Chapter 5.II

PHYSICO-CHEMICAL AND BIOCHEMICAL MECHANISMS OF HORMONAL (ETHYLENE) STIMULATION

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I. INTRODUCTION

The physiological processes which lead to stimulation of latex production are still not fully known, but are far better understood today than they were at the beginning of the first field trials. It is interesting to note that *Hevea* were initially treated in order to enhance renewal of the bark removed during tapping (Chapter 5.I). Bark scraping was thought to improve oxygenation of the underlying productive laticiferous cells. Lavish application of manure and clay on the tapping panel was thought to improve its nutrition. These treatments proved to be useful and able to increase production — the main objective. Researchers became progressively more curious about new, efficient products and about the basic mechanisms of stimulation. This shows that, as often, useful practices can be carried out with inadequate theories. Plant physiologists try to explain the phenomena and in doing so give planters new ideas. New trials lead to new and unknown phenomena and the cycle begins again.

II. STIMULATION AND LATEX RHEOLOGY: EARLY THEORIES

A. Effects on Bark Anatomy

The first hypothesis put forward to account for the effects of stimulation was that of enhancement of the size of the laticiferous tissue which would then allow a larger outflow of latex. De Jonge[120] showed that 2,4-D and 2,4,5-T had a slight, nonsignificant effect on the number of latex vessels in the bark. Hormone-induced increase of the bark width was caused by undifferentiated parenchymatous tissue (Section 7.I). Similar results were obtained by Campaignolle[51] and de Jonge.[122] Gomez[95] found a significant fall in the number of vessel rings in the bark upon treatment. Explanation of stimulation by enhancement of the diameter and/or the number of laticiferous vessels was *a priori* hardly conceivable since the effect of hormonal substances could be detected as early as 24 hr after treatment.[173,201] Faster processes than an increase in the number of laticifers had to be found. Hamzah and Gomez[100] carried out a comprehensive examination of the effects of stimulation on bark anatomy; they noticed that treatments had no significant effects on the physical properties of bark (girth and bark thickness) and nearly all cell characteristics (except for sieve tube size, which appeared to be reduced). The most significant action was on latex vessel volume per specific area (i.e., the first six rows of latex vessels) where two (PB 86 and RRIM 600) of the four clones examined showed significant reduction after application of ethephon.

B. Effects on Latex Flow

It has been observed for a long time that stimulation takes the form of dramatic prolongation of latex flow.[56,104,120,210] Duration of flow can often be doubled or even more.[121] The initial speed of flow is often increased,[121] but Boatman[43] pointed out that this enhancement did not

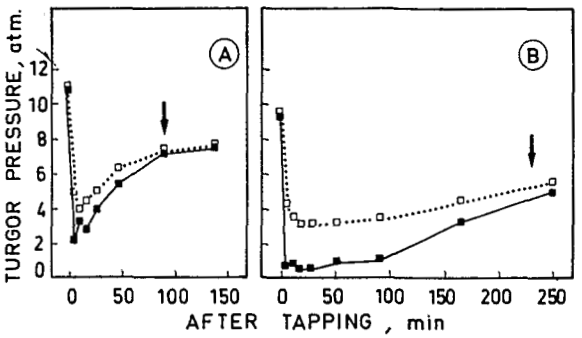

FIGURE 1. Effect of stimulation on turgor pressure. Changes were recorded at 4 cm (■-■) and 32 cm (□-□) before the cut during latex flow. The arrows indicate the cessation of flow. (A) Before stimulation. (B) Same tree, 6th tapping after treatment with 2,4,5-T. (Adapted from Buttery, B. R. and Boatman, S. G., *J. Exp. Bot.*, 18, 644, 1967.)

persist from one tapping to the next, even though total latex yield continued to increase. In some cases[163] there was no variation at all in initial speed of flow.

C. Effects on Latex Viscosity

De Jonge[121] reported that stimulation lowered the dry rubber content of latex. Latex viscosity is related to the dry rubber content,[250] which may account for the increase in flow rate at tapping. Nevertheless, Boatman[43] also eliminated the role of latex viscosity: there was no correlation between this parameter and latex production; erratic variations in viscosity were observed in stimulated trees in spite of a noticeable lowering of the solid content of latex.

D. Blackman's Hypotheses

Blackman[42] (Chapter 5.I) put forward three main hypotheses concerning the mechanism of stimulation by 2,4,5-T: "the internal pressure within the system of latex vessels is increased, the properties governing flow through the vessels or the small orifices of the cut are changed, the severed vessels do not plug up so readily." (1) An increase in turgor pressure was conceivable through a decrease in osmotic potential. (2) The turgor pressure could be increased by a rise in cell wall plasticity (brought about by auxin action) and an increase in the pressure of the surrounding tissue on the latex cell. Blackman was already skeptical about the latter explanation since it was known that auxin had no effect on mature cell walls such as those of latex cells.

E. Increase of Turgor Pressure

With regard to the first hypothesis, Buttery and Boatman[48,49] developed methods for direct measurement of turgor pressure in latex vessels by puncturing the bark and inserting capillary manometers in the phloem (Chapters 4.II and 4.III). After tapping there was a rapid fall in pressure immediately below the cut and there was a slow recovery before flow ceased. In stimulated trees this recovery occurred much later, showing that stimulation could not be ascribed to an increase in turgor pressure. Turgor pressure fell at the beginning of tapping and then rose progressively. In trees stimulated with 2,4,5-T (Figure 1), latex flow was sustained and occurred simultaneously with delayed recovery of the turgor pressure.[50] This observation proved that unlike the idea put forward above, stimulation prolongs flow under low pressure and delays plugging of the latex vessels. The same authors thought that enhancement of flow might rather be explained by a lowering of latex viscosity and also by

decreased plugging of the vessels. It had been previously shown that tapping was followed by trunk shrinkage immediately below the cut (Chapters 4.II and 4.III). Boatman,[43] using careful dendrometric measurements, ruled out the explanation of the stimulation by a greater trunk contraction after the opening of the tapping cut.

Osborne and Sargent[160] proposed a model of the stimulation of latex flow by ethylene based on the predicted effects of this substance on the plasticity and structure of the cell walls of the latex vessels. This theory was based on the effects of ethylene on the cell walls of *Pisum sativum*;[110] the authors concluded that under stimulation *Hevea* should develop wider vessels with thicker, more rigid walls. After tapping, these vessels would lead to reduced shearing forces and prolonged latex flow. In *Pisum* the thickening of the cell wall[161] can readily be measured after 24 hr. The model proposed contains many contradictions and is very unlikely: ethylene does not modify wall thickness in mature pea cells[206] in spite of the fact that mature vessels readily respond to stimulation; furthermore, auxins are known to enhance wall plasticity of young cells alone.

F. Lowering of Osmotic Potential

Variation of osmotic potential upon stimulation was studied by several authors in order to test one of Blackman's hypotheses. Pakianathan et al.[167] found that the osmolarity of the latex was unaffected by stimulation. Even Boatman[43] and Coupé[69] noticed a certain fall (10%) in osmolarity which could indicate a lowering of the total solid content of the latex. The osmolarity of latex exhibited a progressive fall in fractions collected during the same tapping. This dilution pattern was unchanged by stimulation. The decay of osmolarity of collected latex was a little less marked in stimulated trees.[166]

G. Extension of the Drainage Area

The effect of stimulation has been mainly ascribed to an extension of the drainage area for a long period of time.[42,43] De Jonge[120] and especially Lustinec et al.[140] proved that after stimulation there is an extension of the drainage area; this parameter is defined by the zone of the trunk where the total solid content of the latex is lowered after tapping; it is measured on latices sampled by micropunctures at some distance from the tapping cut. Lustinec et al.[140] pointed out that stimulation at 65 cm from the tapping cut resulted in an extension of the zone from which the latex was collected (Figure 2). This observation established quite clearly that one effect of stimulation is an extension of the drainage area. Pakianathan et al.[168] showed that the displacement area, as assessed by variations of turgor pressure, was extended by stimulation (Figure 3). Other reports have ascertained this finding.[211]

Organo-mineral composition and lutoid properties between the first and the last fractions collected during a tapping showed large variations.[195] It would be interesting to determine whether all the fractions have the same properties after stimulation and whether the limit of the drainage area is characterized by changes in the biochemical or biophysical properties of the latex.

H. Boatman Experiment: Reduction of Plugging

The last rheological hypothesis proposed by Blackman[42] concerned the plugging of vessels. In a normally tapped tree the flow stops progressively by a process of coagulation at the cut surface a few hours after the incision of the bark. Boatman[43] tested the hypothesis that stimulation enhances production by improving latex stability thus lengthening duration of flow. He recorded the rate of flow on two similar trees. On the second tapping day, the cuts of both trees were reopened 10 min after the initial tapping and again 10 min later. After this experiment one tree was stimulated and the second served as control. A sequence of normal and repeated tappings is shown in Figure 4. The results for control were very similar to those for the first cycle. Normal tapping of the stimulated tree exhibited the usual

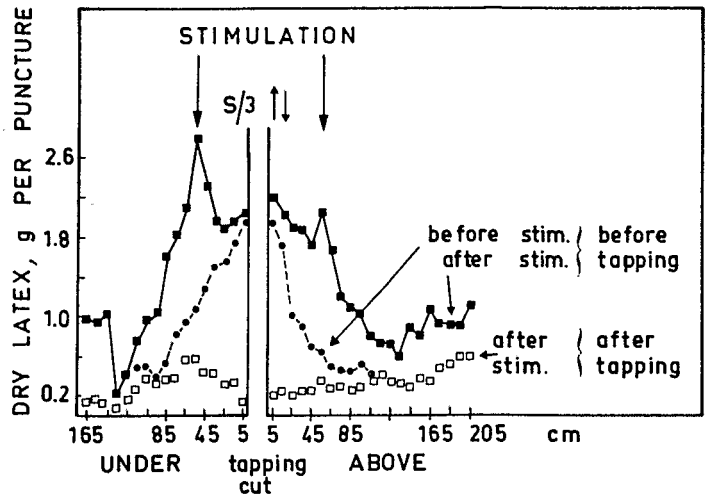

FIGURE 2. Effect of stimulation on the drainage area. Micropuncture latex yield from below and above $^1/_3$ spiral tapped (↑ ↓) GT1 tree, before simulation, before tapping (●); after stimulation by 2,4-D, before tapping (■); and after stimulation by 2,4-D, after tapping (□).(Adapted from Lustinec, J., Langlois, S., Resing, W. L., and Chai Kim Chun, *Rev. Gen. Caoutch. Plast.*, 44, 635, 1967.)

trend: there was a much slower fall-off in the rate of flow. Under these conditions, reopening the cut 10 min after the initial tapping had no apparent effect on the flow rate. There was only a small effect, even after 20 min. These observations indicated that in the stimulated tree no significant obstruction to flow appeared in the last millimeters of tissue. This work provided a major insight into the understanding of stimulation, but, as usual, led to a fresh question: why was the latex apparently more stable after stimulation?

III. STIMULATION AND LUTOID STABILITY

A. The Bursting Index

Lutoids in latex were isolated and described for first time by Homans and Van Gils[106] but their function was unknown. Their role in coagulation was suspected by Paton.[172] Subsequent work demonstrated that destabilization of lutoids had a negative effect on the biosynthesis of rubber and on latex flow, and that stimulation resulted in stabilization of this organelle (Chapter 4.III). Pujarniscle[181,182] showed that lutoids were equipped with a broad variety of hydrolytic enzymes and put them in the same category as lysosomes. He demonstrated that isolated lutoids were sensitive to various destabilizing treatments: detergents, thermal shocks, and especially osmotic shocks. Pujarniscle and Ribaillier[185,195] noticed that there was a direct correlation between the index of stability of lutoids and *in vitro* biosynthesis of rubber. Ribaillier[195] defined the bursting index (BI) as the ratio of free acid phosphatase activity to total acid phosphatase activity (Chapters 4.III and 6.I). The BI is an indicator of the degree of intactness of lutoids. It is precisely the opposite of the index of stability. Ribaillier[192] found that factors favoring production, such as relatively low magnesium content, were negatively correlated with a high BI. Pujarniscle and Ribaillier[186] showed that tapping virgin trees led to simultaneous lowering of the plugging index (PI, see below) and of the BI. Ribaillier[193,194] pointed out that the BI was enhanced in the last fractions collected during tapping and above all that stimulation resulted in considerable lowering of the BI (Figure 5). He also found a close parallel between variations of BI and of PI.

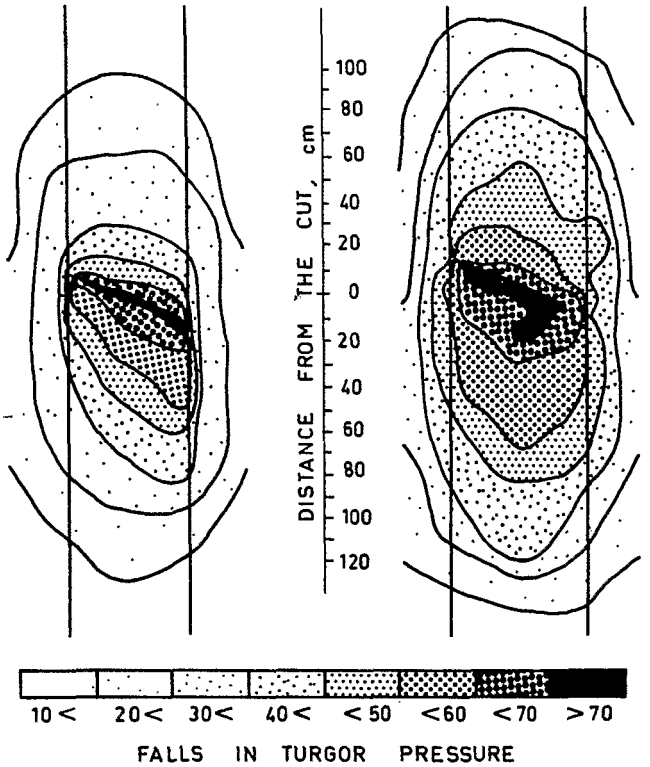

FIGURE 3. Effect of stimulation on the displacement area. Maps of displacement area of a RRIM 600 tree (S/2 tapped) before (left) and after (right) stimulation. Ethephon (10%) was applied to a 3.8-cm band of scraped bark under the cut. The potential displacement area is defined as the area of the bark where pressure falls are observed within 10 to 12 min after tapping. Falls in turgor pressure are expressed as $(P_b - P_a) \times 100/P_b$ where P_b and P_a are, respectively, the turgor pressure measured before and after tapping. (Adapted from Parkianathan, S. W., Wain, R. L., and Ng, E. K., Studies on displacement area on tapping in mature *Hevea* trees, in *Proc. Inst. Rubber Conf. 1975*, Rubber Research Institute Malaysia, Kuala Lumpur, 1976, 225.)

B. The Plugging Index

By this time, Milford et al.[145] had defined a PI related to the speed at which the flow lessens with time. They found a direct correlation between the PI and the response to stimulation; in other words stimulation lengthened the total duration of flow. Pakianathan and Milford[166] showed that when latex was collected in an hypertonic buffer there was very little loss of lutoids (as assessed by comparison of the counts of these particles by microscopic examination) in latex collected *in situ* and latex collected at the spout. The authors reported that the difference between these two counts increased progressively with time after tapping. This may indicate that the lutoids became progressively less stable in the later fractions collected.

Later Ribaillier[194,195] found a close correlation between the BI and the PI. This proved that the slowing of flow was caused by the bursting of the lutoids. Whatever the mechanism of coagulation, it was evident that the instability of lutoids played a prominent role in this process[228,229] (see Chapter 4.III), and it was natural to suppose that the main effect of stimulation consisted of stabilizing the lutoids. Ribaillier[195] showed clearly that for the first ten tappings after stimulation there was a striking increase of the stability of the lutoids, as

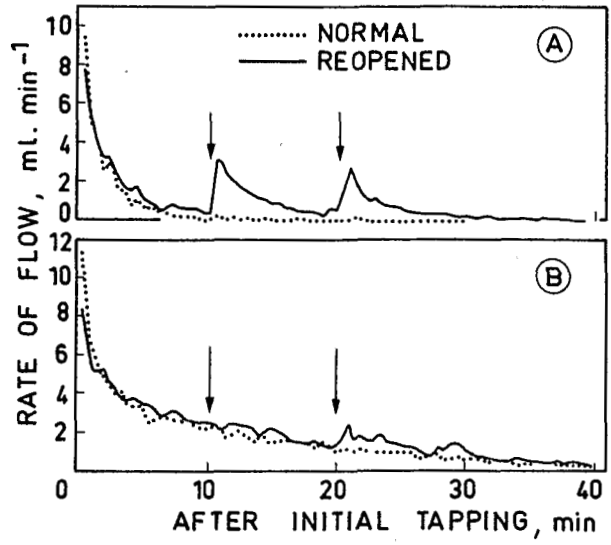

FIGURE 4. Effect of stimulation on plugging. Rate of latex flow recorded at half-minute intervals after tapping for two Tjir 1 trees. (A) Control. (B) Treated with 2,4,5-T. Comparison between normal tapping (. . .) with reopening the cut (—), as indicated by arrows, 10 and 20 min after the initial tapping. (Adapted from Boatman, S. G., *J. Rubber Res. Inst. Malaya*, 19, 243, 1966.)

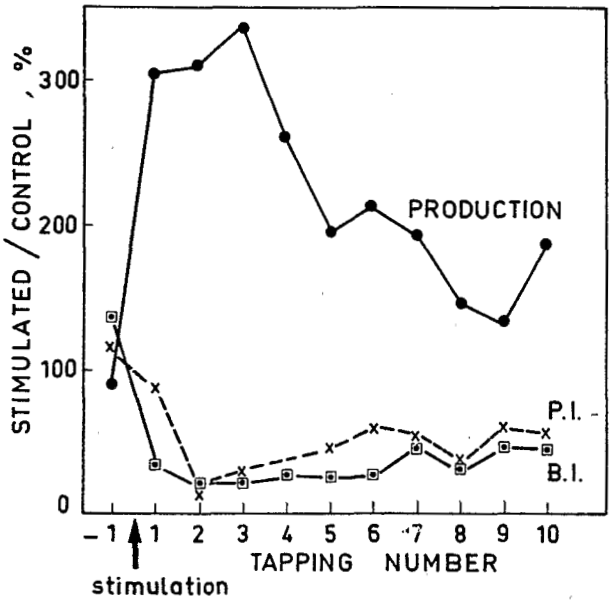

FIGURE 5. Effect of stimulation on production, PI and BI. Production of stimulated tree is expressed as percentage of control. Stimulation was carried out with ethephon, 2.5% on 4 vertical strips (4 cm wide) below the cut.[193,194]

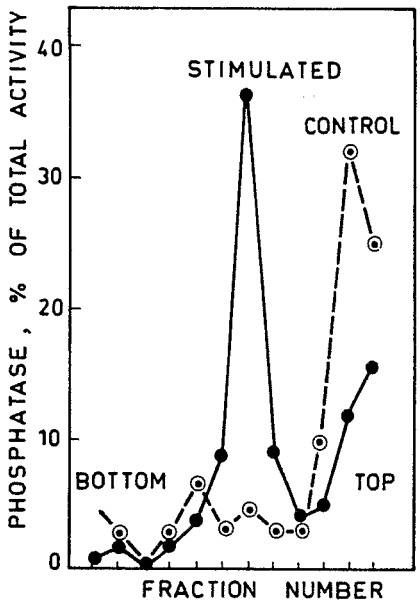

FIGURE 6. Effect of stimulation on lutoid stability. Lutoids were sedimented on a sucrose gradient. Those collected from stimulated trees were more stable and exhibited higher density. (Adapted from Ribaillier, D., Quelques Aspects du Rôle des Lutoides dans la Physiologie et l'Ecoulement du Latex d'*Hevea brasiliensis*, Action de produits Libérant de l'Éthylene, Thèse Doct. Etat Sci. Nat., Université Abidjan, Cote d'Ivoire, 1972.)

measured by the BI. The mechanism by which stimulation affected this stability remained unclear.

C. Stabilization of Lutoids by Stimulation

Ribaillier[195] reported that lutoids from stimulated trees gained new properties as assessed by migration on a sucrose gradient (Figure 6). They were much more resistant to various destabilizing treatments: osmotic lysis and *in vitro* glass homogenizer shearing. He also noticed that the destabilizing activity of lutoid serum was considerably reduced by stimulation. Hanower et al.[101] showed that latex from stimulated trees coagulated less rapidly than latex from unstimulated controls.

Abraham et al.[10] and d'Auzac and Ribaillier[29] discovered the stimulating effect of ethephon. Ribaillier[195] showed that ethylene had a direct effect *in vitro* on the swelling of lutoids. This swelling would modify the permeability of the lutoid membrane. This ethylene-induced swelling of organelles had been reported by Myron and Connelly[147] in rat liver mitochondria. Conversely, Ribaillier[195] found no *in vitro* effect of ethylene treatment on the BI of the lutoids. This indicated that the stabilizing action of ethylene was indirect and probably complex.

Fresh field latex had been shown to exhibit thixotropy when examined in a rolling-ball type microviscometer[226] due to the presence of "active" lutoid particles (Chapter 4.III). This thixotropy was modified by stimulation[258] and thought to indicate lutoids in better condition, and hence probably more stable.

Gomez[96] showed that bark extracts contained latex coagulants. They may act on lutoid stability. Dialysis of these extracts removed their activity. Extracts from ethephon-stimulated trees exhibited smaller activity than those from controls.

D. Stimulation and Lutoid Destabilization

The lutoid destabilization observed in the last fractions[193] of flow has not been fully

explained. It can hardly be attributed to a lowering of the osmolarity of the latex: c in osmolarity during latex flow is slight (approximately 50 mosmol) and now Pujarniscl had proved that lutoids displayed fair stability over a relatively wide range of osmol Finally, Coupé[69] showed that ethephon stimulation did not affect the osmotic pres either C serum or B serum. Obviously the main effect of tapping on the water st. lutoids lies in the rapid fall in the turgor pressure which may lead to bursting of the org especially near the cut.

Conflicting results on the stabilization of lutoids by stimulation were apparently ob by Pakianathan et al.[167] They noticed that stimulation caused the appearance of da lutoids measured by a visual count of the bottom fraction after centrifugation. Thi was standardized by lattices submitted to osmotic shocks. Their results showed h that high scores were never obtained for the first tapping after stimulation when proc was enhanced already, but rather for the third tapping after application of 2,4,5-T. bilization of the lutoids did not occur as a direct result of stimulation. Coupé et al.[7 that after stimulation the BI fell following a transient rise which accompanied the up of production. Chrestin[59] pointed out that there was often a transient rise of the BI lutoids after stimulation; this was linked to a transient increase in the peroxidative pl enon in the latex, and in the case of certain particularly sensitive overstimulated tree was a striking rise in the BI corresponding to the appearance of bark dryness sym These observations show that an enhancement of production may be accompanied increase of the bursting of lutoids. Thus the role of this organelle is complex.

IV. STIMULATION-INDUCED METABOLIC CHANGES

It has been shown (Chapter 5.I) that a very wide variety of substances and treatme stimulate latex production. Some authors supposed that each substance might have a s effect on the *Hevea* cell. In particular, it was interesting to try to find out which mo or enzymes are involved in the improvement of lutoid stability. Archer et al.[24] show *in vitro* acid phosphatase hydrolyzed the key substance necessary for the biosynth rubber. Pujarniscle and Ribaillier[185] stressed that the bursting of lutoids may lead release into the cytoplasmic serum of hydrolases which would reduce biosynthesis of

A. Specific Effect of Copper Sulfate

A mechanism of stimulation by copper sulfate was proposed by Jacob et al.[112,11- authors found in the cytoplasmic serum a specific NADP-phosphatase which was s inhibited by copper salts. As NADP is essential for the biosynthesis of rubber, phosphatase inhibition would enhance the biosynthesis of rubber. The authors prov copper sulfate enhanced this process *in vitro*. More generally, Ribaillier[195] put forw idea that stimulation by copper sulfate was partially mediated by the inhibition phosphatases in latex (which may hydrolyze energy-charged molecules like ATP) an increase the biosynthesis of rubber. He showed that *in vitro* low concentrations of enhance the transformation of labeled acetate into rubber. The latex from copper-stir trees exhibited increased biosynthetic capacity of rubber as assessed by the incorpor this acetate. Another inhibitor of phosphatases such as sodium molybdate[195] displa yield stimulation capacities.

These theories on the mechanism of copper sulfate were only partly satisfactory b they did not explain why this ion led to improved stability of the lutoid and other physio modifications discovered progressively: changes in the organo-mineral composition o and especially of pH and the sucrose concentration.

B. Organo-Mineral Composition

Variations in the organo-mineral composition of latex after stimulation have been de

Table 1
EFFECTS OF STIMULATION ON ATP, ADP, AND TOTAL ADENINE NUCLEOTIDES

Clone		ATP	ADP	Total NA	ATP/ADP
PB 217	Before	84 ± 4	24 ± 2	152 ± 8	3.50
	After	170 ± 3	96 ± 4	341 ± 12	1.77
PB 235	Before	112 ± 2	28 ± 2	189 ± 6	4.00
	After	167 ± 7	78 ± 5	299 ± 14	2.14
GT 1	Before	78 ± 4	24 ± 1	144 ± 7	3.25
	After	144 ± 4	134 ± 7	381 ± 15	1.07
RRIM 600	Before	72 ± 3	26 ± 1	137 ± 7	2.77
	After	170 ± 9	137 ± 7	457 ± 29	1.24
Avros 2037	Before	94 ± 4	22 ± 2	164 ± 7	4.27
	After	159 ± 6	152 ± 5	422 ± 17	1.05

Note: Means and standard errors were obtained from 9 trees for each clone. Assays were performed on latex from 2 tappings before and from the 2nd and 3rd tapping after treatment with ethephon (5%). Concentrations are expressed as μM in the latex. Total NA is the sum of ATP + ADP + AMP concentrations.

for decades, especially in long-term experiments. Their amplitude depend on the clone and the age of the tree (Chapters 5.III and 6.I). An enhancement of the levels of copper,[26,64,104] phosphorus,[27] free amino acids, and SH-containing molecules of low molecular weight was reported.[26,194] Proline became detectable in latex although it was virtually absent in controls; there were increases of glutamic acid in C-serum and of tyrosine in B-serum.[45] Ribailler[195] noticed a distinct decrease (to 50% of the control) in magnesium after ethephon stimulation. Yip and Chin[257] found that stimulation with ethephon resulted in a lowering of the concentrations of calcium and magnesium in latex. Ribailler[195] reported that the distribution of magnesium between C- and B-sera was profoundly affected: there was a striking enrichment of B-serum and a lowering of the concentration in C-serum. He also showed that ethephon stimulation led to doubling of the copper content of latex, corresponding mainly to an enrichment of the B-serum without noticeable modification of C-serum. Variations of ionic concentrations in latex may modify several factors which affect latex flow: when applied to the trees, calcium ions have been shown to lower latex yield;[187] the same ion, together with magnesium ions, have exhibited destabilizing effects on lutoids and on the biosynthesis of rubber *in vitro*,[192] on rubber particles *in vitro*,[103,191] and on whole latex.[101] The calcium ion content fell in B-serum after ethephon treatment,[97] but in some cases[69] stimulation had no effect on this ion. An explanation of stimulation by the lowering of the amounts of certain ions appeared to apply more to magnesium than to calcium.

Enzymes which act on the biosynthesis of rubber and/or cell turnover are also affected by ion contents in latex (Chapter 3.I). It should be pointed out, however, that the main variations observed concern lutoid serum, and that cytoplasmic serum, which contains the main enzymes of anabolism, is not greatly affected by stimulation. Unfortunately several compartments have escaped our investigations: the nucleus and the mitochondria.

Details of the variations in "lutoid serum", which is a blend of true lutoids and Frey-Wyssling particles, has never been assessed. The Frey-Wyssling particles probably play an important role in latex stability since they contain phenoloxidase, whose activity is diminished after stimulation (see below). Studies of these organelles like those carried out on the stability of the lutoids after stimulation would be very useful.

A more general investigation of the effects of stimulation on adenosine phosphate levels in the latex was carried out using five clones (Table 1). A rise in the nucleotide levels can

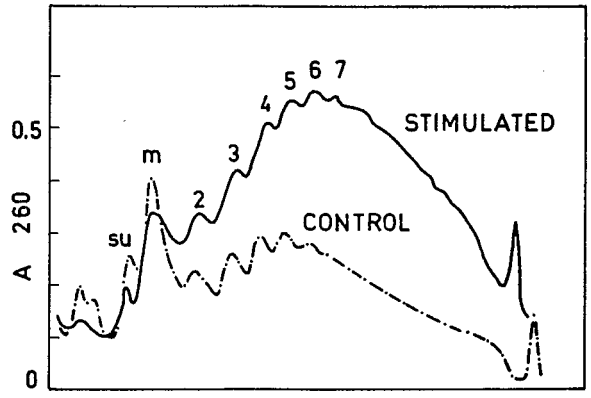

FIGURE 7. Effect of stimulation on polyribosomes. Polyribosome analysis (absorbance at 260 nm) on sucrose gradient 5 days after stimulation. (-·-·-), Control; (—), stimulated; su, subunits; m, monomers; 2, dimers; 3, trimers, etc. (Adapted from Coupé, M. and d'Auzac, J., *Physiol. Vég.*, 12, 1974.)

be observed after treatment. The least responsive clone (PB 235) is a naturally high yielder which displays lower response to hormonal stimulation and is prone to bark dryness. The increased level of ATP favors the anabolic processes, including rubber and nucleic acid biosynthesis. The increased level of ADP would result from these processes.

C. Latex Regeneration

The massive outflow of latex induced by stimulation leads necessarily to an increase in the activities of anabolical physiological processes in the cells, and especially biosynthesis of rubber and of proteins involved in this renewal. Tupý[238] was the first to report on the role of nucleic acids in latex. He showed that high-yielding trees contained more RNA (mainly rRNA) than low yielders. *In vitro* incorporation of phosphorus in nucleic acids was higher in latex from high than from low yielders. Intensive tapping led to an increase in the nucleic acid content. All these facts proved that the nucleic acids played a prominent role in production. Further work by Tupý[243] showed that 2,4-D stimulation led to a lowering of the rRNA/tRNA ratio at the first tapping after treatment; conversely, an increase in this ratio was noticed at the second tapping. He considered these characteristics of RNA metabolism to be a consequence rather than the cause of increased latex production.

Coupé and d'Auzac[68,70] isolated polyribosomes from latex and showed that they were able to incorporate leucine into proteins. Coupé and d'Auzac[71] showed that stimulation by ethephon induced an increase in the polymerization of ribosomes (Figure 7), in the RNA concentration and in the proportion of rRNA. This effect was noticed within the 3 days following stimulation whereas the effect on the production lasted for less than 1 day. These effects could not be ascribed to weaker activity of acid phosphatase or RNase in the cytoplasmic serum. Coupé et al.[72] pointed out that bark scraping, like treatment with ethephon, ANA, 2,4-D, boric acid, copper sulfate, and mercury chloride led simultaneously to increased production and ribosome polymerization. In an improved experiment (all treated trees tapped on the same day), Coupé et al.[74] showed that the variation in ribosome polymerization could be observed 12 hr after ethephon treatment (Figure 8). This shift occurred 12 hr before enhancement of the latex production which closely matched the variations in ΔpH (the difference between the pH of cytoplasmic and lutoid sera). Coupé et al.[75] noticed that repeated stimulation sustained the enhancement of production and ribosome polymerization. These experiments indicate that an increase in the biosynthesis of nucleic acids and proteins is

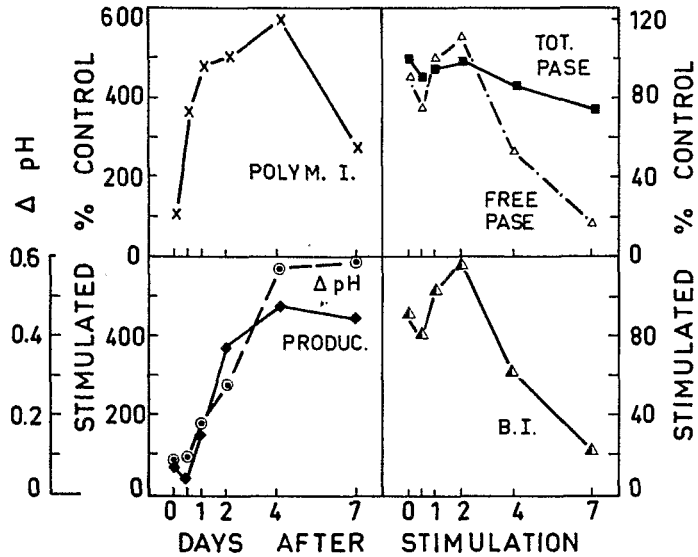

FIGURE 8. Variations of some latex parameters as a function of time between ethephon treatment and tapping. ♦, Rubber production; X, ribosome polymerization index: ratio of heavy (trimers and more) to light particles (subunits, monomers, and dimers); △, free and ■, total acid phosphatases; ▲, bursting index; ●, difference between latex pH before and after stimulation. (Adapted from Coupé, M., Lambert, C., Primot, L., and d'Auzac, J., *Phytochemistry*, 16, 1133, 1977.)

obtained after enhancement of production by various means: bark scraping and stimulating compounds themselves.

D. Phenols and Phenoloxidases

Latex contains a phenoloxidase located inside the Frey-Wyssling particles.[73] Studies by Brzozowska-Hanower et al.[46] showed that phenoloxidases are key enzymes in the coagulation of latex. *In vitro* experiments[101] proved that coagulation is accelerated by oxygen but delayed by nitrogen.

Stimulation by ethephon had striking effects on the phenol metabolism. The response was divided by Cretin[76] into three sequences (Figure 9). The first, short one (first and second tappings after treatment) was characterized by an abrupt rise in the phenolic content of the tapping. The secondary response exhibited a progressive increase of the phenolic content up to 250% of the control. A final, tertiary response corresponded to a progressive return to normal levels of phenolics and of production. Phenoloxidase activity decreased to 25% of control at the second tapping after treatment, and this response was sustained throughout the first two phases of the response. Cretin found a positive correlation between production and the levels of scopoletine, syringic, and sinapic acids and a negative correlation between production and salicylic acid. He concluded that stimulation was characterized by a modification of the phenolic metabolism; in particular, the initial decrease in phenoloxidase might be caused by the synthesis of an inhibitor of this enzyme, leading to the rise in phenol content observed in the latex. Brzozowska-Hanower et al.[46] also showed a definite lowering of *o*-diphenol oxidase activity in lattices at the first tapping after stimulation.

E. Lipid Composition

The role of lipids and proteins in the mechanism of vessel plugging was shown by Sherief and Sethuraj.[213] Lutoid instability, shown by the BI, was negatively correlated with the phospholipid content of the bottom fraction. The neutral lipid content of rubber particles

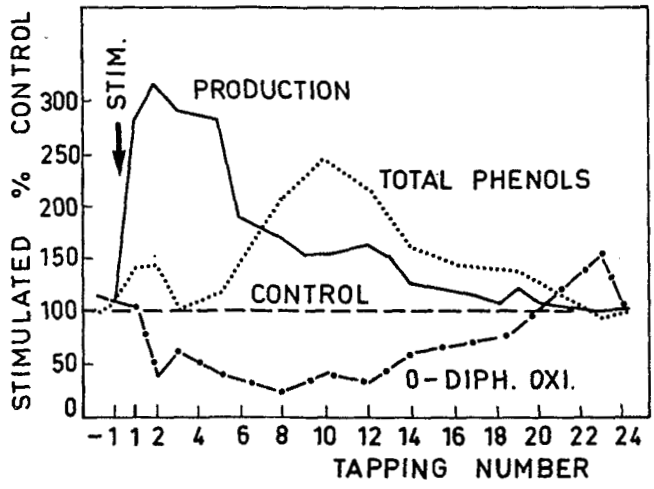

FIGURE 9. Effect of ethephon stimulation on latex production, total phenolic compounds and *o*-diphenol oxidase activity. (Adapted from Cretin, H., Contribution à l'étude des facteurs limitants la production du latex d'*Hevea brasiliensis*, Rapp. d'élève 2ème année ORSTOM, Adiopodoumé, 1978.)

was correlated positively with the colloidal stability of latex. A high ratio of cationic and anionic proteins in the B-serum was related to a high PI. It would be interesting to investigate the effects of stimulation on the above parameters. Very few reports deal with the influence of phytohormones on the lipid metabolism. The gibberellin-induced increase in phosphocholine-cytidyl transferase and phosphocholine-glyceride transferase in the barley aleurone layer was shown by Johnson and Kende.[118] This effect led to an enhancement of lecithin biosynthesis and an increase in the rate of formation of endoplasmic reticulum.

It is known that environmental changes act on the lipid metabolism in higher plants.[127] In particular, the hardening of black locust tree has been correlated with an increase in total phospholipids.[214] As adaptations to environmental stresses are mediated by ethylene, it would appear to be interesting to investigate the effects of this hormone on the lipid metabolism.

F. Sucrose Levels and Metabolism

As shown in Chapter 3.IV, stimulation has a pronounced effect on sucrose levels and metabolism. In previously untapped trees the sucrose content of the latex collected by puncture tapping was dramatically decreased until the 4th day and steadily increased for the next 6 days to reach the initial value (Figure 10). In regularly tapped trees, the evolution of sucrose levels along the bark showed a sharp decrease above the tapping cut and considerable enhancement near it (see Chapter 3.IV, Figure 7). These changes may indicate translocation of sucrose to a sink (the cells near the cut) intensified by stimulation. When sucrose was assayed in fractions collected after tapping (Figure 11), it was noticed that stimulation increased the sucrose level principally in the first fractions. There was even a diminution in the later fractions. These results agree with those obtained by puncture tapping.

According to whether the trees are regularly tapped or not two different events may be observed near the cut after stimulation: a considerable decrease or increase in the sucrose level. The latter is the result of translocation of sucrose from the phloem and from the liberian and medullar rays, and consumption in latex cells. The apparent discrepancy observed may result from differences in the speed of these two phenomena, which may be both affected by stimulation. In particular (Chapter 3.IV), the utilization of sucrose in latex was measured *in vitro*. Stimulation greatly increased this catabolism. Since the quantities of

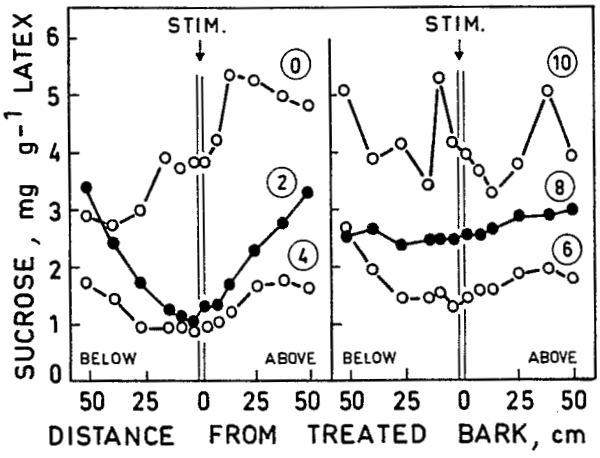

FIGURE 10. Effect of stimulation on latex sucrose content. Changes in the sucrose content in latex collected by micropunctures in untapped bark for 10 days after stimulation with 2-methyl-4-chlorophenoxyacetic acid. Figures enclosed in circles refer to days after treatment. (Adapted from Tupý, J., *Physiol. Vég.*, 11, 633, 1978.)

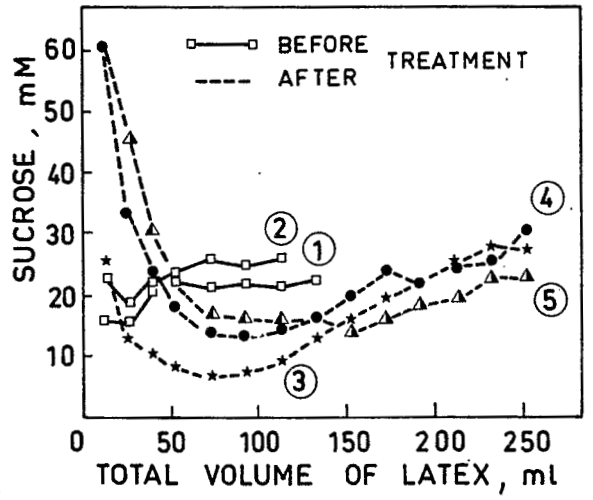

FIGURE 11. Effect of stimulation on latex sucrose content. Effect of 2,4-D applied as a 2-cm strip under the cut, on the sucrose content in successive fractions flowing after tapping. Curve 1 is for latex collected 4 days before stimulation. The day of stimulation, the tree was tapped (curve 2). Curves 3, 4, and 5 refer to latex collected, respectively, 5, 7, and 10 days after stimulation. (Adapted from Tupý, J., *Physiol. Vég.*, 11, 633, 1973.)

invertase and of its effectors were not affected by stimulation, this sucrose degradation appeared to be caused mainly by an increase in invertase activity following a shift of the latex pH toward the optimum for this enzyme. This increase in invertase activity would fuel the biosynthesis of rubber.

In one case (Chapter 3.IV) it was shown that an increase of production occurred 8 hr after stimulation without any rise of the latex pH and at the same time as an enhancement of *in vitro* utilization of sucrose. Since the role of invertase in this process is ruled out owing

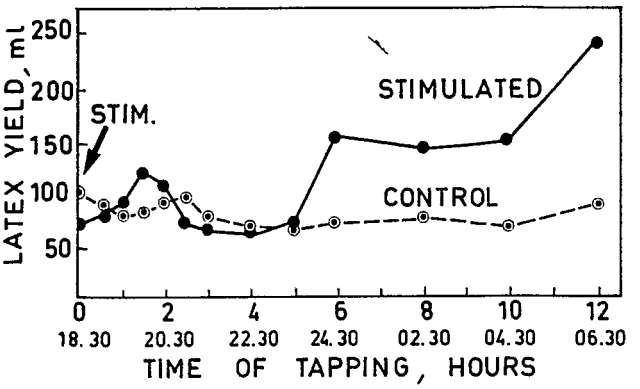

FIGURE 12. Delay of response to stimulation. Ethephon 10% was applied onto a 3.8-cm band of scraped bark at 18.30 hr. (Adapted from Pakianathan, S. W., *J. Rubber Res. Inst. Malays.*, 25, 50, 1977. With permission.)

to the stability of the pH, Tupý and Primot[246] explained the effect of ethylene by a reduction of the real synthesis activity of sucrose synthetase, although proof was indirect.

V. EARLY BIOCHEMICAL EVENTS INDUCED BY STIMULATION

A. Methodological Problems

Before discussing the kinetics of ethephon stimulation of *Hevea*, it should be stressed that comparisons with other data in the literature should be made with caution as it is difficult to assess the speed at which the substance enters the bark of the tree. Certain discrepancies between the effects of stimulation on *Hevea* itself may be due to different bark thicknesses or permeability, the type and concentration of the substance used, and also the different responsiveness of the clones studied.

Kinetic studies of stimulation require specific methods. When analysis can be carried out on tiny quantities of latex, the collection of latex by periodic microtapping (puncture tapping) of the bark is adequate. When larger quantities of latex are necessary, latex is collected in a different way. It consists of time shifting the application of stimulant on the bark of groups of comparable trees. These trees are then all tapped on the same day, thus avoiding any artifact which might be attributed to tapping variations.

B. Increase of Production, pH, and Ribosome Polymerization

The kinetics of evolution of latex parameters upon stimulation display large differences from one experiment to another. Increase in latex production was noticed only 5 to 6 hr after application of ethephon (Figure 12).[165] This took longer in other experiments: 8 to 12 hr[246] and 12 to 24 hr.[74] According to Tupý, the increase in production preceded the cytosol alkalinization. The opposite was observed by Coupé et al.[74] In any case, the variations of these two parameters seem to be closely related.

Tupý[244-246] claimed that the "primary known event in ethephon action was an increase of carbohydrate catabolism resulting from the fall off in synthetic activity of sucrose synthetase." Tupý and Primot[246] showed that 8 hr after treatment the *in vitro* sucrose consumption had increased without any variation of pH being recorded. As the enzyme content was not affected by stimulation, it seemed likely that some factor regulating its activity was modified by ethephon (Chapter 3.IV). It might be interesting to search for this regulator. Studies of purified sucrose synthetase should be carried out with this phenomenon in mind.

It was shown that a rise in the polyribosome content could be observed as early as 12 hr

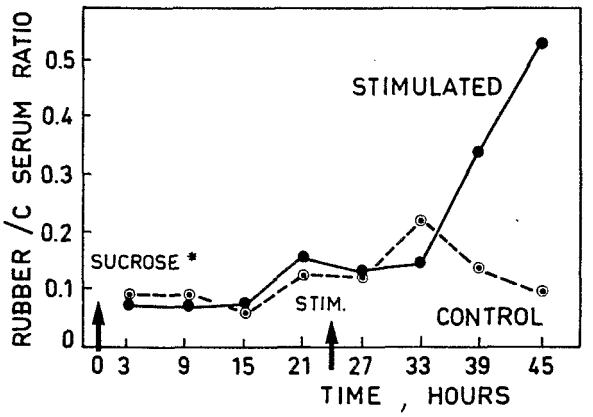

FIGURE 13. Effect of stimulation on incorporation of sucrose in rubber. U^{14}C sucrose was injected into the bark of both trees at time 0. At time 24 hr one tree was stimulated with ethephon while the second served as control. Radioactivity of C-serum and rubber was measured and their ratio plotted as a function of time. (Adapted from Lacrotte, R., Van de Sype, H., and Chrestin, H., *Physiol. Vég.*, 23, 187, 1985.)

after stimulation (Figure 8).[74] This supports a direct role of ethylene in the expression of the genome. Although proofs of this effect are scarce in the literature, it is known that ethylene can actually derepress specific genes in the cell (see below).

C. Multiplicity of Stimulating Compounds

Owing to the multiplicity of the stimulating molecules and the fairly similar nature of the responses, it seemed likely that ethylene was the common agent in stimulation since its biosynthesis in the cells is induced by numerous treatments or substances which cause stimulation of the production such as, for example, mechanical wounding,[254] auxins,[3,146] copper, and various stress factors including phytotoxic chemicals, extreme temperatures and drought, ionizing radiation, diseases, insect damage, and other injury.[1] It was also shown that hormone treatments stimulating yield induced the production of ethylene in excised leaflets and segments[25] and bark discs[170] of *Hevea*. The most effective ethylene inducers are also the best stimulants. No compound which was not a yield stimulant induced the formation of a significant amount of ethylene.

D. Increase of Sugar Loading

Lacrotte et al.[128] studied the influence of ethephon on the sugar content of laticiferous cells in *Hevea*. Injection of U^{14}C-sucrose into the bark *in vivo* resulted in the appearance of radioactivity in the cytosol and in the rubber fractions of the latex collected; this phenomenon was accentuated by ethephon. The comparison of naturally high- and low-yielding trees showed that there was more radioactivity in the rubber from the high yielders. Since Hebant (Chapters 1.I and 1.II), noticed that the latex vessels contained few or no plasmodesmata, the sucrose must have been pumped from the apoplast by active means.

The kinetics of stimulation of sucrose incorporation into rubber are rapid and last less than 12 hr (Figure 13). These kinetics had the same timing as those of the rise of the pH in the latex following stimulation by ethephon. The preceding results indicate that ethephon probably acts on an ATPase proton pump which simultaneously causes a rise in latex pH and pumping of sucrose from the neighboring phloem. The primary effect of ethylene in the laticiferous cell probably lies at this level.

The times observed above can be compared with the ethylene induction period measured

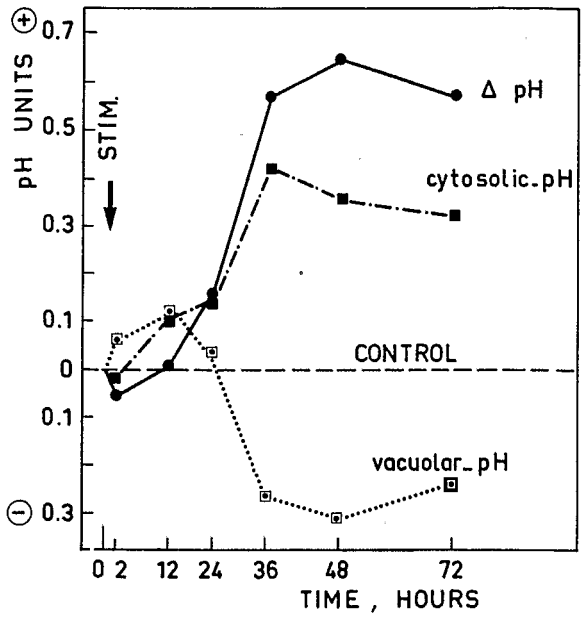

FIGURE 14. Kinetics of ethephon effects on cytosolic and vacuolar pH, and on the resulting transtonoplastic ΔpH. (Adapted from Chrestin, H., *Rev. Gen. Caoutch. Plast.*, 62, 75, 1985.)

by Audley et al.[25] on tissues treated with yield-stimulating compounds. For 2,4-D this period was 110 hr (more than 4 days); similar figures, in the 30- to 120-hr range, were found with other substances. This is much longer than the times observed in field trials. These authors used only 20-m*M* concentrations of stimulants. In field practice 1.5% concentrations, that is to say 3 times the preceding figure, are commonly used. In addition, the foliar and stem tissues used by these authors may have different responses to those of the trunk.

E. Compartmental pH Changes

It was then shown that the alkalinization of the latex cytosol induced by treatment with ethephon occurred in parallel with acidification of lutoids.[47,59,74] These opposite pH variations of the two major cell compartments of latex after hormone stimulation led to seeking to observed activation of transtonoplast proton fluxes, as induced *in vivo* by ethylene. It was therefore attempted to find out whether the functioning of the tonoplast proton pump was involved in these compartmental pH changes.

Figure 14 describes some kinetic aspects of the effects of ethephon on changes of cytosol and lutoid pH in latex vessels. It can be seen that the response to ethephon treatment can be divided into two distinct phases:

1. An initial stage (lasting about 21 hr), characterized by slight, slow alkalinization of the cytosol and a slight transient rise in the intravacuolar pH (maximum after 13 hr). During this early stage, the pH response of both compartments varied in the same way and to the same extent. The transtonoplast pH gradient (ΔpH) then remained constant for at least 13 hr.
2. A delayed response which exhibited simultaneous alkalinization of the cytosol and acidification of the lutoids. Cytosol pH increased by 0.42 unit compared to control (maximum after 33 hr) and then remained 0.3 unit higher for more than 2 days. At the same time, the intravacuolar pH decreased by 0.2 to 0.3 unit below control (Figure 14 and 15).

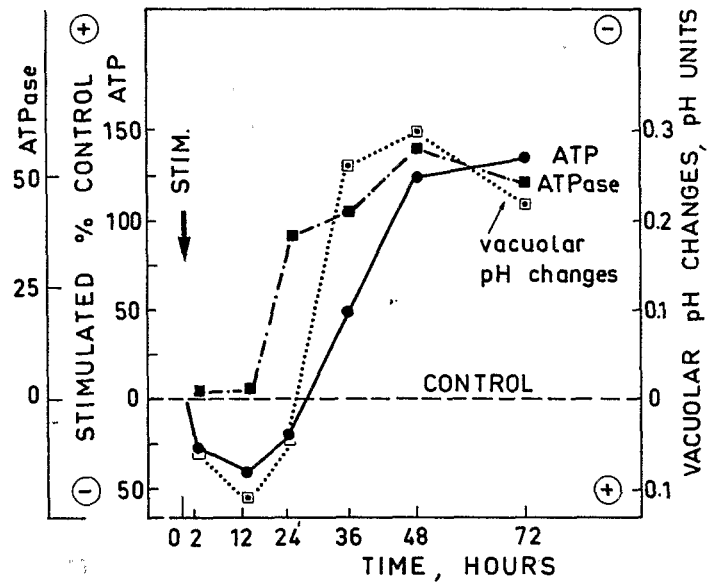

FIGURE 15. Kinetics of ethephon effects on tonoplastic ATPase activity, cytoplasmic ATP level, and vacuolar pH changes. (Adapted from Chrestin, H., Gidrol, X., Marin, B., Jacob, J. L., and d'Auzac, J., *Z. Pflanzenphysiol.*, 114, 269, 1984.)

The resulting transtonoplastic ΔpH increased by 0.57 pH unit (33 hr) and then 0.55 pH unit above control. Although the pH of the two compartments moved in symmetrically opposite directions, the cytosol pH was shown to be more affected than the intravacuolar pH. This may be accounted for by the greater buffer capacity of intralutoid serum.[59]

Figure 15 shows that 21 hr after treatment, ethephon induced a sharp increase in tonoplast ATPase activity as measured *in vitro* on intact lutoids. The same activity measured on purified tonoplast and under optimal pH and substrate conditions (Figure 16A) was shown to be four times higher in the membranes from trees treated with ethephon than in membranes from control trees.

Kinetic studies (Figure 16B) indicated that the K_m of the enzyme for Mg-ATP remained unchanged whereas increase in V_{max} could be interpreted as an increase in the number of catalytic sites on the tonoplast.[92,93] This was assumed to result from activation of *de novo* synthesis of the enzyme.

F. Increase in Protein Synthesis

Experiments were performed in the field, on whole latex collected directly into an incubation medium containing labeled amino acids and the classic proteosynthesis effectors. Figure 17 shows that *in vitro* the incorporation of exogenous amino acids was strongly enhanced in the acid-precipitable cytosol fraction and in the particulate membrane from the latex of stimulated trees. This activation of protein synthesis reached a maximum in both cytosol and membrane fraction about 12 hr after application of ethephon. It then remained stable in the membrane (+40 to 55%) but decreased in the cytosol. These results are consistent with those of Coupé et al.,[74] who observed a considerable increase in the polymerization index of ribosomes as soon as 12 hr after the application of stimulants.

Exogenous RNases had strong inhibitory effects (−73%) on this incorporation of amino acids, whereas polyuridylic acid was at least a weak activator (+34%). Moreover, the fact that chloramphenicol had no effect demonstrated that this proteosynthesis could not be ascribed to bacterial contamination. Alkaline or enzymatic (exogenous protease) hydrolysis

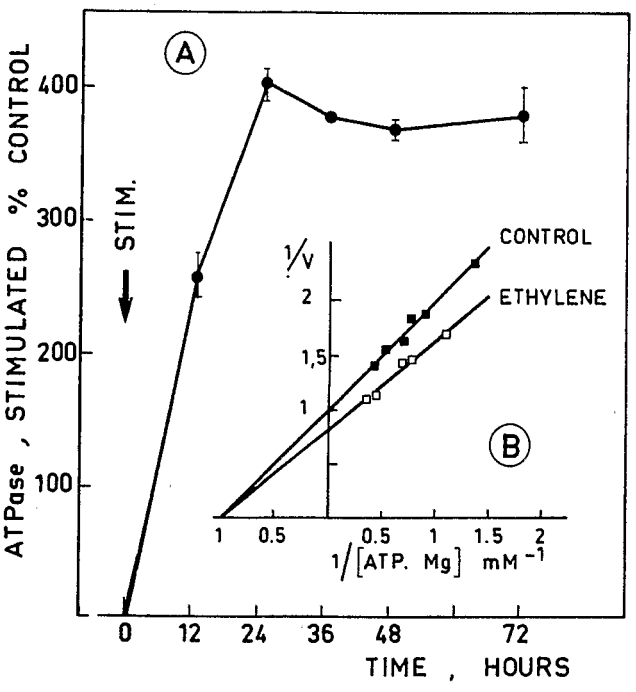

FIGURE 16. Effects of ethephon treatment on potential activity and kinetics parameters of tonoplastic ATPase. (Adapted from Gidrol, X., Caracterisation de l'ATPase Tonoplastique de la Cellule Laticifère *d'Hevea brasiliensis*, Thèse Doct. 3ème cycle, Université Aix-Marseille II, 1984.)

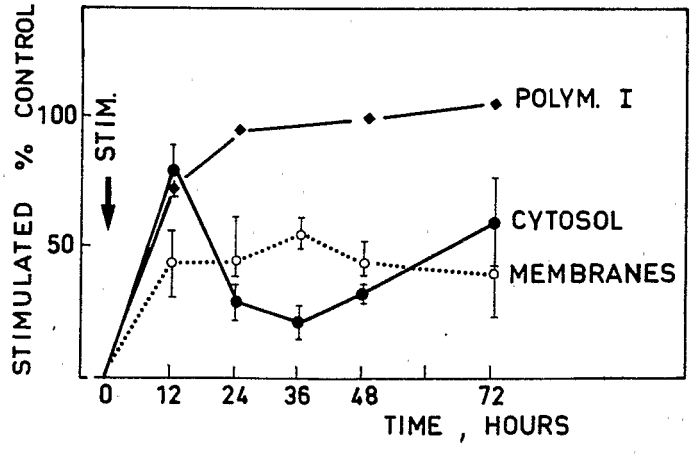

FIGURE 17. Effect of stimulation on protein synthesis. Kinetics of ethephon treatment on incorporation of amino acids into tonoplastic (o..o), and cytosolic (•—•) proteins. Vertical bars show the maximal amplitude of observed variations. Polymerization index of ribosomes (♦—♦) is from Coupé et al.[74] (Adapted from Gidrol, X., Caracterisation de l'ATPase Tonoplastique de la Cellule Laticifère d'*Hevea brasiliensis*, Thèse Doct. 3ème cycle, Université Aix-Marseille II, 1984.)

Table 2
ACTIVATION OF THE TONOPLASTIC ATPase BY A CYTOSOLIC FACTOR FROM STIMULATED TREES

Lutoid origin	Incubation medium	ATPase activity	Activation (%)
Control trees	Isotonic buffer	0.38	0
	Control cytosol	0.66	+74
	Stimulated cytosol	0.98	+118
Stimulated trees	Isotonic buffer	0.84	+121
	Control cytosol	0.88	+132
	Stimulated cytosol	1.17	+207

Adapted from Gidrol, X., Caractérisation de l'ATPase Tonoplastique de la Cellule Laticifère d'*Hevea brasiliensis*, Thèse Doct 3ème cycle, Université Aix-Marselle II, 1984.

induced the release of soluble radioactivity from the acido-precipitable fraction.[92] This proved that the acido-precipitable radioactivity measured could effectively be ascribed to the incorporation of labeled amino acids in the protein fraction and thus to protein synthesis.

All these results, and in particular the fact that the increase in specific ATPase activity (with unchanged K_m), measured on purified tonoplast after hormone stimulation was shown to be accompanied by enhancement of the ribosomal polymerization index and protein synthesis at tonoplast level, strongly support the hypothesis that at least part of activation of the tonoplast proton pumping ATPase may be accounted for by a certain amount of *de novo* synthesis of ATPase catalytic sites.

Tupý[244a] showed recently that variation of rRNA and of poly $(A)^+$RNA were associated with variations in the level of latex sucrose. Correlations of rRNA and poly $(A)^+$RNA with latex pH also appeared. Bark treatment with ethephon, increasing latex pH, sucrose utilization, and latex yield, increased the levels of rRNA and particularly of poly $(A)^+$RNA.

G. Appearance of Soluble Activators of ATPase

Enhancement of tonoplast ATPase activity was not only ascribable to an increase in the number of catalytic sites in the tonoplast. Table 2 displays the results of crossed incubation experiments in which lutoids from stimulated and control trees were incubated in artificial medium and in ultrafiltered latex cytosol from stimulated and control trees.

The results show that tonoplast ATPase activity was enhanced in ultrafiltered cytosol in all cases, but that latex cytosol from trees treated with ethephon had a greater stimulating effect than cytosol from control. It can therefore be concluded that cytosol contains one or more activating compounds and that hormone stimulation caused an increase in the amount of such a preexisting activator and/or the appearance of some new ATPase-stimulating factor and/or the disappearance of an ATPase inhibitor.

Activating compounds obtained from ultrafiltrated cytosol have a low molecular weight (< 10,000) and were shown to be heat resistant. Specific chelation of ions, ion-exchanger treatment, and neutralization of ultrafiltered boiled cytosol showed that the activator was anionic. It was also found that hormone stimulation simultaneously induced the appearance of weak inhibitory cations in the cytosol. These results are consistent with those found *in vitro* with regard to the stimulating effects of anions and the depressive effects, on ATPase activity, of certain cations such as Cu^{2+}.[92] However, the activators which appeared in latex cytosol after hormone treatment were shown to considerably outweigh the inhibitory cations; tonoplast ATPase activity measured in stimulated trees is thus greatly stimulated.

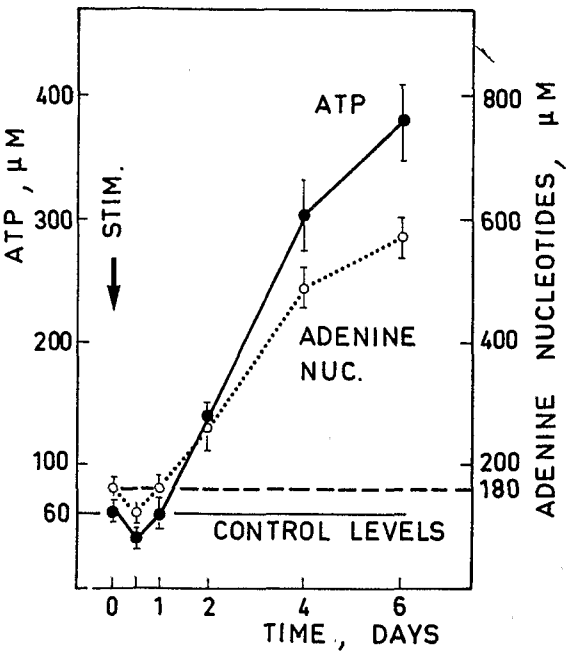

FIGURE 18. Kinetics of ethephon effects on ATP and total adenine nucleotides pools. Stimulation was performed on virgin bark 1.5 m above the tapping cut. Latex samples were obtained by micropunctures 10 cm above and under the treated bark and directly collected in a fixative medium. Control levels refer to unstimulated trees. (Adapted from Chrestin, H., *Rev. Gen. Caoutch. Plast.*, 62, 75, 1985.)

H. Increase in ATP and Total Adenine Nucleotides Pools

Stimulation of regularly tapped trees induced a variation of ATP content of latex cytosol as soon as 2 hr after treatment. In stimulated trees, it fell to 30% below control and then decreased to reach 47% after 13 hr. After this transient decrease, the ATP content increased rapidly, exceeding control level up to 240%, 71 hr after treatment.

Regular tapping is known to bring about activation of the entire mechanism involved in latex regeneration; this is thought to determine certain specialized metabolite fluxes and metabolic balances in the tapped bark.[242] Virgin bark, i.e., bark outside the area activated by regular tapping,[139] was therefore treated with ethephon to assess the response of latex cells which had never been tapped. Furthermore, the latex was collected by puncture-tapping with needles to prevent modifications to the latex metabolism in the activated area after large-scale sampling of latex. Only the few drops of latex strictly necessary for the adenine assay were collected.

These experiments showed that the adenine pools in latex from virgin bark treated with ethephon (Figure 18) displayed similar kinetic variations, with somewhat different amplitudes, to the results obtained in experiments on regularly tapped bark (Figure 15). Two contrasting phases were observed once again. ATP and total adenine nucleotide pools displayed significant decreases during the 12 hr following treatment (33% below control) followed by a considerable gradual increase of both pools lasting for at least 6 days. Both ATP and total adenine contents remained fairly constant throughout the experiment in latex from nonstimulated control trees.

These results showed that ethephon induced biochemical events even in preexisting latex of resting laticiferous cells in virgin bark. It can therefore be assumed that the changes

induced by ethephon in ATP and total adenine nucleotide pools are a true, direct effect of hormone stimulation.

In addition, it was seen in fact that the total adenine nucleotides pool (ATP + ADP + AMP) closely followed kinetic variations of ATP content alone (Figure 18), resulting in scarcely detectable changes in the energy charge or ATP:ADP ratio in latex cytosol.[59] This led to concluding that *de novo* synthesis of adenine nucleotides was caused by ethylene.

I. Conclusion

It can be seen in Figure 15 that the intravacuolar pH closely followed variations in latex ATP content. Low concentrations of ATP were associated with a weak alkalinization of the lutoids; high ATP concentrations were accompanied by considerable acidification of the vacuolar compartment.

These data (Figure 15) clearly show that the marked early activation of the tonoplast proton-pumping ATPase (between 13 and 21 hr) did not immediately result in any vacuolar acidification that might be expected. This may be accounted for by the transient relative lack of availability of substrate for the H^+-pumping ATPase (i.e., ATP) as an early side effect of treatment with ethylene releaser. Variations in the total adenine nucleotide pool, and in particular the decrease in ATP content, are the earliest biochemical characteristics of latex known to be affected by ethephon treatment. Early disturbance of the adenine nucleotide pool might result from enhancement of the utilization of triphosphate nucleotides in the synthesis of nucleic acids and proteins. The report by Coupé et al.[72,74] that treatment of *Hevea* bark with ethephon (or with auxin-like substances) induced an increase in total RNA and polyribosome contents of latex during the 12 hr which follow treatment supports such an hypothesis. It is therefore possible that ethylene induces true *de novo* adenosine nucleotide synthesis in less than 24 hr, and that the total adenine nucleotide pool will be rebalanced continuously. The most striking biochemical event after ethephon treatment is the sharp increase in tonoplast ATPase activity after 13 to 21 hr. This was observed in vitro in intact washed lutoids and also in purified lutoid tonoplast,[78,92] hence in the absence of any possible soluble cytosolic activator. This increase in ATPase-specific activity was shown to be accompanied by significant stimulation of protein synthesis in lutoid tonoplast.[92] Thus the tonoplast of lutoids from stimulated trees keeps an indelible print of ethylene treatment. The fact that only V_{max}, but not K_m for Mg-ATP was modified means that enhancement of ATPase activity is due to an increase in the number of ATPase catalytic sites in the tonoplast ascribable to *de novo* ATPase synthesis. This is among the earliest biochemical events induced by ethylene. The simultaneous opposing changes in cytosol and vacuolar pH, together with the satisfactory stoicheiometry of estimated transmembrane H^+ fluxes (depending on respective buffer capacities of the compartments)[59] suggest some stimulation of tonoplast proton pumps by ethylene. Parallel increases in intravacuolar acidification and cytosolic ATP content leads to concluding that transtonoplast H^+ fluxes depend on lutoid ATPase activity, as controlled by the availability of ATP. ATPase K_m for Mg-ATP was found to be about 0.5 to 0.7 m*M* when measured under physiological conditions,[92,94] whereas the mean ATP content of cytosol remained less than 0.25 m*M* in the control.[59]

Tonoplast H^+-pumping ATPase thus always operates at far less than its maximum potential in vivo, and its real activity depends in a linear manner on cytosol ATP content. The initial decrease in latex ATP content (30 to 40% less than control) causes a decrease in ATPase activity and thus vacuolar alkalinization. In contrast, increase in cytosolic ATP activates the H^+-pumping ATPase and accounts for the rise in the transtonoplast ΔpH and alkalinization of latex cytosol. It has been established that the two opposing proton pumps on the lutoid tonoplast (ATPase and NADH cytochrome *c* reductase) can operate as a biophysical pH-stat under in vivo conditions (Chapter 3.III). The differential functioning of the two moieties is probably controlled by the availability of the respective substrates (ATP and NADH).

This has been demonstrated in particular for proton-pumping ATPase both in vitro and in vivo.[59,92,93] The ATPase moiety of the tonoplast pH-stat was shown to be activated by exogenous ethylene because of at least three early biochemical events: (1) increase in potential ATPase activity probably through *de novo* synthesis of the enzyme; (2) increase in real ATPase activity through a marked increase in the ATP content of the cytosol; (3) increase in real ATPase activity through the appearance of an anionic activator in the cytosol.

The combination of these phenomena induced by ethylene leads to effective activation of the tonoplast proton-pumping ATPase, resulting in an increase in transtonoplast ΔpH (favoring detoxification of the cytosol) both of which favor the latex metabolism and hence the production of rubber (Chapter 3.III and 6.I).

VI. STIMULATION MECHANISMS

This section is partly conjectural since data concerning early events after stimulation are lacking. After application of ethephon to the tree, there is a lag of at least 6 hr before any effect of the substance is noticed. This is long enough for many types of biosynthesis to be activated.

After the period of latency (6 to 8 hr), production increases and at almost the same time latex pH rises, sucrose synthetase falls, utilization and incorporation of sucrose in rubber is enhanced, and the polyribosome level rises. Since all these biochemical events have similar timings it is difficult to identify which of them is the very first effect of ethylene. The variations in the total adenine nucleotide pool and especially the decrease in the ATP content are among the earliest known biochemical characteristics affected by ethephon stimulation. Early disturbance of the adenine nucleotide pool might result from the enhancement of the utilization of triphosphate nucleotides for the synthesis of nucleic acids. Comparison with data found in the literature helps to understand the mechanisms involved in stimulation. We review the effects of ethylene on growth, protein synthesis, permeability, and respiration.

First of all, it is legitimate to ascribe ethephon stimulation to ethylene since many similarities have been found between the effects of both of these plant growth regulators.[133,157] However, possible side effects of the products of the degrading of ethephon are not excluded.

A. Growth

In most cases ethylene inhibit growth, although it can be increased in certain plants,[249] in particular in hydrophytes.[67] Osborne[159] pointed out the importance of the target cell in the response to ethylene.

Growth of *Hevea* is little affected by stimulation.[100] Obviously the mature trunk cannot display the same responses as young seedlings. Its longitudinal growth is hampered by lignification. Only sieve tube size is reduced by stimulation. Unpublished observations by de Faÿ[261] show that the main effect of ethephon is obtained on the outer tissues of the phloem, leading to hypertrophy and hyperhydricity. The bias of experiments dealing with the effects of simulation on tree growth must be remembered. Treatments are designed to increase latex production and they thus take photosynthates from the tree. The strict effect of ethylene on tree growth cannot be observed under these conditions.

B. Nucleic Acid Synthesis

In the hook zone of pea, ethylene was shown to inhibit DNA synthesis;[20,22] very small amounts were found to be effective.[207] RNA synthesis is not affected by this gas in subapical tissue. In *Hevea*, stimulation takes the form of a large increase in the polyribosome population, showing that mRNA synthesis is enhanced. The same effect has been observed in many plants.[63,98,125,142,260] A direct effect of ethylene on gene expression was demonstrated by Nichols and Laties.[155,156] Mitochondrial DNA and RNA polymerase increase in response to exposure of whole potato tuber to this gas.[23]

C. Enzyme Synthesis

In many types of tissue, the promotion of biosynthesis of mRNA takes the form of an increase of certain enzymes such as cellulase in the abscission zone,[98,107] phenylalanine ammonia lyase,[53,84,198] polygalacturonase in fruits,[204] peroxidases in sliced or wounded tissue.[40,41,108] In bean leaves,[44] induction of chitinase was caused by ethylene after a time lag of 6 hr, which was close to the period recorded in *Hevea* for the synthesis of the ATPase proton pump.

D. Permeability, Secretion, and Transport

Another type of effect of ethylene is ascribed to its actions on permeability and transport. Direct effects were noticed on mitochondrial membrane permeability,[126,144] but they were proved to be unspecific and required high levels of gas. Inversely ethylene-induced changes in cell permeability of *Narcissus tazetta* were attained after 5 hr of exposure to very low concentrations of ethephon.[171] The time lag observed suggest that ethylene may trigger the biosynthesis of proteins modifying the cell membrane permeabilities.

Ethylene also increases secretory processes such as the release of α-amylase from barley half seeds,[119] of peroxidase from peas,[197] and of cellulase in the abscission zone.[2] All these effects on permeability and secretion must be borne in mind in order to understand the effect of ethylene on *Hevea*. In bark, numerous exchanges may be modified during the 6 hr prior to the enhancement of the latex flow. Lacrotte et al.[128] showed that stimulation led to an increased flux of sucrose into the latex.

E. Respiration and Translocation

The ability of ethylene to stimulate the respiration of fruits and other plant tissues, including dormant potato tubers, is well documented.[1,189,190] This phenomenon may arise, among other reasons, because of the increased activity of respiratory enzymes and/or improved supply of substrates to treated tissue.

Ethylene promotes the translocation of sugars and inorganic material from petals of carnation to the ovary.[152-154] These authors used exogenously applied ^{14}C sugars to study the source-sink relationship between the flower parts. Their results provided evidence for the hypothesis that ethylene promotes mobilization of substrates and an outflow of material from petals to other flower parts.

The favorable role of ethylene in onion and leek bulb formation may be due to its effect on the translocation of the assimilates.[132] The promotive effect of ethylene on fruit growth is probably due to the same causes.[208] Enhancement by ethylene of tuber growth in radish seedlings is probably due to a changing of assimilate partitioning rather than induction of cambial activity.[252]

Distribution of assimilates in cut carnation flowers is affected by ethylene.[251] After cutting, translocation of assimilates to the petals is sustained for 5 days and stops with the surge of ethylene accompanying the wilting of the flower. Treatment with silver thiosulfate, which is an ethylene antagonist, prevents wilting and allows continuous translocation towards the petals.

Elongation of rice coleoptile is promoted by ethylene.[67] The gas increases the transport of uranine, a fluorescent dye, through the coleoptile.[111] It is likely that enhancement of growth is due to improved translocation of sugars from the endosperm.

Nonclimacteric organs such as potato tubers exhibit a climacteric rise when exposed to ethylene.[1] This shift is associated with an increased activity of mitochondrial enzymes. It was shown in potato tuber[23] that cytochrome oxidase activity and respiration was enhanced upon ethylene treatment after a time lag of 6 hr which is close to the latency period observed in *Hevea* after application of ethephon. It can be postulated that respiration and thus nucleotide production is activated in the same way in the latex cell.

The increase of sucrose in the zone of application of ethephon in *Hevea* is probably caused by ethylene-stimulated transport from leaves or medullar rays.

F. Lutoid Properties

A troublesome observation can be made concerning lutoids. Their major role in coagulation has been firmly established. Their stabilization by stimulation is distinct, but occurs clearly after the enhancement of latex flow. Even an increase of the BI was noticed the day after treatment. Likewise, this index remains low a long time after stimulation when production has started to decrease. This makes it possible to think that the BI does not reflect all the properties of this organelle. It is conceivable that ethylene causes rapid modifications of the membrane properties of the lutoids or of rubber and Frey-Wyssling particles (surface charge for example) which results in stabilization of the latex and decreased plugging.

The lutoid membrane has a special composition: it is characterized by an unusual phosphatidic acid content.[83] Comparison between lutoid membranes from control and stimulated trees has been made without clear-cut results.[262]

VII. CONCLUSIONS

A direct and rapid action of ethylene on membranes is not excluded. Modifications of lutoid characteristics (surface potential for example) by ethylene may lead to a modification of rheological properties of the latex. Direct actions of ethylene on enzymes have never been established. Activation of proton pump ATPases by plant hormones are scarcely documented. Hager et al.[99] hypothesized an IAA activation of the plasmalemmic ATPase in elongating cells. This was indirectly proved since vanadate, a well-known inhibitor of plasmalemmic ATPase, counteracts the IAA-induced elongation. IAA could increase the affinity of plasmalemmic ATPase for its substrate in vitro,[209] but failed to activate Ca^{2+}-ATPase in vitro, whereas enzyme was markedly stimulated when leaf fragments were incubated with IAA, prior to enzyme isolation.[143]

Ethylene activation of ATPase synthesis have not been reported since the work of Gidrol.[92] Conversely, ACC (a direct precursor of ethylene) treatment of carnations during senescence induced a reduction of ATPase activity and of sucrose uptake.[17]

It can be supposed that upon ethylene treatment, gene derepression leads to increased synthesis of other proteins, especially plasmalemmic ATPase and mitochondrial enzymes. The following events (Figure 6, Chapter 3.III) would result.

The functioning of both ATPases lead to an alkalinization of the cytosol and acidification of the lutoidic serum. At the same time active pumping of sucrose into the latex cell is promoted. Cytoplasmic alkalinization activates the functioning of invertase, which is the key enzyme of glycolysis in the latex cell. Activation of glycolysis leads to overproduction of pyruvate and ATP. The availability of these molecules allows the enhancement of mevalonate production and ultimately of rubber biosynthesis. Pyruvate is readily oxidized in the mitochondrion, producing ATP which fuels the ATPases.

Two feedback mechanisms regulate this phenomenon: a lutoidic NADH cytochrome *c* reductase which can expel protons from lutoids,[62] and a Davies-type biochemical pH-stat.[113,115] The ultimate response against stimulation is an autolysis carried out by an NADH quinone reductase. This enzyme produces toxic oxygen which may destroy lutoidic membranes by lipid peroxidation, unless this toxic oxygen is quenched by protective enzymes like superoxide dismutase.[61,62,77]

Other effects of ethylene are mentioned in the literature: Apelbaum et al.[21] found evidence proving that ethylene directly inhibited synthesis of polyamines by inhibition of the arginine decarboxylase. Polyamines may be a kind of second hormonal messenger. In most cases, the lower the ethylene concentration, the higher the polyamine content and the higher the DNA multiplication because polyamines are involved in its synthesis. The relevance of this observation for the latex cell is questioned.

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