

Physiological Basis for Latex Diagnosis of the Functioning of the Laticiferous System in Rubber Trees

J.L. JACOB, J.M. ESCHBACH, J.L. PREVOT, D. ROUSSEL AND R. LACROTTE
IRCA-CIRAD, Paris, France

H. CHRESTIN
ORSTOM, Abidjan, Ivory Coast

AND

J. D'AUZAC
*Universite des Sciences et Techniques du Languedoc,
Montpellier Cedex, France*

Abstract in Bahasa Malaysia

Lateks ialah sejenis sitoplasma sel. Pengetahuan tentang ciri-ciri biologinya yang utama membolehkan keadaan fisiologi sistem latisifer dinilai dan selanjutnya menentukan dayanya untuk menghasilkan dan meregenerasikan kandungan selnya pada ketika yang tertentu.

Beberapa penyelidikan biologi dan fisiologi yang dijalankan dalam dua puluh tahun kebelakangan ini telah mendedahkan parameter-parameter lateks tertentu yang mengawal pengeluaran secara langsung atau tidak langsung dan yang boleh menyumbang kepada penentuan 'diagnosis lateks'. Parameter-parameter ini adalah parameter fizik (pH, potensi redoks), kimia (kandungan fosforus bukan organik dan magnesium) dan biokimia atau enzim (kandungan pepejal lengkap, kandungan sukrosa dan tiol, keaktifan fosfatase untuk pengiraan indeks pecahan).

Ditunjukkan bahawa dalam keadaan percubaan yang tertentu, semua parameter ini mempunyai korelasi yang amat nyata dengan pengeluaran. Korelasi-korelasi ini mungkin positif dan mungkin negatif. Oleh itu adalah logik bahawa parameter-parameter ini mungkin menjadi faktor-faktor yang menghadkan pengeluaran, baik dalam bidang aliran lateks mahupun dalam bidang regenerasi sel antara dua torehan. Contoh diberi bagi setiap kriterium dan peranan fisiologinya dibincangkan. Perubahan musim bersama dengan kesan penggalakan dan/atau jenis klon mesti diberi pertimbangan dalam pemeriksaan nilai parameter-parameter ini. Keadaan sistem latisifer yang tidak dieksploit sepenuhnya dan yang dieksploit dengan keterlaluan boleh dianggarkan dengan menggunakan parameter-parameter ini.

Abstract

Latex is a cellular cytoplasm. Knowledge of some of its important biological characteristics should therefore make it possible to assess the physiological condition to the laticiferous system and hence its ability for producing and regenerating its cellular content at a given moment.

A considerable amount of biological and physiological research carried out during the last twenty years has revealed certain parameters of latex which control production directly or indirectly and which can contribute to the determination of a 'latex diagnosis'. These parameters are physical (pH, redox potential), chemical (inorganic phosphorus and magnesium contents) and biochemical or enzymatic (total solid content, sucrose and thiols contents, phosphatase activities for bursting index determination).

It has been shown that under certain experimental conditions all these parameters can be correlated with production in a highly significant manner. The correlations may be positive or negative. In these cases, it is logical that the parameters might be limiting factors with regard to production, either in the field of latex flow, or in the field of cellular regeneration between two tappings. An example is given for each criterion and their physiological roles are discussed. Seasonal variations, together with the effect of stimulation and/or clone type, must be taken into account in the examination of the values of all these parameters. Definition of an under-exploited condition and an over-exploited condition of the laticiferous system is envisaged using these parameters.

Many different research studies¹⁻⁴ have led to extensive knowledge of the cytology of the laticiferous system in *Hevea*. The latex of *Hevea brasiliensis* is without doubt, a cytoplasm in the strict sense of the term. It is easy to tap this cytoplasm with no prior destruction of cells. Its characteristics are very close to those of *in situ* latex except that some of the constituents of latex (mitochondria and nuclei) are not collected at tapping¹.

The cytoplasmic nature of latex leads to the belief that analysis of certain biochemical and physicochemical parameters of this latex should provide knowledge about the state of health of the laticiferous system in the same way that analysis of the various physiological fluids provides information about man. Associated with knowledge of ecoclimatic conditions, the tapping history of the panel and the phytosanitary state of the tree, these criteria measured in the latex should make it possible to draw up a 'latex diagnosis'. The purpose of this, is to optimise exploitation (tapping method, hormone stimulation, etc.) by avoiding the exhausting of the laticiferous system while exploiting the maximum production potential of a given clone under given ecoclimatic conditions.

Choice of the latex parameters to be analysed depends on the degree of correlation which can be established between these parameters and production under appropriate conditions.

A large amount of research has made it possible to make such a selection of parameters. The aim of the present paper is to make a synthesis of all this work and thus to define the physiological significance of the various criteria retained in relation to *Hevea* production.

MATERIAL AND METHODS

Collection of Latex

Studies of the flow of latex during tapping have shown that there is elastic expansion of the latex tubes during the early minutes of tapping^{5,6} accompanied by a substantial drop in turgor pressure which causes considerable damage to organelles such as lutoids and Frey-Wyssling particles⁷⁻¹¹.

Consequently, the latex which flows during the first 5 min is set aside and the fraction flowing between the fifth and thirty-fifth minutes of tapping is collected in a container packed in ice. In addition, collection in ice (at a temperature of less than 5°C) leads to total blocking of the metabolism which normally continued *in vitro*, at ambient temperature.

Collection of a 30-min fraction of tapping flow has the advantage of ensuring adequate compensation of the variations of composition that occur during flow^{12,13}.

The latex thus collected is carefully homogenised. It is used for analysis of pH, redox potential (RP) and the bursting index of lutoids (BI). It is also used for the preparation of a trichloroacetic extract (TCA) (9 parts of 2.5% TCA to 1 part of latex). The TCA serum thus obtained enables the measurement of inorganic phosphate, Mg, thiols and sucrose¹⁴.

It is well known there are considerable seasonal variations in the composition of latex^{15,16}. For this reason, samples of latex were taken when latex parameters were subjected to the least seasonal variations, *i.e.* from September to December in the climatic conditions of the Ivory Coast¹⁶.

The various analytical methods used have already been described¹⁴.

Statistical Interpretation of the Results

Analysis was carried out either on the latex from thirty trees of the same clone or on three mixtures of latex, each from ten trees, that were also representative of a clone grown in a given plot.

The result obtained were subjected to various statistical analyses such as single and multiple correlation, Fisher and Student tests, principal component analysis and hierarchical ascending classification¹⁷.

RESULTS AND DISCUSSION

Physiological justification of the various biochemical and physico-chemical parameters of latex are given below.

pH

The pH of the total latex was generally determined, although in certain specific cases the cytosolic pH and the intra-lutoid pH were also measured.

The largest part of the metabolism, and in particular the anabolic process of regeneration of rubber, takes place in the cytosol or in contact with it¹⁸. It was soon observed that the relative alkalisation of the medium (pH 7.0 - 7.5), activates glycolysis, essentially by means of the invertase which appeared as the key enzymes in the functioning of glycolysis^{19,20,21}. This enzyme is extremely sensitive to physiological variations of pH (6.6 - 7.4). The same applies to phosphoenol-pyruvate carboxylase (PEP-Case)²² which can divert a flow of substrates from its main purpose of isoprenic synthesis. Among other enzymes which are sensitive to physiological variations of pH are glyceraldehyde-phosphate dehydrogenase²³ (GAP decarboxylase), a generator of NAD(P)H source of reducing capacity, and pyruvate decarboxylase^{19,24}, which controls the production of acetate, the first step in isoprenic anabolism (*Figure 1*).

It was possible in certain cases to correlate the cytosolic pH (in fact, when it was a limiting factor), important in metabolism regulation, directly and highly significant with production (*Figure 2*)^{13,25-27}. The logic of such correlation is obvious: when it occurs it probably indicates the limiting nature of the regeneration of cell material — and hence of rubber — between two tappings, notably by the slowing of glucidic catabolism for low pH.

The regulation mechanism of the pH of cytosol, which is of great importance²⁸, involves two major phenomena. The first is a biochemical pH-stat as described by Davies²⁹. It originates in the successive functioning of PEP-Case, of malate dehydrogenase and malic enzyme³⁰. The second mechanism involves a bio-osmotic pH-stat^{31,32}. The main constituent element of this mechanism is the tonoplast ATPase discovered by d'Auzac³³ and which functions as a proton pump^{34,35,36} from the cytosol to the lutoids. This pH-stat also implies a proton pump operating in the opposite direction and energised by an NADH oxido-reduction system³⁷. The various elements of the regulation of the pH of the cytosol are shown in *Figure 3*. By means of these pumps, the protons brought by the acids produced by the catabolism of carbohydrate can be ejected into the vacuole and into the apoplast thus contributing to the homeostasis of the cytosol³¹. The setting up of this proton gradient energises active transport to the vacuole compartment (citrate, basic amino acids, calcium, etc.)³⁸ and very probably the influx of sucrose from the apoplast to the laticiferous cytosol³⁹ (*Figure 3*). It should be noted that citrate, Ca²⁺ and Mg²⁺ concentrated in lutoid serum, are inhibitors of certain cytosolic enzymes which are also often regulated by the pH (*Figure 4*).

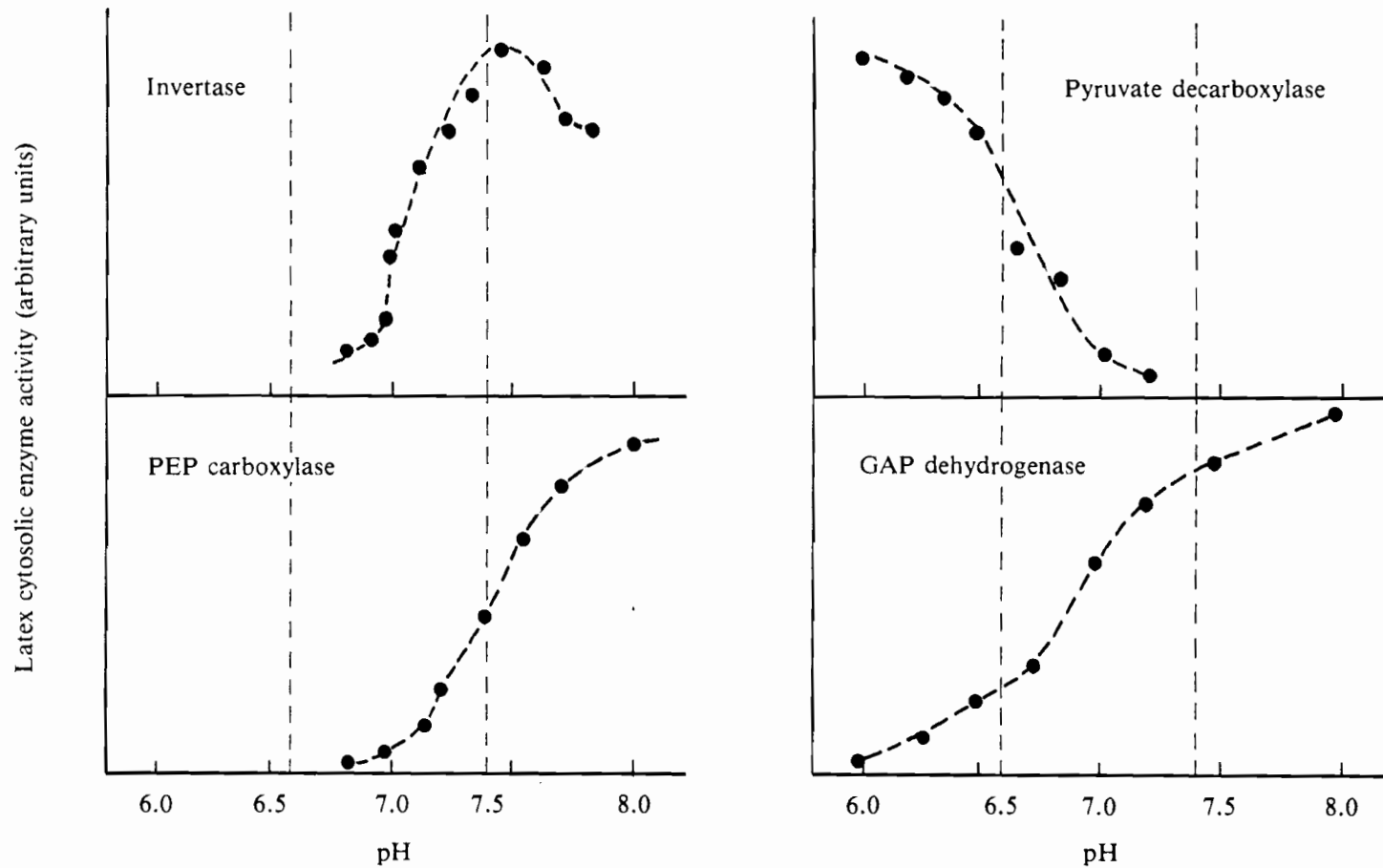


Figure 1. pH influence on the activity of important enzymatic steps in latex glycolytic pathway. The vertical dotted lines indicate the pH physiological range of latex.

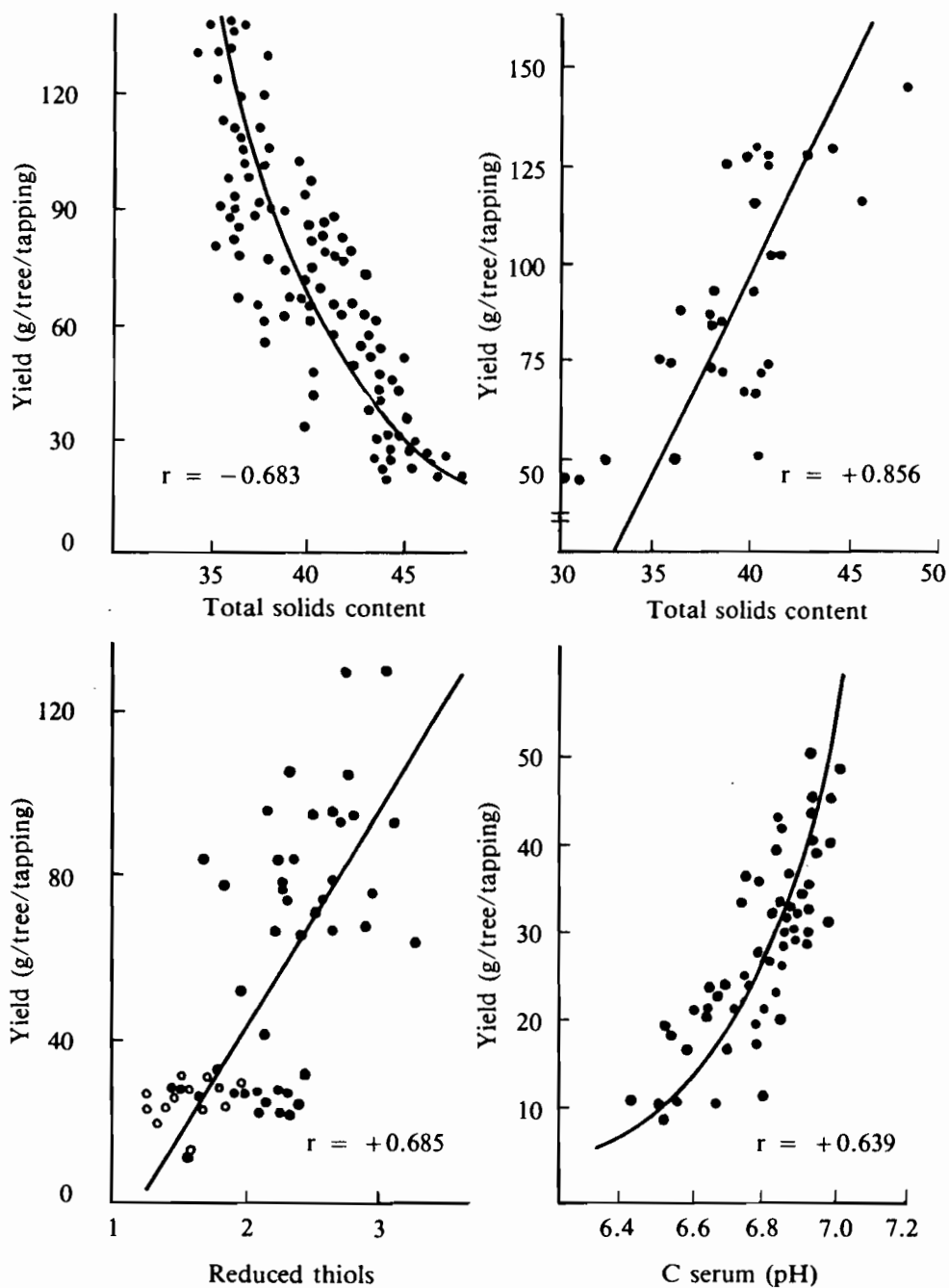


Figure 2. Relationships between total solids content, reduced thiols content, pH of latex and yield. All the correlations are very highly significant, $P < 0.001$.

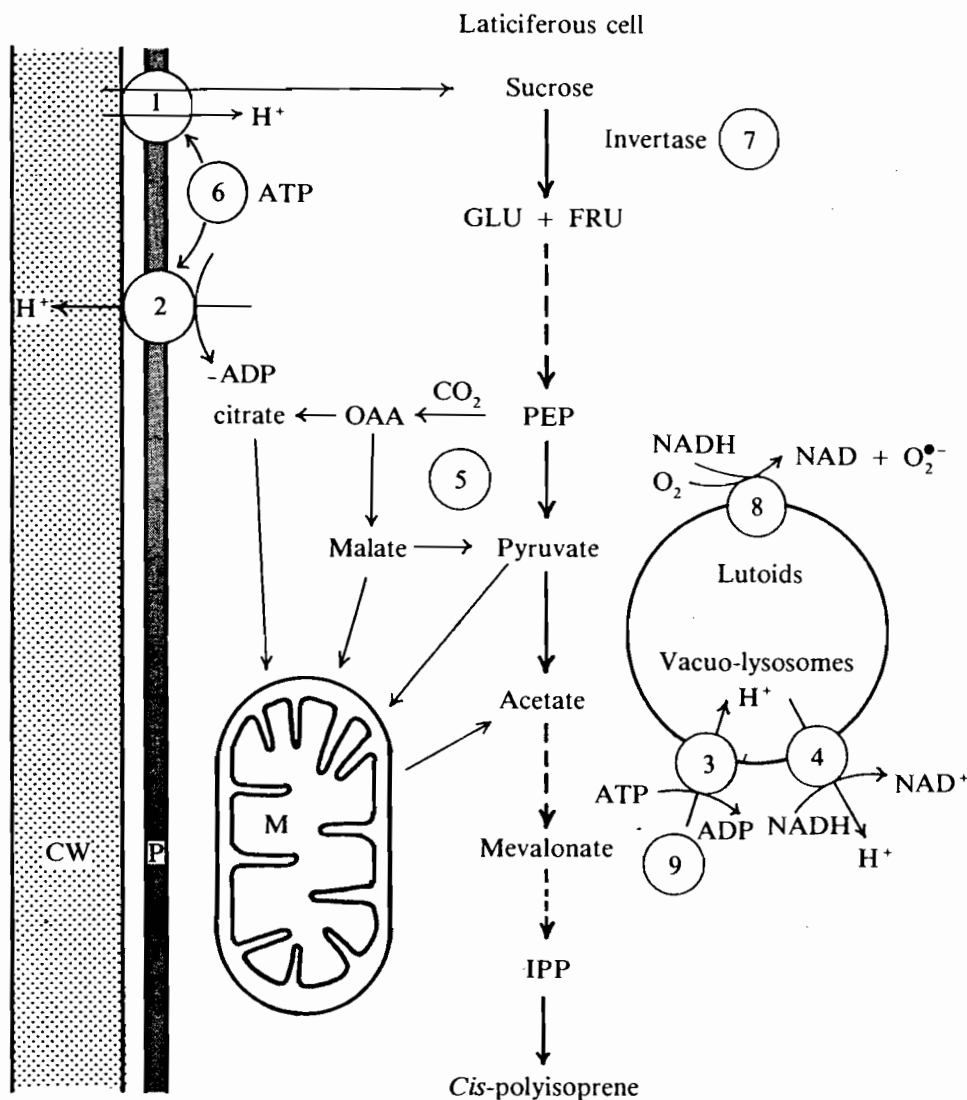


Figure 3. Absorption of sucrose into laticiferous cells, isoprenic anabolism and regulation of the cytosolic pH.

CW: cell wall; P: plasmalemma; M: mitochondria; GLU: glucose; FRU: fructose; IPP: isopentenyl-pyrophosphate; PEP: phosphoenol-pyruvate.

1: Symport proton-sucrose; 2, 3 and 4: Proton-pumps acting as a biophysical pH-stat; 5: Biochemical pH-stat; 6: Active influx of sucrose into latex cell which may be activated by ethylene; 7: Invertase step pH-regulated and limiting factor of sucrose catabolism; 8: Production of toxic oxygen leading to a degradation of lutoids and a malfunctioning of cytosol metabolism; 9: Specific activity of tonoplasmic ATPase increases 12 h after ethephon treatment causing a cytosol alkalisation, an increase of the glycolytic activity and (?) an acceleration of H^+ -sucrose influx.

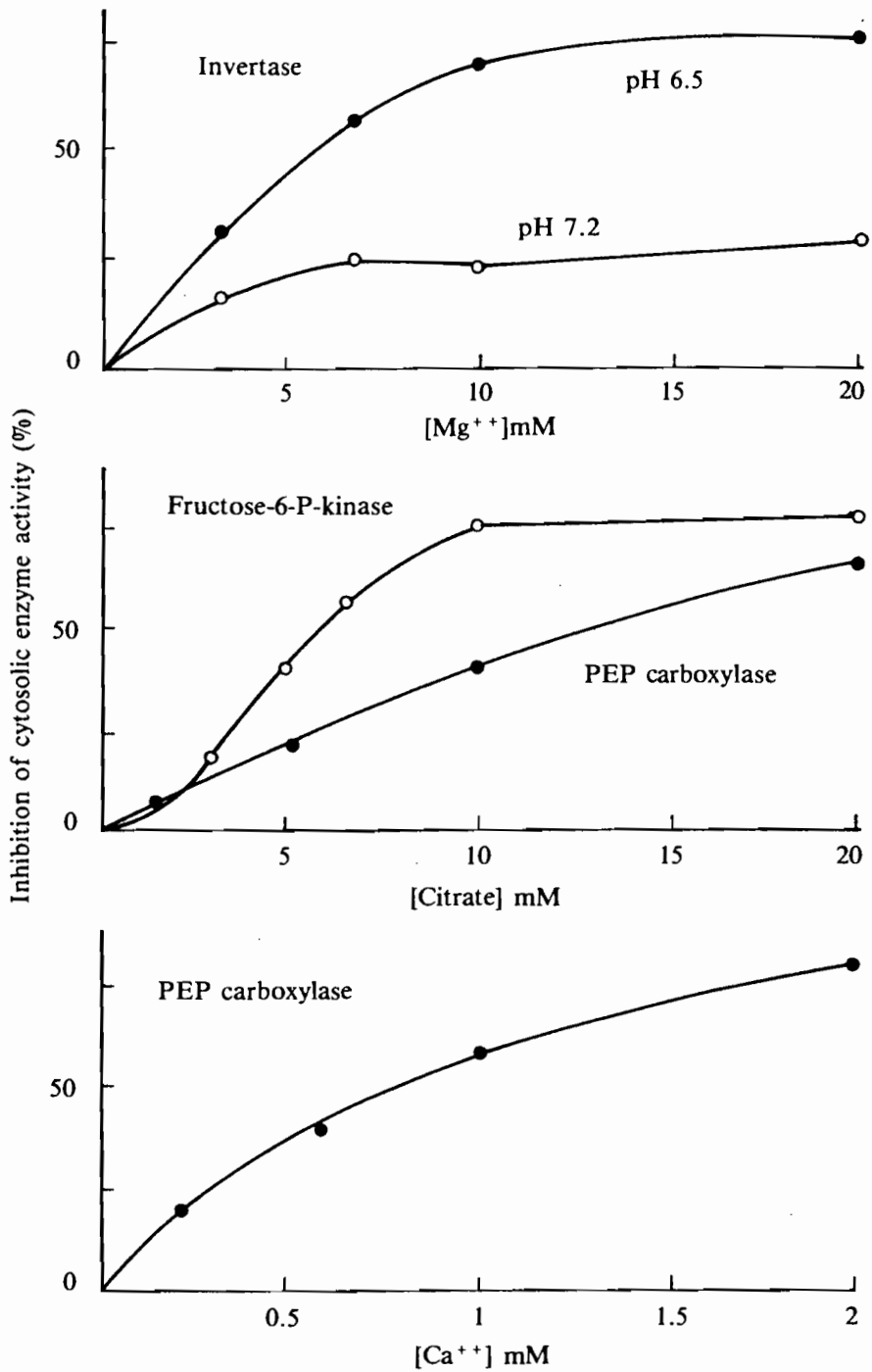


Figure 4. Influence of Mg^{2+} , Ca^{2+} and citrate (molecules accumulated essentially in the luteoids) on glycolytic enzymes in *Hevea latex*.

It has been known for a long time that the stimulation of latex production by substances such as 2,4-D, ANA and CuSO_4 and ethephon (which was introduced by Abraham *et al.*⁴⁰, and d'Auzac and Ribaillier⁴¹), is accompanied by an alkalisation of the latex and particularly of the cytosol which is roughly proportional to the increase in production^{20,24,26,42}. Such alkalisation of the cytosol constitutes logically one of the factors that may explain the over-production linked with stimulation. Another cause of over-production is to be sought in activation of proteosynthesis detected globally 12 h after the application of ethephon²⁶ and then observed more specifically⁴³ at the level of the synthesis of tonoplast (lutoid) ATPase. If ATP is not a limiting factor, such synthesis accounts for the activation of an influx of intra-lutoid protons and hence the alkalisation of the laticiferous cytosol. Finally, very recent results lead also to suggesting activation of a probably active influx of sucrose at the level of the laticiferous plasmalemma³⁹.

It should also be noted that the pH of latex is at least, to a certain extent, a clonal characteristic¹⁴. In addition, the appearance of at least some types of bark dryness is accompanied by a significant fall in the pH of the latex⁴⁴. There can be two reasons for this. The first probably results from inadequate functioning of the ATPases causing the outflux of protons from the cytosol; the second is certainly the degradation of lutoids leading to the release of H^+ concentrated in the vacuole, into the cytosol^{7,45,46}. A mechanism explaining the degradation of the lutoid membrane and the release of intra-lutoidic protons has been proposed by Chrestin¹⁷.

Experiments showing a significant negative correlation between the pH of latex and the BI have been carried out⁴⁷. Lutoids burst more extensively causing a rise in the BI, and the cytosol thus becomes relatively more acidified.

It can thus be generalised that when the pH is low, the carbohydrate catabolism will be weak, isoprenic synthesis will be reduced and hence the rubber content and production will be small (and *vice versa*).

Sucrose

In all plants, sucrose originating in photosynthesis is the basic molecule involved in all anabolic synthesis, whether concerning starch, cellulose, membrane or reserve lipids or numerous secondary metabolites of the plant kingdom. *Hevea* is no exception to this rule, and the secondary metabolite *cis*-polyisoprene comes directly¹⁸ or indirectly⁴⁸ from sucrose.

Numerous authors^{12,13,20,21,24,25,49-51} have shown the primordial role played by sucrose in the production of latex in *Hevea*. Under conditions in which sugar may be a limiting factor, direct correlations appear between the sugar content of latex and the rubber production of the tree^{14,49}. A high sugar content in latex may signify good supply to the laticiferous cells associated with good utilisation in the metabolism of these cells²⁴. However, a high sugar content in latex may also signify a high or low rate of supply which is nevertheless associated with low metabolic utilisation of the sugar and leading finally to low productivity. The latter possibility is revealed

in particular by a negative sugar-pH relationship: high sugar levels correspond to a low pH⁴⁷ in the latex indicating slow carbohydrate catabolism.

The phenomenon may also account for the fact that the sugar content of latex from trees affected by bark dryness is sometimes higher than that of latex from healthy control trees^{44,52}. The cytological^{53,54} and biochemical⁵⁵ disorganisation caused by bark dryness may also imply metabolic malfunctioning of the laticiferous system.

It is also accepted that hormone stimulation accentuates, in a transitory manner, the sink effect of sucrose in latex^{13,14,56}. On the other hand, the effect of stimulation of the yield is linked with the extent of the sink effect^{14,21,25}, in other words, the availability of sucrose in the areas of bark next to the tapping panel is of prime importance. There are two explanations to account for the increase of the sink effect. The first might involve considerable activation of carbohydrate catabolism; part of the sugars might be used either by the chain of mitochondrial cytochromes or by alternate cyano-resistance respiration which gives poor yield as regards production of ATP^{57,58}. The second explanation may involve direct activation of the active influx of sucrose at the level of the laticiferous plasmalemma^{39,59} (*Figure 3*).

From a practical point of view, it will be noted that hormone stimulation is effective *a priori* in increasing production when the sugar content of the latex is naturally high and the pH of the latex low (activation of carbohydrate catabolism). On the other hand, stimulation may have practically no positive effect on production if:

- too intensive exploitation has led to a fall in the sugar content of the tapping panel, or
- if the bark renewal panel located above the tapping cut is too large (this is the panel blockage phenomenon well known in GT 1)¹⁴, or
- if the starting of peroxidative phenomena which are typical of a certain category of bark dryness occurs⁶⁰, or finally
- if phenomena such as the invasion of the laticiferous tubes by thylloids leads to the *in situ* coagulation of latex⁶¹ or if the appearance of gum in the laticiferous tissue⁶² results in bark dryness which may or may not be different from the preceding type.

Total Solids Content of Latex

This parameter is very easy to measure and is of definite value. However, as is the case for sugar, its value may result from antinomic phenomena.

Low rubber content values may result from inadequate regeneration of *cis*-polyisoprene between two tappings. This appears to be the case of PB 86 whose enzymatic system for the biogenesis of rubber appears to be limited¹⁴. It is normal in such cases to obtain positive correlations between this parameter and production⁴⁷; this type of correlation (*Figure 2*) was also found by Milford *et al*⁶³. It is also accepted that at least in certain types of over-exploitation, there are significant drops in the TSC accompanied by the appearance of bark dryness^{27,64}.

On the other hand, high TSC values may indicate a high rate of regeneration (thus *a priori* satisfactory), but at the same time these values may limit production and in particular the flow of the latex owing to the high viscosity caused^{27,63} (*Figure 2*). It should be specified in the latter case that the phenomenon will *a priori* be all the more marked if the availability of water is a limiting factor⁶⁵. That is to say that inadequate hydric transfer from parenchymatic tissue to the laticiferous tissue will prevent latex from being diluted by water. Such demand for water is a classic phenomenon in laticiferous tissue during tapping¹⁰, but this demand may be slowed down in the case of dry bark which has not been induced by over-exploitation and where cell permeability may be reduced^{61,62}. The result may be an increase in TSC. This phenomenon has been observed by Sivakumaran and Pakianathan⁶⁶ and by Prevot *et al.* (unpublished results).

Stimulation with ethephon probably plays a role in hydric transfer. This may explain the drop in TSC that it causes^{27,42} and also, in part, the considerable ease of flow observed after treatment. It is also known that the availability of water can greatly limit response to stimulation⁶⁷.

Thiols

The thiols in latex consist of cysteine, methionine and above all of glutathion⁶⁸. They play an important and complex role.

Several authors^{14,47,69} have thus succeeded in showing the existence of highly significant positive correlations between the thiol content and production (*Figure 2*). This means, classically, that low thiol contents can limit production in certain cases.

Two major roles can be attributed to latex thiols which, together with ascorbic acid^{17,70}, make up quantitatively the main reducing molecules in latex. Their presence is indispensable in all the cells since these molecules can neutralise various forms of toxic oxygen which are the classic by-products of any cellular metabolism^{71,72}.

It is now commonly accepted that the oxido-reduction chains operating in the chloroplasts, and also in the mitochondria (cytochrome chain and cyano-resistant respiration), may produce as by-products, varying amounts of hydrogen peroxide (H_2O_2) and superoxide ions (O_2^-)⁷³. Such molecules are usually neutralised by enzyme systems such as catalase and superoxide dismutase or by reducing agents (antioxidants) such as thiols and ascorbic acid. This production of toxic oxygen remains small but real when the metabolism is normal, but can reach considerable proportions when cells are subjected to stress, whatever the reason might be^{72,73}. This is the case with bark dryness resulting from traumatism caused by over-exploitation of *Hevea*⁶⁰. The thiol groups (together with the other antioxygens) are then used up and the reducing capacity of the latex is sometimes not sufficient for their regeneration. Such regeneration involves the functioning of enzymes such as glutathion reductase which is present in latex⁷⁴.

It must be remembered that the harmfulness of toxic forms of oxygen acts at the level of the degradation of genes, and, even more important, can cause cellular decompartmentalisation following the peroxidative degradation of membrane phospholipids⁷⁵.

Thiols can also operate in a different way in cell metabolism. It is thus accepted today that at least six enzymes of the Calvin cycle in photosynthesis, are considerably more active when the R-S-S-R groups which make up their basic structure are reduced to R-SH by reducing capacity which depends on the photoreduction of ferredoxin⁷⁶. If ferredoxin is not present in the latex, it seems that reducing capacity from glucidic catabolism^{19,27} is capable of maintaining a reduced degree of glutathion and the latter, in its turn, may activate certain enzymes either by reducing the R-S-S-R groups of certain enzymes or by means of another mechanism. This is the case of invertase⁷⁸ and pyruvate-kinase⁷⁹ and, it would appear, of HMGC_oA reductase⁸⁰ which in an activated form allows more active functioning of cell metabolism.

Bursting Index of Lutoids

The bursting index (BI) of lutoids indicates the wholeness of lutoids (vacuolysosomes) in latex^{45,81,82}. The test was proposed by Ribaillier⁸³. It is determined by measuring the ratio of free acid phosphatase activity to the total phosphatase activity of latex. Given the high coagulant capacity of lutoids shown by Southorn and Edwin⁸⁴, a high BI signifies the partial destabilisation of the latex which may induce micro-coagulations. The latter occurs at the open extremities of the laticiferous tubes. It results in a limit to the duration of flow which can also be characterised using the plugging index defined by Milford *et al*⁸³.

The destabilising effect of lutoids as regards the colloidal suspension that is formed by latex⁸⁵ is explained by the release of lutoid H⁺ ions into cytosol with a low buffer capacity. Positively charged bivalent cations such Ca²⁺ and Mg²⁺ are also involved in this phenomenon. The release of lutoidic peroxidases⁸⁶ which react with their cytosolic phenolic substrates, and the release of polyphenoloxidases from the Frey-Wyssling particles⁹, are also involved in the coagulation of latex^{86,87}.

It is clear that in addition to these destabilising effects connected with the bursting of lutoids, there is also a slowing of the metabolism following the acidification of the cytosol and possibly the inhibition of certain key cytosolic enzymes by certain substances accumulated in the lutoids [inhibition of invertase by Mg²⁺⁸⁸ or Cu²⁺⁸⁹, inhibition of phosphofructokinase and PEP carboxylase by citrate¹⁹ (*Figure 4*)].

It is therefore normal in certain cases, to encounter highly significant inverse correlations between BI and production^{14,47} (*Figure 5*) like the inverse correlations shown between the plugging index (PI) and production^{15,63}. The inverse correlation between BI and production can be accompanied in particular by an inverse correlation between BI and pH⁴⁷ indicating that greater bursting of lutoids may effectively induce cytosol acidification.

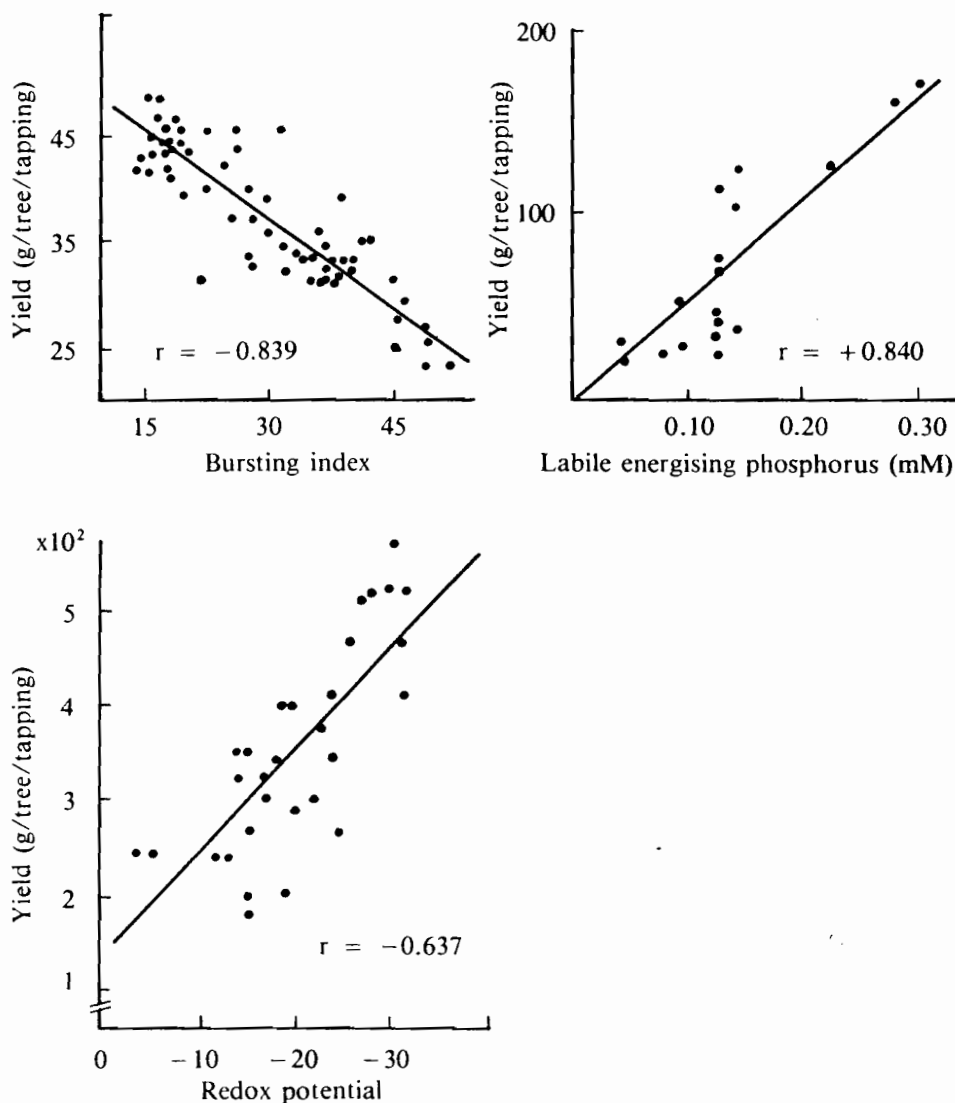


Figure 5. Relationships between bursting index, labile energising phosphorus, redox potential of latex and yield. All the correlations are very highly significant, $P < 0.001$.

In parallel, Chrestin⁶⁹ has shown a very close direct correlation between the activity of reactions producing toxic oxygen and BI. Finally, in certain cases of bark dryness, a direct relationship can be observed between the appearance of the disease (percentage of length of dry bark) and BI⁴⁴.

However, bark dryness in *Hevea* is a disease which may be a result of various causes^{91,61}. The mechanism of the appearance of bark dryness linked with peroxida-

tive phenomena as proposed by Chrestin¹⁷, is one of the types of bark dryness referred to as physiological fatigue. Bark dryness caused by pathological factors probably exists; the vectors remain to be discovered⁶¹. In addition, the over-exploitation which causes considerable physiological modifications of the laticiferous system does not appear necessarily to cause substantial modifications to PI or BI^{64,92}. Nevertheless, Yeang and Paranjothy⁶⁴ observed considerable reduction in the volume of the bottom fraction and the polyphenoloxidase activities which can result from different biological mechanisms. The last case brings to mind the appearance of physiological fatigue countering the regeneration of subcellular organites and the enzymatic activities connected with the biosynthesis of rubber and the limiting of intercompartmental transfers.

However, a high BI measured when latex is collected indicates cell decompartmentalisation which is unfavourable to the flow of latex and to the regeneration of the contents between two successive tappings.

Inorganic Phosphate

Free inorganic phosphate (Pi) in the cytosol may reflect the energy metabolism. In 1964, d'Auzac⁹³ demonstrated the highly significant positive relationship in PR 107 latex between the availability of labile energising phosphate (LEP) and the biosynthetic activity measured *in vitro* as regards acetate-2¹⁴C on the one hand, and the same LEP and production of the trees on the other (*Figure 5*).

Significant correlations have been shown more recently between ATP latex content, ATP/ADP latex ratio and yield⁹⁴.

Eschbach *et al.*¹⁴ and Subronto⁹⁵ also showed direct correlations between the total Pi content of latex and the production of certain clones. The significance of such correlations should perhaps be sought in an inadequate supply of Pi in the latex of the clones in question.

It has been known since 1959⁹⁶ that stimulation — which plays an obvious role in the activating of the laticiferous metabolism—often results in an increase in the phosphate content of latex, whatever the stimulation agent; this result has since been confirmed^{14,97}. It is probable that a laticiferous system operating weakly has less Pi in its latex (since the molecule is closely linked with the energy metabolism) than a laticiferous system subjected to more intensive exploitation and whose metabolism is therefore more active.

Magnesium

When magnesium in latex is discussed, the total magnesium usually assayed by complexometry is meant. It would certainly be more judicious for Mg²⁺, as for Pi, which are known to accumulate considerably in lutoids⁸¹, to assay the Mg²⁺ and Pi in the cytosol. Nevertheless, Mg²⁺ plays several roles in latex. These are:

- the Mg^{2+} positively charged bivalent cation leads, when the cation is present at a high enough concentration, neutralising the negative charges of the colloidal suspension which forms latex. The bursting of the laticifers, laticifers which form a 'reservoir' of Mg^{2+} , contributes to this neutralisation and hence to the coagulation of the latex^{8,85}. This is encountered in latex with a very high Mg^{2+} content e.g. in clones Gl 1, AVROS 308, *etc.*⁹⁸
- Mg^{2+} is an activator which is indispensable to the functioning of numerous enzymes in latex: APTases in general³³, various kinases such as phosphofructokinase¹⁹, pyruvate kinase⁷⁹, PEP carboxylase³⁰, *etc.*
- Mg^{2+} also inhibits other enzymes such as invertase⁸⁸. This explains why it must be partially eliminated from the cytosol by being trapped in the laticifers⁸¹ where it contributes by itself to the trapping of citrate, another cytosolic inhibitor⁹⁹.

This diversity in the role of Mg^{2+} is shown again here by an equilibrium phenomenon, and it can be expected to find a relationship with production in the form of a 'bell-shaped curve'.

In fact, Subronto *et al.*⁹⁵ have shown the existence of a significant inverse correlation between the Mg^{2+} content and production. However, on the contrary, Eschbach *et al.*¹⁴ were able to show a positive correlation between Mg^{2+} and production; this means that in these last experiments the biological role of Mg^{2+} , as an activator of cytosol enzymes, is greater than its role as a destabiliser.

The Redox Potential of Latex

The latex tubes are the sites of an intense metabolism connected with the partial regeneration of their content between two tappings. Expressed as a simplification, distinction can be made between oxidation (catabolism) of sucrose, producing energy, reduction capacity and acetate, on one hand and the anabolism process based on the reduction of intermediate metabolites by means of cofactors reduced by catabolism on the other.

The importance of oxidation and peroxidation phenomena as regards the stability of the membranes of subcellular organites is also known¹⁷.

Measurement of the redox potential (RP) of latex and/or cytosol should lead logically to integrated measurement of the redox phenomena in the laticiferous cells. As the redox potential of cytosol is always reducing (redox potential less than 0) and that of laticifer serum is always oxidant (redox potential greater than 0)⁴⁷, measurement of the RP of latex could also give an idea of the compartmentalisation in the latex. Prevot *et al.*⁴⁷ were able to show inverse correlations between the RP and production (*Figure 5*): the greater the reducing value of the RP (less than 0), the greater the production. In such cases, a low RP indicates the satisfactory functioning of the laticiferous cells (good compartmentalisation), active laticifers and available reduction capacity. An increase in the RP is the sign of a certain compartmental

and metabolic degradation, and in particular a lack of available reduction capacity. It should also be noted that latex from trees affected by partial bark dryness possess a significantly higher RP (less reductive) than that of latex from healthy control trees¹⁰⁰.

It therefore seems logical that a low RP (reducing) indicates a medium in which the regenerative processes of anabolism are potentially active. An RP which becomes less reductive should be considered to be the sign of a metabolic modification or of compartmental degradation which is harmful as regards productivity.

CONCLUSION

With the aim of establishing a diagnosis of the physiological state of the laticiferous system, all the criteria described were selected for ease of measurement and in relation to the importance of their role in the physiology of productivity — whether this be in regards to the flow of latex or its regeneration. This being so, the various parameters probably do not all have the same 'weight' as regards productivity.

Other parameters are still being sought. The PI of Milford *et al.*⁶³ is of undoubted interest although it is slow to use. Various scientists have proposed modified versions which are easier to handle^{101,102,103}.

Analysis of the numerical values of the parameters is complex since they may:

- not be connected or on the contrary correlated positively or negatively with production, depending on the circumstances, or
- vary, particularly in absolute terms, with the vegetative cycle of *Hevea* and with climatic conditions, or
- be clonal characteristics of different extents.

Taking into account a single parameter is inadequate. Thus, although a high sucrose value is satisfactory *a priori*, other criteria must be taken into account at the same time. For example, in this case, a low pH may indicate poor use of this sucrose and a high BI may indicate certain limitations in regard to carbohydrate catabolism.

Nevertheless, when values of the parameters and of the individual production of some thirty trees of the same clone and from the same experimental plot are known, the establishment of correlations that may appear between some of the parameters will make interpretation easier.

It is possible that during practical experimental work some of the parameters described here may be discarded or replaced by others that turn out to be better correlated with production or more descriptive of an important aspect of exploitation physiology, particularly with regard to availability of water.

Nevertheless, in spite of obvious difficulties, the experimental work in progress seems promising; over- and under-exploitation definition tests have already been

attempted (Tables 1 and 2). The result of the diagnosis should make it possible to optimise exploitation intensity by modifying the length of tapping cut and frequency of tapping, the position of the cut with regard to possible panel blockage and the intensity of stimulation (concentration of ethephon, frequency of application, etc.). One can thus hope to attain the *potential optimum production* of a given clone in specific ecoclimatic conditions. In addition, the physical and biochemical parameters described here are already being used for the early selection of new clones created by IRCA¹⁰⁴.

TABLE 1. PHYSIOLOGICAL PARAMETERS OF LATEX ACCORDING TO CONDITIONS OF *HEVEA* EXPLOITATION⁹⁷

Parameter	Under-exploitation		Over-exploitation	
pH	(>7.05)	high	(<6.80)	low
TSC	(>35)	high	(<30)	low
Suc	(>16)	high	(<5)	low
Pi	(<8)	low	(>20)	high
Mg/Pi	(>1.5)	high	(<0.5)	low
RP		(reducing) low		(oxidising) high
RSH	(<0.40)	low	(>0.90 or <0.40)	low or high
Dry bark		low		high

The values within brackets are provided for GT 1 in Cameroon (concentrations expressed in mM).

TABLE 2. CORRELATIONS BETWEEN LATEX PARAMETERS AND YIELD IN THE CASE OF OVER-EXPLOITATION⁹⁷

Correlations between yield and latex parameters	Correlations between latex parameters
<u>Yield — TSC</u> > 0 after stimulation	<u>TSC — Suc</u> > 0 after stimulation
<u>Yield — Suc</u> > 0 after stimulation	<u>TSC — RSH</u> > 0 after stimulation
Yield — BI < 0 after stimulation	TSC — BI < 0 after stimulation
Yield — Pi > 0 after stimulation	Suc — Mg > 0 after stimulation
Yield — RSH > 0 before stimulation	

Very frequent and high correlations are underlined.

Finally, there is no doubt that the defining of the parameters described here has considerably aided understanding of the physiology of the laticiferous system and hence to precise some important limiting factors in the production of *Hevea* latex.

REFERENCES

1. DICKENSON, P.B. (1965) The Ultrastructure of the Latex Vessel of *Hevea brasiliensis*. *Proc. nat. Rubb. Prod. Res. Ass. Jub. Conf. Cambridge 1984* (Mullins, L. ed), p. 52. London: Maclaren and Sons Ltd.
2. HEBANT, C. AND DE FAY, E. (1980) Functional Organization of the Bark of *Hevea brasiliensis* (Rubber Tree). *Z. Pflanzenphysiol.*, **97**, 391.
3. HEBANT, C. (1981) Laticiferous Ontogeny of the Primary System of *Hevea brasiliensis*: An Ultrastructural and Cytochemical Study. *Cab. Bot.*, **59(6)**, 974.
4. GOMEZ, J.B. AND MOIR, G.F.J. (1979) *The Ultracytology of Latex Vessels in Hevea brasiliensis*. *Monograph No. 4*, pp. 48. Kuala Lumpur: Malaysian Rubber Research and Development Board.
5. BUTTERY, B.R. AND BOATMAN, S.G. (1967) Effect of Tapping, Wounding and Growth Regulators on Turgor Pressure in *Hevea brasiliensis* Mull. Arg. *J. exp. Bot.*, **18** 644.
6. LIORET, C., RIBAILLIER, D. AND COMBE, J.C. (1978) Cinétique de l'écoulement du latex d'*Hevea brasiliensis* après la saignée. *Rev. gen. Caout. Plast.*, **55(578)**, 91.
7. SOUTHORN, W.A. (1969) Physiology of *Hevea* (Latex Flow). *J. Rubb. Res. Inst. Malaya*, **20(4)**, 187.
8. RIBAILLIER, D. (1972) Quelques aspects du rôle des lutoides dans la physiologie et l'écoulement du latex d'*Hevea brasiliensis* (Kunth) Mull. Arg., Action de produits libérant de l'éthylène. Thesis (Th. Etat), Abidjan Université (C.N.R.S.-A.O.7716).
9. COUPE, M., PUJARNISCLE, S. AND D'AUZAC, J. (1972) Compartimentation de diverses oxydo-reductases (peroxydases, o-diphénol-oxydase et malate deshydrogénase), dans le latex d'*Hevea brasiliensis* (Kunth) Mull. *Agr. Physiol. Veg.*, **10(3)**, 459.
10. PAKIANATHAN, S.W., BOATMAN, S.O. AND TAYSUM, D.H. (1966) Particule Aggregation following Dilution of *Hevea* Latex: A Possible Mechanism for the Closure of Latex Vessels after Tapping. *J. Rubb. Res. Inst. Malaya*, **19(5)**, 259.
11. PAKIANATHAN, S.W. AND MILFORD, G.F.S. (1973) Changes in the Bottom Fraction Contents of the Latex during Flow in *Hevea brasiliensis*. *J. Rubb. Res. Inst. Malaya*, **23(5)**, 391.
12. D'AUZAC, J. (1965) Sur quelques relations entre la composition, l'activité biochimique du latex et la productivité de l'*Hevea brasiliensis*. Thesis (Th. Etat 2nd. part), Paris Université (C.N.R.S. A.O.5447).
13. TUPY, J. (1973) The Level and Distribution Pattern of Latex Sucrose along the Trunk of *Hevea brasiliensis* as Affected by the Sink Region Induced by Latex Tapping. *Physiol. Veg.*, **11(1)**, 1.
14. ESCHBACH, J.M., ROUSSEL, D., VAN DE SYPE, H. AND JACOB, J.L. (1984) Relationships between Yield and Clonal Physiological Characteristics of Latex from *Hevea brasiliensis*. *Physiol. Veg.*, **22(3)**, 295.
15. YEANG, H.Y. AND PARANJOTHY, K. (1982) Some Primary Determinants of Seasonal Yield Variation in Clone RRIM 623. *J. Rubb. Res. Inst. Malaysia*, **30(1)**, 131.
16. VAN DE SYPE, H. (1985) Variations saisonnières du latex chez le clone GT 1 (*Hevea brasiliensis*). *Rev. gen. Caout. Plast.* (in press).
17. CHRESTIN, H. (1984) Le comportement vacuolysosomal (les lutoides) du latex d'*Hevea brasiliensis*. Son rôle dans le maintien de l'homéostasie et dans les processus de sénescence des cellules laticifères. Thesis (Th. Etat), Montpellier Université.
18. LYNEN, F. (1969) Biochemical Problems of Rubber Synthesis. *J. Rubb. Res. Inst. Malaya*, **21(4)**, 389.
19. JACOB, J.L. (1970) Particularités de la glycolyse et de sa régulation au sein du latex d'*Hevea brasiliensis*. Thesis (Th. Etat) Orsay Université (C.N.R.S. A.O.4129).

20. TUPY, J. (1969) Stimulatory Effects of 2,4-Dichlorophenoxyacetic Acid and 1-Naphtylacetic Acid on Sucrose Level, Invertase Activity and Sucrose Utilization in the Latex of *Hevea brasiliensis*. *Planta*, **88**, 144.
21. TUPY, J. (1973) The Regulation of Invertase Activity in the Latex of *Hevea brasiliensis*. The Effect of Growth Regulators, Bark Wounding and Latex Tapping. *J. exp. Bot.*, **24(80)**, 516.
22. JACOB, J.L., PREVOT, J.C. AND PRIMOT, L. (1979) Purification et etude de la phosphoenolpyruvate carboxylase du latex d'*Hevea brasiliensis*. *Physiol. Veg.*, **17(3)**, 501.
23. JACOB, J.L. AND D'AUZAC, J. (1972) La glyceraldehyde-3-phosphate deshydrogenase du latex d'*Hevea brasiliensis*. Comparaison avec son homologue phosphorylante. *Eur. J. Biochem.*, **31**, 255.
24. TUPY, J. AND PRIMOT, L. (1976) Control of Carbohydrate Metabolism by Ethylene in Latex Vessels of *Hevea brasiliensis* Mull. Arg. in Relations to Rubber Production. *Biol. Pl.*, **18(5)**, 373.
25. TUPY, J. (1973) The Activity of Latex Invertase and Latex Production in *Hevea brasiliensis* Mull. Arg. *Physiol. Veg.*, **11(4)**, 633.
26. COUPE, M. (1977) Etudes physiologiques sur le renouvellement du latex d'*Hevea brasiliensis*. Action de l'Ethylene. Importance des polyribosomes. *Thesis (Th. Etat)*, pp. 171-179. Montpellier Universite.
27. BROZOWSKA-HANOWER, J., CRETIN, H., HANOWER, P. AND MICHEL, P. (1979) Variations de pH entre compartiments vacuolaire et cytoplasmique au sein du latex d'*Hevea brasiliensis*. Influence saisonniere et action du traitement par l'ethrel generateur d'ethylene. Repercussion sur la production et l'apparition d'encoches seches. *Physiol. Veg.*, **17(4)**, 851.
28. SMITH, F.A. AND RAVEN, J.A. (1979) Intracellular pH and its Regulation. *Ann. Rev. Pl. Physiol.*, **30**, 289.
29. DAVIES, D.D. (1973) Control of and by pH. *Soc. Exp. Biol.*, **27**, 513.
30. JACOB, J.L., PREVOT, J.C. AND D'AUZAC, J. (1983) Physiological Regulation of Phosphoenolpyruvate Carboxylase from a Non-chlorophyllian System: the *Hevea* Latex. *Physiol. Veg.*, **21(5)**, 1013.
31. CHRESTIN, H., GIDROL, X., MARIN, B., JACOB, J.L. AND D'AUZAC, J. (1984) Role of Luteoidic Tonoplast in the Control of the Cytosolic Homeostasis within the Laticiferous Cells of *Hevea*. *Z. Pflanzenphysiol. B.*, **114.S**, 269.
32. CHRESTIN, H., GIDROL, X., D'AUZAC, J., JACOB, J.L. AND MARIN, B. (1985) Cooperation of a 'Davies type' Biochemical pH-stat and the Tonoplastic Bioosmotic pH-stat in the Regulation of the Cytosolic pH of *Hevea* Latex. *Biochemistry and Function of Vacuolar Adenosine-triphosphatase in Fungi and Plants (Marin, P. ed)*, p. 245. Berlin, Heidelberg, New York. Tokyo: Springer verlag.
33. D'AUZAC, J. (1975) Caracterisation d'une ATPase membranaire en presence d'une phosphatase acide dans les lutoides d'*Hevea brasiliensis*. *Phytochem.*, **14(3)**, 671.
34. CRETIN, H. (1982) 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.*, **65**, 175.
35. MARIN, B. AND BLASCO, F. (1982) Further Evidence for the Proton Pumping Work of Tonoplast ATPase from *Hevea*. *Biochem. biophys. Res. Commun.*, **XX(105)**, 354.
36. MARIN, B. AND GIDROL, X. (1985) Chloride Ion Stimulation of the Tonoplast H⁺ Translocating ATPase from *Hevea brasiliensis* (Rubber Tree) Latex. *Biochem. J.*, **266(1)**, 85.
37. CRETIN, H. (1983) Efflux transtonoplastique de protons lors du fonctionnement d'un systeme transporteur d'electrons (la NADH-cytochrome c reductase) membranaire des vacuo-lysosomes du latex d'*Hevea brasiliensis*. *C.R. Acad. Sci.*, Paris (serie III), **296**, 137.
38. MARIN, B., CRETIN, H. AND D'AUZAC, J. (1982) Energization of solute transport and accumulation at the tonoplast in *Hevea* latex. *Physiol. Veg.*, **20(2)**, 333.

39. LACROTTE, R., VAN DE SYPE, H. AND CHRESTIN, H. (1985) Influence de l'éthylène sur l'utilisation du saccharose exogène par les laticifères d'*Hevea brasiliensis*: proposition d'un mécanisme d'action. *Physiol. Veg.*, **23**(2).
40. 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**(5), 291.
41. D'AUZAC, J. AND RIBAILLIER, D. (1969) L'Éthylène, un nouveau stimulant de la production de latex chez l'*Hevea brasiliensis*. *C.R. Acad. Sci., Paris (serie D)*, **268**, 3046.
42. PRIMOT, L., JACOB, J.L., D'AUZAC, J. AND PREVOT, J.C. (1979) Evolution chronologique après stimulation de quelques caractéristiques du latex d'*Hevea brasiliensis*. *Rev. gen. Caout. Plast.*, **56**(587-588), 63.
43. GIDROL, X. AND CHRESTIN, H. (1984) Lutoidic ATPase Functioning in Relation to Latex pH Regulation and Stimulation Mechanisms. *C.R. Coll. Expl. Physiol. Amelior. Hevea Montpellier 1984*, 81.
44. ESCHBACH, J.M., VAN DE SYPE, H., ROUSSEL, D. AND JACOB, J.L. (1983) The Study of Several Physiological Parameters of Latex and their Relationships with Production Mechanisms. *Int. Rubb. Res. Dev. Bd Symp. Peking 1983*.
45. PUJARNISCLE, S. (1968) Caractère lysosomal des lutoides du latex d'*Hevea brasiliensis*. *Physiol. Veg.*, **6**(1), 27.
46. GOMEZ, J.B. (1983) *Physiology of Latex (Rubber) Production. Monograph No. 8*. Kuala Lumpur: Malaysian Rubber Research and Development Board.
47. PREVOT, J.C., JACOB, J.L. AND VIDAL, A. (1984) The Redox Potential of Latex Criterion of the Physiological State of Laticiferous System. *C.R. Coll. Expl. Physiol. Amelior. Hevea Montpellier 1984*, 227.
48. BEALING, F.J. (1975) Quantitative Aspects of Latex Metabolism: Possible Involvement of Precursors other than Sucrose in the Biosynthesis of Hevea Rubber. *Proc. Int. Rubb. Conf. Kuala Lumpur 1975*, **2**, 543.
49. D'AUZAC, J. AND PUJARNISCLE, S. (1961) Aperçu sur l'étude des glucides de l'*Hevea* et sur leur variation. *Rev. gen. Caout.*, **38**(7-8), 1131.
50. LOW, F.C. (1978) Distribution and Concentration of Major Soluble Carbohydrates in *Hevea* Latex: The Effects of Ethephon Stimulation and the Possible Role of These Carbohydrates in the Latex Flow. *J. Rubb. Res. Inst. Malaysia*, **26**(1), 21.
51. LOW F.C. (1981) The Role of Carbohydrates in the Exploitation and Latex Flow of *Hevea*. Doctor of Science Thesis, Ghent University, Belgium.
52. PREVOT, J.C. AND JACOB, J.L. (1985) Unpublished data.
53. DE FAY, E. AND HEBANT, C. (1980) Étude histologique des écorces d'*Hevea brasiliensis* atteints de la maladie des encoches sèches. *C.R. Acad. Sci., Paris, serie D*, **291**, 865.
54. PARANJOTHY, K., GOMEZ, J.B. AND YEANG, H.Y. (1975) Physiological Aspect of Brown Bast Development. *Proc. Int. Rubb. Conf. Kuala Lumpur 1975*, **2**, 181.
55. CHRESTIN, H., BANGRATZ, J., D'AUZAC, J. AND JACOB, J.L. (1984) Role of the Lutoidic Tonoplast in the Senescence and Degeneration of the Laticiferous of *Hevea brasiliensis*. *Z. Pflanzenphysiol. Bd.*, **114**S., 261.
56. LOW, F.C. AND GOMEZ, J.B. (1982) Carbohydrate Status of Exploited *Hevea*. I. The Effect of Different Exploitation Systems on the Concentration of the Major Soluble Carbohydrates in Latex. *J. Rubb. Res. Inst. Malaysia*, **30**(1), 1.
57. FAN SIWEI, YANG SHAOQIONG, YANG WANNIAN AND GONG JIAMIN (1984) Preliminary Study of Cyanide Resistant Respiration in *Hevea brasiliensis*. *C.R. Coll. Expl. Physiol. Amel. Hevea Montpellier 1984*, 185.

58. D'AUZAC, J. (1985) Private communication. Universite des Science et Techniques du Languedoc, Montpellier, France.
59. TUPY, J. (1985) Some Aspects of Sucrose Transport and Utilization in Latex-producing Bark of *Hevea brasiliensis*. *Biol. Plant.* (in press).
60. CHRESTIN, H. (1985) La stimulation a l'Ethrel de l'*Hevea*. Jusqu'ou aller trop loin? *Rev. gen. Caout. Plast.*, **62(647-648)**, 75.
61. DE FAY, E. (1981) Histophysiologie compare des corces saines et pathologiques (maladie de l'encoche seche) de l'*Hevea brasiliensis*. *Thesis (Thse, Doc. 3e cycle)* Montpellier Universite, France.
62. BEALING, F.J. AND CHUA, S.E. (1972) Output Composition and Metabolic Activity of *Hevea* Latex in Relation to Tapping Intensity on the Onset of Brown Bast. *J. Rubb. Res. Inst. Malaya*, **23(3)**, 204.
63. MILFORD, G.F.J., PAARDEKOOPER, E.C. AND HO, C.Y. (1969) Latex Vessel Plugging-its Importance to Yield and Clonal Behaviour. *J. Rubb. Res. Inst. Malaya*, **21(3)**, 274.
64. YEANG, H.Y. AND PARANJOTHY, K. (1982) Initial Physiological Changes in *Hevea* Latex and Latex Flow Characteristics Associated with Intensive Tapping. *J. Rubb. Res. Inst. Malaysia*, **30(1)**, 31.
65. BUTTERY, B.R. AND BOATMAN, S.G. (1976) Water Deficits and Flow of Latex. *Water Deficit and Plant Growth*, (Koslowky, T.d.T. ed), Vol. IV, p. 233. New York, San Francisco, London: Academic Press.
66. SIVAKUMARAN, S. AND PAKIANATHAN, S.W. (1984) Studies on Tree Dryness. I. A Simple and Rapid Method of Inducing Dryness in *Hevea* Trees. *J. Rubb. Res. Inst. Malaysia*, **31(2)**, 88.
67. NINANE, F. (1970) Les aspects cophysiologiques de la productivite chez *Hevea brasiliensis* au Cambodge. *Thesis (Th. Doc. Agro.)* Louvain Universite, Belgium.
68. McMULLEN, A.I. (1960) Thiols of Low Molecular Weight in *Hevea brasiliensis* Latex. *Biochem. Biophys. Acta*, **41**, 341.
69. CHRESTIN, H. AND BANGRATZ, J. (1983) Une activite enzymatique endogene NAD (P)H dependante, responsable de la degradation peroxydative des organites membranaires et de la coagulation proce, ou *in situ* du latex d'*Hevea brasiliensis*. *C.R. Acad. Sci.*, Paris (serie III), **296**, 101.
70. ARCHER, B.L., AUDLEY, B.G., McSWEENEY, G.P. AND TAN CHEE HONG (1969) Studies on Composition of Latex Serum and Bottom Fraction Particles. *J. Rubb. Res. Inst. Malaya*, **21(4)**, 560.
71. FRIDOVICH, I. (1978) The Biology of Oxygen Radicals. *Science*, **201(8)**, 875.
72. TARBELL, D.S. (1961) The Mechanism of Oxidation of Thiols to Dissulfides. *Organic Sulphur Compounds (Klorash, N. ed)*, p. 97. New York: Pergamon Press.
73. ELSTNER, E.F. (1982) Oxygen Activation and Oxygen Toxicity. *Ann. Rev. Plant. Physiol.*, **33**, 73.
74. PREVOT, J.C., CHRESTIN, H. AND JACOB, J.L. (1984) Mise en vidence d'une glutathion reductase dans le serum cytoplasmique du latex d'*Hevea brasiliensis*. *C.R. Acad. Sci.*, Paris (serie III), **298**, 35.
75. SLATER, T.F. (1984) Free-radical Mechanism in Tissue Injury. *Biochem. J.*, **222(1)**, 1.
76. BUCHANAN, B.B. (1980) Role of Light in the Regulation of Chloroplast Enzymes. *Ann. Rev. Pl. Physiol.* **31**, 341.
77. ARREGUIN, B. AND ROCK, M.C. (1967) Ciclo de la pentosas en el latex d'*Hevea brasiliensis*. *J. Bot. Inst. Quim. Mexico*, **19**, 58.
78. JACOB, J.L., PREVOT, J.C. AND D'AUZAC, J. (1982) Physiological Activators of Invertase from *Hevea brasiliensis* Latex. *Phytochem.*, **21(4)**, 851.
79. JACOB, J.L., PREVOT, J.C. AND PRIMOT, L. (1981) La pyruvate kinase du latex d'*Hevea*. *Rev. gen. Caout. Plast.*, **58(612)**, 89.

80. SIPAT, A.B. (1982) 3-Hydroxyl-3-methylglutaryl CoA Reductase (NADPH) in the Latex of *Hevea brasiliensis*. *Phytochem.*, **21**(11), 2613.
81. RIBAILLIER, D., JACOB, J.L. AND D'AUZAC, J. (1971) Sur certains caracteres vacuolaires des lutoides du latex d'*Hevea brasiliensis*. *Physiol. Veg.*, **9**(3), 1091.
82. D'AUZAC, J., CHRESTIN, H., MARIN, B. AND LIORET, C. (1982) A Typical Vegetal Vacuolar System: The Lutoids from *Hevea brasiliensis* Latex. *Physiol. Veg.*, **20**(2), 311.
83. RIBAILLIER, D. (1968) Action *in vitro* de certains ions mineraux et composes organiques sur la stabilite des lutoides du latex d'*Hevea*. *Rev. gen. Caout. Plast.*, **45**(489), 1395.
84. SOUTHORN, W.A. AND EDWIN, E.E. (1968) Latex Flow Studies. II. Influence of Lutoids on the Stability and Flow of *Hevea* Latex. *J. Rubb. Res. Inst. Malaya*, **20**(2), 187.
85. SOUTHORN, W.A. AND YIP, E. (1968) Latex Flow Study III. Electrostatic Considerations in the Colloidal Stability of Fresh *Hevea* Latex. *J. Rubb. Res. Inst. Malaysia*, **20**(2), 201.
86. HANOWER, P. BRZOZOWSKA, J. AND LIORET, C. (1976) Etude du mecanisme de la coagulation du latex d'*Hevea brasiliensis*. I. Facteurs agissant sur la coagulation. *Physiol. Veg.*, **14**(4), 677.
87. BRZOZOWSKA-HANOWER, J., HANOWER, P. AND LIORET, C. (1978) Etude du mecanisme de coagulation du latex d'*Hevea brasiliensis*. II. Phenoloxydases. *Physiol. Veg.*, **16**(2), 231.
88. PRIMOT, L. (1975) L'invertase. *Colloque sur la physiologie du latex d'Hevea brasiliensis*. Travaux et documents de l'ORSTOM No. 68, p. 79. Paris: ORSTOM.
89. CONDURU, N., JACOB, J.L., PREVOT, J.C. AND VIDAL, A. (1984) Some Properties of a Key Enzyme in the Metabolism of Latex: Invertase. *C.R. Coll. Expl. Physiol. Amelior. Hevea Montpellier 1984*, 121.
90. COMPAGNON, P., TIXIER, P. AND ROUJANSKY, G. (1953) Contribution a l'etude des accidents physiologiques de saigne. *Archs. Rubb. Cult.*, 54.
91. GOMEZ, J.B. AND YEANG, H.Y. (1976) Physiological Aspects of Brown Bast Development. *Proc. Int. Rubb. Conf. Kuala Lumpur 1975*, 2, 181.
92. SETHURAJ, M.R., USHA NAIR, N., GEORGES, M.J. AND MANI, K.T. (1977) Physiology of Latex Flow in *Hevea brasiliensis* as Influenced by Intensive Tapping. *J. Rubb. Res. Inst. Sri Lanka*, **54**, 221.
93. D'AUZAC, J. (1964) Disponibilit en phosphore energetique, biosynthese du caoutchouc et productivite de l'*Hevea brasiliensis*. *C.R. Acad. Sci. Paris*, **258**, 5091.
94. CHRESTIN, H. AND BANGRATZ (1984) Private communication. Office de la Recherche scientifique et technique Outre-Mer, Abidjan, Cote d'Ivoire.
95. SUBRANTO (1978) Correlation Studies of Latex Flow Characters and Latex Minerals Content. *Int. Rubb. Res. Dev. Bd Symp. Kuala Lumpur 1978*.
96. D'AUZAC, J. AND PUJARNISCLE, S. (1959) Sur les differentes formes de phosphores presentes dans le latex d'*Hevea*. *Rev. gen. Caout.*, **36**, 862.
97. ROUSSEL, D. (1984) Private Communication. Institut Recherches Agronomy of Cameroun, Ekona.
98. D'AUZAC, J. (1960) Sur la signification du rapport Mg/P des latex frais vis-a-vis de leur stabilite. *Oper. Techn. Inst. Rech. Caout. Vietnam*, **40/60**, 1.
99. MARIN, B. AND CHRESTIN, H. (1984) Citrate Compartmentation in the Latex from *Hevea brasiliensis*. Relationship with Rubber Production. *C.R. Coll. Expl. Physiol. Amelior. Hevea Montpellier 1984*, 169.
100. PREVOT, J.C. (1984) Private communication. Institut Recherches Caoutchouc, CIRAD, Montpellier.
101. SOUTHORN, W.A. AND GOMEZ, J.B. (1970) Latex Flow Studies. VII. Influence of Length of Tapping Cut on Latex Flow Pattern. *J. Rubb. Inst. Malaysia*, **23**(1), 15.

102. SETHURAJ, M.R., SUBRANTO, SULOCHANAMMA, S. AND SUBBARAYALU, G. (1978) Two Indices to Quantify Latex Flow Characteristics in *Hevea brasiliensis*. *Indian J. agric. Sci.*, **48(9)**, 521.
103. YEANG, H.Y. AND MOHD NAPI DAUD (1984) Quantification of Latex Vessel Plugging by 'Intensity of Plugging'. *J. Rubb. Res. Inst. Malaysia*, **32(2)**, 164.
104. DINTINGER, J., NICOLAS, D. AND NOUY, B. (1981) Nouveaux criteres de selection precoce de l'*Hevea*. Definition, premiers resultats. *Rev. gen. Caout. Plast.*, **58(609)**, 85.

DISCUSSION

J.B. GOMEZ (Rubber Research Institute of Malaysia, Kuala Lumpur, Malaysia)

One graph in *Figure 2* shows negative correlation between yield and total solids content while the other shows a positive correlation. Is there a mistake?

H. CHRESTIN

There is no mistake; in the two cases, there are two different metabolisms. In the first one, the limiting factor is the high viscosity of latex associated with high TSC. So, if the viscosity of latex is high, the production is low. Thus, while the regeneration of latex is good, the limiting factor is the viscosity. In the second case, the regeneration of latex is a limiting factor for the production.

ASRIL DARUSSAMIN (Balai Penelitian Perkebunan Sungei Putih, Indonesia)

What is the interaction between lutoids and mitochondria, focussing your answer on ATPase activity?

H. CHRESTIN

There is no clear evidence of involvement of large numbers of mitochondria in the function of latex vessels. Further, we have shown that the enzymatic systems we studied were not mitochondrial in origin because they were not inhibited by the classical inhibitors of the mitochondrial and plasmalemma ATPase systems. Instead, they were powerfully inhibited by typical tonoplasmic ATPase inhibitors. So we conclude that there is no contamination by mitochondrial effects in our experimental conditions.

LEONG TAT THIM (Guthrie Research Chemara, Seremban, Negri Sembilan, Malaysia)

Have you studied the levels of sucrose, N, P, K and Mg contents from the latex of the partial dry trees and normal dry trees?

H. CHRESTIN

Yes, we have done a lot of measurements on sucrose content of latex in relation to dryness. It appears that initially the sucrose content of latex decreases at the beginning of dryness but later the sucrose content increases once the metabolic machinery is impaired. Mg^{++} and inorganic phosphate were shown to decrease in the latex in dry trees. N and K were not analysed in the experiment.

Jacob J.L., Eschbach J.M., Prévot J.L., Roussel D., Lacrotte R., Chrestin Hervé, Auzac J. d'. (1986).

Physiological basis for latex diagnosis of the functioning of the laticiferous system in rubber trees.

In : Rajarao J.C. (ed.), Amin L.L. (ed.)
International rubber conference 1985 :
proceedings. Kuala Lumpur : Rubber Research
Institute of Malaysia, 43-65.

International Rubber Conference, Kuala
Lumpur (MYS), 1985/10/20-25.