Phytochemistry, 1977, Vol. 16, pp. 455–458. Pergamon Press. Printed in England.

ABSORPTION OF CITRATE BY THE LUTOIDS OF LATEX AND RUBBER PRODUCTION BY *HEVEA*

MICHEL COUPÉ and CLAUDE LAMBERT
Laboratoire de Physiologie Végétale, Faculté des Sciences—B.P. 4322, Abidjan, Cote d'Ivoire

(*Received* 28 *June* 1976)

Key Word Index—*Hevea brasiliensis*; Euphorbiaceae; lysosome; absorption; rubber production; ethrel.

Abstract—Correlations were established between the variations in rubber production by *Hevea* and the pH of the lutoids (lysosomes with vacuolar characteristics) and the pH of latex cytoplasm. Correlation was also noted between production and the pH difference of the two compartments. Analogous relationships were found between citrate absorption by lutoids and the pH values of latex sera. Hormonal stimulation of production is accompanied by an increased citrate absorption by the lutoids.

INTRODUCTION

Rubber production by *Hevea* can be greatly increased by the application of various stimulatory agents on the tapping panel of the tree. An increase in the pH of the latex is systematically observed, whether the stimulatory agent be auxinomimetic [1, 2], 2-chloroethanephosphonic acid (ethrel) [1, 2], or inorganic compounds such as $CuSO_4$ (2, 3), H_3BO_3 and $HgCl_2$ (2). The pH rise is detectable 24 hr after the application of ethrel and closely follows the increase in rubber production [4].

The increase in the pH of latex favors carbohydrate catabolism and stimulates, sometimes very significantly, certain enzymatic activities such as invertase [5], pyruvate kinase [6], phosphofructokinase [7], and glyceraldehyde-3-phosphate dehydrogenase [7, 8].

In addition, considerable research has demonstrated the influence of the lutoids on determining latex flow. Lutoids, which are vacuolar vesicles [9] of 2–10 μm diameter, have a lysosomal nature [10]. Their internal pH is acidic, between 5.5 and 6.0 [11] like other plant vacuoles. The fact that certain ions and metabolites are accumulated inside them confirms their vacuolar nature [9, 12, 13]. During stimulation, it was shown by Ribailler [14] that the stability of the lutoids increases considerably and that under these conditions, liberation of hydrolases in the latex results in a prolonged flow. The lutoids can also intervene at the level of variations in pH of the latex, observed during stimulation, due to exchange mechanisms occurring between these organelles and the cytoplasm.

It is observed in plantations that morphologically identical trees can be either high or low latex producers. It was thus of interest to examine the relationships which could exist between rubber production, the pH of the latex and certain characteristics of the lutoids, such as their pH, ion content and capacity for citrate absorption.

RESULTS

Production

The relationship between production and pH was studied in a series of 18 trees of clone GT1, grouped in threes in such a way that there were important variations in production among the different groups. Six consecutive tappings were performed and the results, shown in Fig. 1, indicate a significant correlation between production and the pH values of cytoplasmic serum ($p < 0.02$)

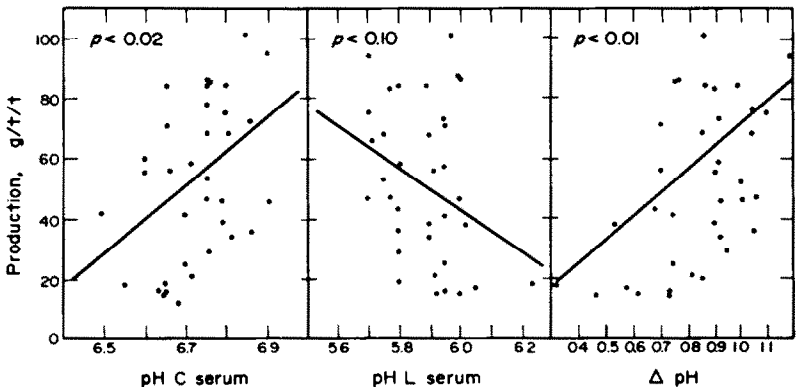

Fig. 1. Variations in production as a function of cytoplasmic and lutoidic sera pH as well as their difference (ΔpH). Production is expressed as g/tree/tapping.

Table 1. Comparison of various characteristics of high and low producing trees of clone PR 107. The average values presented correspond to results obtained during four successive tappings. Production = g/tree/tapping. Absorption = nmol citrate absorbed/30 min/mg protein. p = probabilities calculated by the 't' test, of non-significance of differences of the means

	High producers	Low producers	p
Production	102.6 ± 38.1	52.1 ± 16.1	0.001
Absorption	30.7 ± 11.4	20.3 ± 9.3	0.02
pH of cytoplasmic serum	6.60 ± 0.05	6.53 ± 0.06	0.005
pH of lutoidic serum	5.60 ± 0.08	5.71 ± 0.07	0.001
ΔpH	1.00 ± 0.11	0.82 ± 0.06	0.001

and lutoidic serum ($p < 0.1$). In addition, the correlation between production and ΔpH is highly significant ($p < 0.01$). Linear regression equations between production and ΔpH, obtained with the method of least squares, give the following results:

For clone PR 107 Production = 108 ΔpH − 8, 3
For clone GT 1 Production = 64 ΔpH − 1, 5

It can be seen (Table 1) that for clone PR 107 the differences between high production and low production (HP–LP) are translated as differences in pH. The pH of cytoplasmic serum of HP trees is higher than that of LP trees and the inverse relationships applies for lutoidic serum pH. The overall result is a clear difference in ΔpH values of the two groups of trees. The Student's t test shows these differences to be highly significant. Thus, the characteristics of the latex studied permits the classification of the trees in two different categories.

During stimulation, an increase in both production and pH of the latex is noted. In Table 2 is shown the results obtained after stimulation of GT 1 trees: a noticeable pH rise occurs accompanied by a 3-fold greater production for both LP and HP trees. Linear regression equations between production and pH show that a ΔpH of 0.2 corresponds to an increased production of 13 g/tree/tapping. The increases in production observed (Table 2) indicate that the cytoplasmic pH rise,

Table 2. Averages of production and ΔpH of high and low producers of clone GT 1. Untreated trees were merely scraped 3 cm below the tapping panel; stimulated trees were similarly scraped, followed by an application of ethrel (see Methods). Production = g/tree/tapping

Trees		High producers Before treatment	High producers After treatment	Low producers Before treatment	Low producers After treatment
Unstimulated	Production	86	73	19	16
	ΔpH	0.85	0.89	0.65	0.64
Stimulated	Production	68	190	43	120
	ΔpH	0.85	1.05	0.68	0.87

which is essentially responsible for the ΔpH, cannot be the only determining factor for the observed overproduction.

Citrate absorption by lutoids

The same batches of latex used for determination of the correlations between production and ΔpH were the source of lutoids used for the measurement of citrate absorption. The results obtained concerning the relationship between citrate absorption and pH are shown in Fig. 2. If the direct relation between absorption and pH is examined, the least squares method shows a significant correlation ($p < 0.05$) between absorption and pH of cytoplasmic and lutoidic sera. Further, the correlation between ΔpH and absorption is highly significant ($p < 0.01$). A better fit is observed between ΔpH and an exponential function of absorption. In this case, the linear regression between ΔpH and ln (absorption) yields an exceedingly significant correlation ($p < 0.001$). The equation of linear regression between $\ln_{absorption}$ ($\ln(A)$) and ΔpH is:

$$\ln(A) = 2{,}11\ \Delta pH + 0{,}58$$

It was observed that the citrate absorbing capacity of

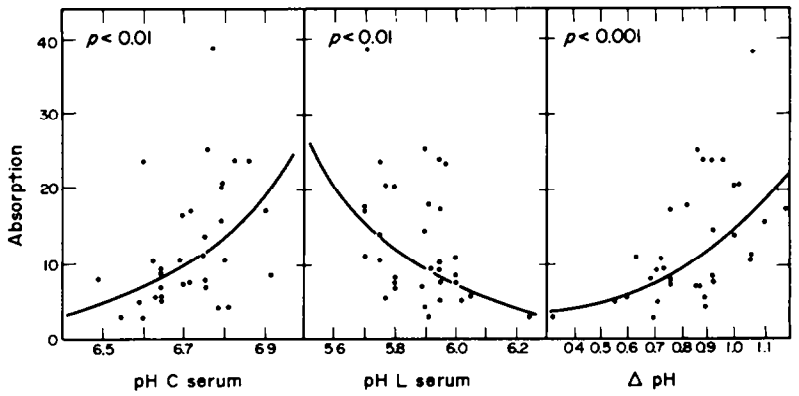

Fig. 2. Variations in citrate absorption by lutoids as a function of cytoplasmic and lutoidic sera pH, as well as their difference (ΔpH). Absorption is expressed as nmol citrate/30 min/mg protein.

Table 3. Comparison of citrate absorption by lutoids from high and low producing trees. Absorption = nmol citrate absorbed/30 min/mg protein. p = probability, calculated by the 't' test, of non-significance of differences of the mean

Clone		High producers	Low producers	p
PR 107	Absorption	17.4 ± 5.7	13.1 ± 5.2	0.02
	ΔpH	0.97 ± 0.11	0.82 ± 0.06	0.001
GT 1	Absorption	12.2 ± 5.4	7.9 ± 2.2	0.05
	ΔpH	0.91 ± 0.15	0.74 ± 0.12	0.05

the lutoids is a function related to the HP or LP nature of the trees. This is clearly shown in Table 3, where are depicted the average values of citrate absorption by lutoids of HP and LP trees of the GT 1 and PR 107 clones. In addition, it appears that citrate absorption can be used to characterize a given tree; whatever fluctuations are observed from one tapping to the next, a tree consistently retains the capacity to absorb either a little or a lot of citrate.

The effect of stimulation on citrate absorption by lutoids of HP and LP trees was also studied. The first tapping after stimulation results in an increase in citrate absorption by lutoids *in vitro*, reaching a maximum on the second tapping for PR 107 trees, and on the third tapping for GT 1 trees. The values, represented in Table 4, show that stimulation of both HP and LP trees leads to a considerable increase in citrate absorption, indicating that important modifications of the properties of lutoids have occurred.

CONCLUSIONS

A correlation was noticed between rubber production and the pH of cytoplasmic serum as well as the ΔpH of latex. Various explanations of these observations can be given. Cytoplasmic serum is the site of carbohydrate catabolism as well as rubber biosynthesis. Numerous enzymes participating in these pathways have an alkaline pH optimum [5–8]. This is thus consistent with the observation that HP trees have a cytoplasmic serum whose pH is higher than that of LP trees. The correlation between production and an elevated ΔpH is meaningful in the light of observations by Pujarniscle and Ribaillier [15], who demonstrated a relationship between the stability of lutoids and the capacity of latex to transform acetate into rubber *in vitro*. Ribaillier observed a correlation between production and the breakage index of lutoids. It can be supposed that the ΔpH as well as the pH of cytoplasmic serum are related to the integrity of lutoids.

Table 4. Citrate absorption by lutoids from unstimulated and stimulated trees. Treatment as described in legend to Table 2

		Citrate absorption (nmol/30 min/mg protein)			
		GT 1		PR 107	
		Before treatment	After treatment	Before treatment	After treatment
High producers	Unstimulated	7.6	15.4	17.8	12.3
	Stimulated	11	75.2	18.8	46.5
Low producers	Unstimulated	6.8	8.8	12.6	8.2
	Stimulated	8	22.1	15.5	36.6

The pH of lutoid serum is acidic and so the pH of cytoplasmic serum will be lower in proportion to the amount of lutoids which burst in the latex. We observed that the pH of cytoplasmic serum varied from 6.4 to 7.0 (0.6 pH units) which is 10 times higher than the variation expected from the breakage index of lutoids. This leads to the conclusion that if the lutoids of the latex participate in the productivity of the tree, this effect is not as much due to the acidity which would be liberated as it is to the hydrolytic enzymes liberated [10]. We have as yet no explanation as to why the lutoids of HP trees are stable and the cytoplasmic pH is elevated; this is perhaps a characteristic of the tree.

The present results confirm previously-acquired data [16] that the intralutoidic pH influences citrate absorption. Stimulated trees yield lutoids whose internal pH is slightly lowered, 0.05 units, on average. Citrate absorption by lutoids from stimulated trees is, at its maximum, 500% that of organelles from non-stimulated trees. This is consistent neither with theoretical results calculated from correlation curves, nor with results obtained *in vitro* by decreasing the intralutoidic pH with ATP (100% increase in absorption for a drop of 0.3 pH units).

A very important absorption of malate and succinate by lutoids is also observed during stimulation; thus the effects studied in the present report are a reflection of a general phenomenon. It can thus be suggested that there is a regulatory role of lutoids during stimulation which is based on important modifications of transmembrane transport. Metabolism would be regulated by controlling exchanges between the two principal compartments of latex.

EXPERIMENTAL

Latex used was from *Hevea brasiliensis* (Kunth) Müll. Arg. comprising two different clones, GT1 and PR 107. In order to have the most homogenous groups possible, 8-yr-old trees were selected by determining trunk diameter at a height of 1.5 m from the ground; rubber production was determined over the course of 10 successive trappings. Latex was harvested in a vessel chilled on ice and then centrifuged for 30 min at 20000 *g*. After centrifugation the rubber was discarded and the remaining fractions consisted of clear cytoplasmic serum and the pellet of lutoids. The latter was divided in two portions, one immediately frozen, the other used for the measurement of citrate absorption. The frozen lutoids were burst by sonication at 20 kHz for 3 min; centrifugation of this brei for 30 min at 20000 *g* yielded a clear lutoidic serum. Production was determined as g of rubber/tree/tapping.

The technique for measuring citrate absorption has been previously described [12]. Briefly, lutoids and incubation medium, containing citrate-[1,5^{14}C] (sp. act. 30 mCi/mmol), were mixed at 25° in a ratio of 1:2.5 (v/v). After 30 min, during which time uptake is linear, citrate content of the lutoids was determined by lysing the separated organelles with Triton X-100 and determining the radioactivity present in the resulting serum. Results are expressed as nmol citrate absorbed/30 min/mg protein. Protein was determined with the method of ref. [17]. Stimulation was obtained by scratching the bark and applying 5% ethrel in glycerol.

REFERENCES

1. Tupý, J. (1973) *J. Exp. Botany* **24**, 516.
2. Coupé, L., Lambert, C. and d'Auzac, J. (1976) *Physiol. Vég.* (in press).
3. Tupý, J. (1973) *Physiol. Vég.* **11**, 633.
4. Coupé, M., Primot, L., Lambert, C. and d'Auzac, J. *Phytochemistry* (in press).
5. Tupý, J. (1969) *Planta* **88**, 144.
6. Jacob, J-L. and d'Auzac, J. (1969) *Bull. Soc. Chim. Biol.* **51**, 511.
7. d'Auzac, J. and Jacob, J-L. (1969) *J. Rubb. Res. Inst. Malaya* **21**, 417.
8. Jacob, J-L. and d'Auzac, J. (1972) *European J. Biochem.* **31**, 255.
9. Ribaillier, D., Jacob, J-L. and d'Auzac, J. (1971) *Physiol. Vég.* **9**, 423.
10. Pujarniscle, S. (1968) *Physiol. Vég.* **6**, 27.
11. Southorn, W. A. and Edwin, E. (1968) *J. Rubb. Res. Inst. Malaya* **20**, 187.
12. d'Auzac, J. and Lioret, C. (1974) *Physiol. Vég.* **12**, 617.
13. Brzozowska, J., Hanower, P. and Chezeau, R. (1974) *Experientia* **30**, 894.
14. Ribaillier, D. (1970) *Rev. Gén. Caout. Plast.* **47**, 305.
15. Pujarniscle, S. and Ribaillier, D. (1966) *Rev. Gén. Caout. Plast.* **43**, 226.
16. Lambert, C. (1975) *C.R. Acad. Sci. Paris* **281**, Série D, 1705.
17. Lowry, O. H., Rosebrough, N. I., Farr, A. L. and Randall, R. J. (1961) *J. Biol. Chem.* **193**, 265.