Plant Cell*. D. MARME, E. MARRE and R. HERTEL, ed., Elsevier/North Holland Biochemical Press. Amsterdam, 201-207.

CZANINSKI Y. and CATESSON A., 1969. — Localisation ultrastructurale d'activités peroxydasiques dans les tissus conducteurs au cours du cycle annuel. *J. Microscopie*, **8**, 875-888.

DAVIES D. D., 1977. — Control of pH and glycolysis. In *Regulation of enzyme synthesis and activity in higher plants*. H. SMITH, ed., Academic Press, London.

DICKENSON P. B., 1960. — Preliminary electron microscope observations on the ultrastructure of the latex vessels and its contents in young tissue of *Hevea brasiliensis*. *Proc. Nat. Rubb. Res. Conf. Kuala Lumpur*, 756-765.

DICKENSON P. B., 1964. — Ultrastructure of latex vessel of *Hevea brasiliensis*, *Proc. Nat. Rubb. Proc. Res. Ass. Jub. Conf.*, L. MULLINS, ed., Cambridge, 52-62.

DICKENSON P. B., 1969. — Electron microscopical studies of latex vessel system of *Hevea brasiliensis*. *J. Rubb. Res. Inst. Malaya.*, **21**, 543-549.

DOLL S., RODIER F. and WILLENBRINK J., 1979. — Accumulation of sucrose in vacuoles isolated from red beet tissue. *Planta*, **144**, 407-411.

DUNLOP J., 1976. — The electrical potential difference across the tonoplast of root cells. *J. Exp. Bot.*, **27**, 908-915.

DUPONT J., MOREAU F., LANCE C. and JACOB J. L., 1976. — Phospholipid composition of the membrane of lutoids from *Hevea brasiliensis*. *Phytochemistry*, **15**, 1215-1217.

DE DUVE C., 1959. — Lysosomes: a new group of cytoplasmic particles. In *Subcellular particles*, T. HAYASHI, ed., The Ronald Press Co., New York, 128-159.

DE DUVE C., 1966. — Functions of lysosomes. *Annu. Rev. Physiol.*, **28**, 435-492.

FAIRBAIRN J. W., HAKIM F. and EL KHEIR Y., 1974. — Alkaloidal storage metabolism and translocation in the vesicles of *Papaver sommiferum* latex. *Phytochemistry*, **13**, 1133-1139.

DE FAY E., 1981. — Histophysiologie comparée des écorces saines (Maladie des encoehes sèches) de l'*Hevea brasiliensis*. *Thèse Doct. 3e cycle*, Université des Sciences et Techniques du Languedoc, Montpellier.

FOSTER J. G. and HESS J. L., 1980. — Responses of superoxide-dismutase and glutathione

reductase activities in cotton leaf tissue exposed to an atmosphere enriched in oxygen. *Plant Physiol.*, **66**, 482-487.

FREY-WYSSLING A., 1929. — Microscopisch endersoch haar het rerkomen van harsen in latex van *Hevea. Arch. Rubber Cult.*, **13**, 394-434.

GOMEZ J. B. and YIP E., 1975. — Microhelices in *Hevea* latex. *J. Ultrastruct. Res.*, **52**, 76-84.

GOMEZ J. B. and MOIR G. G. J., 1979. — The ultracytology of latex vessels in *Hevea brasiliensis*. Malaysian Rubber Research and Development board. Monogr. n° 4.

GROB K. and MATILE P., 1980. — Compartmentation of ascorbic acid in vacuoles of horseradish root cells. Note on vacuolar peroxydase. *Z. Pflanzenphysiol.*, **98**, 235-243.

HALL J. L. and SEXTON R., 1972. — Cytochemical localization of peroxidase activity in root cells. *Planta*, **108**, 103-120.

HANOWER P., BRZOZOWSKA J. and LIORET C., 1976. — Étude du mécanisme de la coagulation du latex d'*Hevea brasiliensis*. I. Facteurs agissant sur la coagulation. *Physiol. Vég.*, **14**, 677-693.

HANOWER P., BRZOZOWSKA J. and NIAMIEN N'GORAN M., 1977. — Absorption des acides aminés par les lutoïdes du latex d'*Hevea brasiliensis. Physiol. Plant*, **39**, 299-304.

HANOWER P., BRZOZOWSKA-HANOWER J., CRÉTIN H. and CHÉZEAU R., 1979. — Composés phénoliques du latex d'*Hevea brasiliensis;* aglycones. *Phytochemistry*, **18**, 686-687.

HÉBANT C., 1981. — Ontogénie des laticifères du système primaire de l'*Hevea brasiliensis* : une étude ultrastructurale et cytochimique. *Can. J. Bot.*, **59**, 974-985.

HO C. C., SUBRAMANIAN A. and YONG W. M., 1975. — Lipids associated with the particles in *Hevea* latex. *Proc. Int. Rubb. Conf.* Kuala Lumpur, **11**, 441-456.

HOLLEMANS M., 1981. — The intralysosomal pH. *Ph. D. Thesis*, Université d'Amsterdam.

JACOB J. L., RIBAILLIER D. and D'AUZAC J., 1970. — La NADP-phosphatase du latex d'*Hevea brasiliensis*. Inhibitions, interférences. *Physiol. Vég.*, **8**, 247-262.

JACOB J. L. and D'AUZAC J., 1972. — La glycéraldehyde-3-phosphate déshydrogénase du latex d'*Hevea brasiliensis*. Comparaison avec son homologue phosphorylante. *Eur. J. Biochem.*, **31**, 255-265.

JACOB J. L. and SONTAG N., 1973. — Une enzyme nouvelle : la 2'-nucléotidase du latex d'*Hevea brasiliensis, Eur. J. Biochem.*, **40**, 207-214.

JACOB J. L., MOREAU E., DUPONT J. and LANCE C., 1975. — Some characteristics of the lutoids in *Hevea brasiliensis* latex. *Proc. Int. Rubb. Conf.* Kuala Lumpur, 470-483.

JACOB J. L., PRIMOT L. and PRÉVOT J. C., 1979. — Purification et étude de la phosphoénolpyruvate carboxylase du latex d'*Hevea brasiliensis. Physiol. Vég.*, **17**, 501-516.

JACOB J. L., PRÉVOT J. C. and PRIMOT L., 1980. — Une enzyme importante de la biosynthèse du caoutchouc : la pyruvate kinase. *Symposium I.R.R.D.B. Kottayam* (Indes), décembre 1980.

JACOB J. L., PRÉVOT J. C. and D'AUZAC J., 1982. — Physiological activators of invertase from *Hevea brasiliensis* latex. *Phytochemistry*, **21**, 851-853.

JACOB J. L. and PRÉVOT J. C., 1981. — Mise en évidence d'une enzyme malique dans le latex d'*Hevea brasiliensis. C.R. Acad. Sc.*, **293**, série III, 309-312.

KUO LAN FONG, MC CAY P. B., POYER J. L., KEELE B. B. and MISRA H., 1973. — Evidence that peroxidation of lysosomal membranes is initiated by hydroxyl free radicals produced during flavin enzyme activity. *J. Biol. Chem.*, **248**, 7792-7797.

LAMBERT C., 1975. — Influence de l'ATP sur le pH intralutoïdique et sur la pénétration du citrate dans les lutoïdes du latex d'*Hevea brasiliensis. C.R. Acad. Sc.*, **281**, série D, 1705-1708.

LEIGH R. A. and BRANTON D., 1976. — Isolation of vacuoles from root storage tissue of *Beta vulgaris. Plant Physiol.*, **58**, 656-662.

LEIGH R. A., AP REES T., FULLER W. A. and BANFIELD J., 1979. — The location of acid invertase and sucrose in the vacuoles of storage roots of beet root (*Beta vulgaris*). *Biochem. J.*, **178**, 539-547.

LIN W., WAGNER G. J., SIEGELMAN W. and HIND G., 1977. — Membrane bound ATPase of intact vacuoles and tonoplasts isolated from mature plant tissues. *Biochem. Biophys. Acta*, **465**, 110-117.

MAC ROBBIE E. A. C., 1974. — Ion Uptake. In *Algal physiology and biochemistry*, W. D. P. STEWART, ed., Blackwell Scientific Publish., Oxford, 676-713.

MARIN B., 1976. — Les problèmes posés par l'existence d'acides ribonucléiques dans les compartiments lysosomaux végétaux. *Ann. Sci. Nat., Bot.*, **17**, 361-374.

MARIN B., 1978. — Ribosomes in the lutoid fraction (lysosomal compartment) from *Hevea brasiliensis* latex. *Planta*, **138**, 1-14.

MARIN B., 1981. — Le fonctionnement du transporteur tonoplastique du citrate du latex d'*Hevea brasiliensis* : relations avec l'activité ATPase membranaire. *Thèse Doct. Etat*, Université des Sciences et Techniques du Languedoc, Montpellier.

MARIN B., TROUSLOT P. and PUJARNISCLE S., 1974. — Some evidence for the occurrence of ribonucleic acid in the lutoid fraction (lysosomal compartment) from *Hevea brasiliensis* latex. *Biochem. J.*, **143**, 479-481.

MARIN B. and TROUSLOT P., 1975. — The occurrence of ribonucleic acid in the lutoid fraction (lysosomal compartment) from *Hevea brasiliensis* Kunth. (Mull.-Arg.) latex. *Planta*, **124**, 31-41.

MARIN B., CRÉTIN H. and D'AUZAC J., 1982. — Energization of solute transport and accumulation in the *Hevea* latex vacuome. In *Plasmalemma and Tonoplast: Their Functions in the Plant Cells*. D. MARMÉ, E. MARRÉ and R. HERTEL, ed., Elsevier/North Holland Biochemical Press, Amsterdam, 209-215.

MARTINOIA F., HECK U. and WIEMKEN A., 1981. — Vacuoles as storage compartment for nitrate in barley leaves. *Nature*, **282**, 292- .

MARTY F. and BRANTON D., 1980. — Analytical characterization of beet root vacuole membrane. *J. Cell. Biol.*, **87**, 72-83.

MARTY F., BRANTON D. and LEIGH R. A., 1980. — Plant vacuoles. In *The Biochemistry of Plants*, **1**, N. E. TOLBERT, ed., Academic Press, New York, 625-658.

MATILE P., 1975. — *The lytic compartment of plant cells*. Springer-Verlag. Vienna, New York.

MATILE P., 1978. — Biochemistry and function of vacuoles. *Annu. Rev. Plant. Physiol.*, **29**, 193-213.

MATILE P., JANS B. and RICKENBACHER R., 1970. — Vacuoles of *Chelidonium* latex. Lysosomal properties and accumulation of alcaloids. *Biochem. Physiol. Pflanzen*, **161**, 447-458.

MATILE P. and WINKENBACH F., 1971. — Function of lysosomes and lysosomal enzymes in the senescing corolla of the morning glory (*Ipomea purpurea*). *J. Exp. Bot.*, **22**, 759-771.

MEYER K., 1948. — *Modern Trends in Ophtalmology*, **2**, A. SORSBY, ed., Butterworth, London, p. 71.

MILANEZ F. R., 1946. — Nota privia sobre os laticiferos of *Hevea brasiliensis*. *Arquivios do servico florestre do Brazil*, **2**, 39.

MILFORD G. F. J., PAARDEKOOPER E. C. and HO CHAI YEE, 1969. — Latex vessel plugging: Its importance to yield and clonal behaviour. *J. Rubb. Res. Inst. Malaya*, **21**, 274-282.

MONTARDY M. C. and LAMBERT C., 1977. — Diverses propriétés de l'absorption du citrate, du malate et du succinate du latex d'*Hevea brasiliensis*. *Phytochemistry*, **16**, 677-680.

MOREAU F., JACOB J. L., DUPONT J. and LANCE C., 1975. — Electron transport in the membrane of lutoids from the latex of *Hevea brasiliensis*. *Biochim. Biophys. Acta*, **396**, 116-124.

PAKIANATHAN S. W., BOATMAN S. G. and TAYSUM D. H., 1966. — Particle aggregation following dilution of *Hevea* latex: A possible mechanism for the closure of latex vessels after tapping. *J. Rubb. Res. Inst. Malaya*, **19**, 259-271.

PETCH T., 1921. — Non parasitic diseases. In *The diseases and pests of the rubber tree*. Proc. R.R.I.M. Planter's conf., 74-90.

Poux N., 1969. — Localisation d'activités enzymatiques dans les cellules du méristème radiculaire de *Cucumis sativus. J. Microscopie*, **8**, 855-856.

Primot L., Coupé M., Jacob J. L. and D'Auzac J., 1978. — Relations between latex pH and yield. Action of stimulation. *Symposium I.R.R.B.*, Kuala-Lumpur, mai 1978.

Pujarniscle S., 1965. — Étude préliminaire sur l'activité enzymatique des lutoïdes du latex d'*Hevea brasiliensis*. Analogie avec les lysosomes. *C. R. Acad. Sc.*, **261**, série D, 2127-2130.

Pujarniscle S., 1966. — Étude préliminaire sur l'activité enzymatique des lutoïdes du latex d'*Hevea brasiliensis*. Distribution de la phosphatase acide de la β-glucosidase et de la cathepsine dans le latex. *C. R. Acad. Sc.*, **262**, série D, 923-925.

Pujarniscle S., 1968. — Caractère lysosomal des lutoïdes du latex d'*Hevea brasiliensis. Physiol. Vég.*, **6**, 27-46.

Pujarniscle S., 1969. — Étude de quelques facteurs intervenant sur la perméabilité et la stabilité de la membrane des lutoïdes du latex d'*Hevea brasiliensis. Physiol. Vég.*, **7**, 391-403.

Pujarniscle S. and Ribaillier D., 1966. — Étude préliminaire sur les lutoïdes du latex et leur possible intervention dans la biosynthèse du caoutchouc. *Rev. Gén. Caout. Plast.*, **43**, 226-228.

Rabinowitch H. D. and Sklan D., 1980. — Superoxide dismutase: a possible protective agent against sunscald in tomatoes (*Lycopersicum esculentum*). *Planta*, **148**, 162-167.

Rands R. D., 1921. — Brown-bast disease of plantation rubber. Its cause and prevention. *Arch. Rubbercult. Ned. Indie*, **5**, 223-278.

Reijngoud D. J., 1978. — The pH inside lysosomes. *Ph. D. Thesis*, Université d'Amsterdam.

Reijngoud D. J. and Tager J. M., 1975. — Effects of ionophore and temperature on intralysosomal pH. *FEBS Lett.*, **54**, 76-79.

Ribaillier D., 1968. — Action *in vitro* de certains ions minéraux et composés organiques sur la stabilité des lutoïdes du latex d'*Hevea. Rev. Gén. Caout. Plast.*, **45**, 1395-1398.

Ribaillier D., 1972. — Quelques aspects du rôle des lutoïdes dans la physiologie et l'écoulement du latex d'*Hevea brasiliensis*. Action de produits libérant de l'éthylène. *Thèse Doct. État*, Université d'Abidjan.

Ribaillier D., Jacob J. L. and D'Auzac J., 1971. — Sur certains caractères vacuolaires des lutoïdes du latex d'*Hevea brasiliensis. Physiol. Vég.*, **9**, 423-437.

Roe C. P. and Ewart R. H., 1942. — An electrophoretic study of the proteins in rubber latex serum. *J. Am. Chem. Soc.*, **64**, 2628-2632.

Rona J. P., 1973. — Premières mesures du potentiel électrique sur des protoplastes et des vacuoles isolés d'*Acer pseudoplatanus. C. R. Ac. Sc.*, **277**, série D, 185-188.

Rona J. P., van de Sype G., Cornel D., Grignon C. and Heller R., 1980 *a*. — Plasmolysis effect on electrical characteristics of free cells and protoplasts of *Acer pseudoplatanus. Bioelectrochemistry and Bioenergetics*, **7**, 377-391.

Rona J. P., Pitman M. G., Lüttge U. and Ball E., 1980 *b*. — Electrochemical data on compartmentation into cell wall, cytoplasm and vacuole of leaf cells in the CAM genus *Kalanchoë. J. Memb. Biol.*, **57**, 25-35.

Sanderson A. and Sutcliffe H., 1921. — *Brown Bast*. The rubber growers' association, London.

Saunders J. A., 1979. — Investigations of vacuoles isolated from tobacco: I. Quantitation of nicotine. *Plant Physiol.*, **64**, 74-78.

Saunders J. A. and Conn E. E., 1978. — Présence of the cyanogenic glucoside dhurrin in isolated vacuoles from *Sorghum. Plant Physiol.*, **61**, 154-157.

Schneider D. L., 1979. — The acidification of rat liver lysosomes *in vitro*: a role for the membranous ATPase as a proton pump. *Biochem. Biophys. Res. Commun.*, **87**, 559-565.

Sheldrake A. R. and Moir G. F. J., 1970. — A cellulase in *Hevea* latex. *Physiol. Plant.*, **23**, 267-277.

SMITH R. H., 1954. — The phosphatides of the latex of *Hevea brasiliensis*. *Biochem J.*, **57**, 140-144.

SOUTHORN W. A., 1964. — A complex sub-cellular component of widespread occurrence in plant latices. *J. Exp. Bot.*, **15**, 616-621.

SOUTHOURN W. A., 1968. — Latex flow studies. I. Electron microscopy of *Hevea brasiliensis* in the region of the tapping cut. *J. Rubb. Res. Inst. Malaya*, **20**, 176-186.

SOUTHOURN W. A., 1969. — Physiology of *Hevea* (latex flow). *J. Rubb. Res. Inst. Malaya*, **21**, 494-512.

STEWART R. O. and BEWLEY J. D., 1980. — Lipid peroxidation associated with accelerated aging of soybean axes. *Plant Physiol.*, **65**, 245-248.

TAN CHEE HONG and AUDLEY B. G., 1968. — Ergothioneine and hercynine in *Hevea brasiliensis* latex. *Phytochemistry*, **7**, 109-118.

TATA S. J., DOYCE A. N., ARCHER B. L. and AUDLEY B. G., 1976. — Lysozymes: major component the sedimentable phase of *Hevea brasiliensis* latex. *J. Rubb. Res. Inst. Malaysia*, **24**, 233-236.

TUPY J. and RESING W. L., 1968. — Anaerobic respiration in latex of *Hevea brasiliensis* substrate and limiting factors. *Biol. Plant.*, **10**, 72-80.

TUPY J., 1969. — Stimulatory effects of 2-4-dichlorophenoxyacetic acid and of 1-naphtylacetic acid on sucrose level. Invertase activity and sucrose utilization in the latex of *Hevea brasiliensis*. *Planta*, **88**, 144-153.

TUPY J., 1973 *a*. — The regulation of invertase activity in the latex of *Hevea brasiliensis*. The effects of growth regulators, bark wounding and latex tapping. *J. Expt. Bot.*, **24**, 516-524.

TUPY J., 1973 *b*. — The activity of latex invertase and latex production in *Hevea brasiliensis*. *Physiol. Vég.*, **11**, 633-644.

WAGNER G. J. and SIEGELMAN H. W., 1975. — Large-scale isolation of intact vacuoles and isolation of chloroplasts from protoplasts of mature plant tissue. *Science*, **190**, 1298-1299.

VAN DER WILDEN, MATILE P., SCHELLENBERG M., MEYER J. and WIEMKEN A., 1973. — Vacuolar membranes: isolation from yeast cells. *Z. Naturforsch., C*, **28**, 416-421.

WIEMKEN A. and NURSE P., 1974. — Isolation and characterization of the amino-acid pools located within the cytoplasm and vacuoles of *Candida utilis*. *Planta*, **109**, 293-306.

WIEMKEN A. and DURR M., 1974. — Characterization of amino-acid in the vacuolar compartment of *Saccharomyces cerevisiae*. *Arch. Microbiol.*, **101**, 45-57.

WIERSUM L. K., 1957. — Enkele latex problemen. *Vakbl. Biol.*, **37**, 17-26.

YIP E. and SOUTHOURN W. A., 1968. — Latex flow studies. VI. Effects of high pressure gradients on flow of fresh *Hevea* latex in narrow bore capillaries. *J. Rubb. Res. Inst. Malaya*, **20**, 248-256.