CHAPTER 14

CERTAIN ASPECTS OF PHYSIOLOGY AND BIOCHEMISTRY OF LATEX PRODUCTION

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The production of rubber is largely dependent upon (a) volume and type of laticiferous tissues in which latex is stored, (b) capacity of storage vessels (c) physiological and biochemical processes controlling latex flow and (d) capacity of the tree to resynthesise latex and other organic constituents within the drained area (for a detailed treatment of biosynthesis of rubber, please see Chapter 6, Ed.).

The organic non-rubber constituents such as proteins, carbohydrates, lipids, phospholipids, nucleic acids play a significant role in the re-synthesis of latex to replace that which had been drained off through tapping.

NATURE AND COMPOSITION OF LATEX

Organic non-rubber constituents of latex

The Hevea latex, as it flows out of the tree, is a complex cytoplasm containing a suspension of rubber and non-rubber particles in an aqueous medium (Frey-Wyssling, 1929; Southorn, 1961; Archer et al. 1969). Using high speed centrifugation (59,000 g), Cook and Sekhar (1953) separated latex into four fractions. These were: an upper white fraction of rubber cream, an orange or yellow layer containing Frey-Wyssling complexes, a colourless serum named C-serum and a greyish yellow gelatinous sediment the 'bottom fraction' consisting mainly of lutoids (Dickenson, 1969; Southorn, 1966).

Moir (1959) using differential staining and high speed centrifugation techniques, showed that the sedimentable material in latex did not consist wholly of one species of particle. By treating the latex with trace amounts

of Janus Green B or neutral red before centrifugation he obtained eleven zones. Zone 1 corresponded to the 'top whitish fraction' of Cook and Sekhar (1953) which consists mainly of hydrocarbon particles. Zone 2 was a much smaller, translucent layer situated under the lowest end of Zone 1. Zone 3 was a suspension of rubber particles in the serum. Zone 4 was the yellow and orange layer of Cook and Sekhar (1953). The aqueous Zone 5 corresponded to C-serum and Zones 6 - 11 together were broadly equivalent to the 'bottom fraction'.

Pujarniscle (1968) later extended Moir's results and showed by isopycnic centrifugation of fresh latex on sucrose gradients that latex could be distinguished by thirteen fractions. However, the lutoids, as obtained by the above technique were found to be either damaged or seriously aggregated (Low, 1976). Ficoll was later proved to be a more superior gradient medium than sucrose. Diluted fresh latex was successfully separated on linear Ficoll gradients with minimal damage to the lutoids (Low and Wiemken, 1982; 1984).

Though 'bottom fraction' contains particles other than lutoids, it was observed that the main protein constituents of the 'bottom fraction' originated from the lutoids (Southorn and Edwin, 1968). By repeated freezing and thawing or by ultrasonication of the bottom fraction, the lutoids can be disrupted. A further centrifugation of this disrupted bottom fraction, yields another serum, the B-serum. This represents the original fluid within the lutoids with perhaps minor contributions from the entrapped C-serum and from particles other than the lutoids (Southorn and Edwin, 1968).

The organic non-rubber constituents of fresh latex are presented in Table 1. Bearing in mind the great variability of latex, these organic non-rubber constituents may vary both in composition and concentration, depending on various physiological and physical parameters.

Proteins

Of the several non-rubber constituents of latex, the one which has received most attention is proteins. The early literature on the proteins of Hevea has been reviewed recently (Tata, 1975; 1980b). The earliest report of the presence of proteins in Hevea latex was by Spencer (1908) who detected peroxidase and catalase activities in dialysed aqueous extracts of rubber sheets, and subsequently, in dialysed latex. Even though enzymes play a major role in the biological function in Hevea latex, discussions on the enzymology of Hevea latex are kept to a minimum in this chapter.

The total protein content in latex has been estimated to be about 1% (Archer and McMullen, 1961; Archer et al. 1963b; Tata, 1980a). However,

TABLE 1

Organic non-rubber constituents of latex.

FRESH LATEX

Rubber Phase

Proteins (0.26)
Phospholipids (0.43-0.50)
Glycolipids
Pigments, Sterol esters, Fatty acid esters, Waxes (0.15)
Triglycerides (0.38)
Sterols, mainly β-sitosterol (0.11)
Free fatty acids, Tocotrienols (trace) (0.07)
Phenolic compounds (0.06)
Diglycerides, Monoglycerides, Alcohols (0.05)
Furanoid fatty acid
(esterified as triglyceride)

FW Complexes

Carotenoids
Plastochromanol
Phospholipid
Glycolipids (mainly sterol glucosides, free and esterified)

Serum

Proteins (0.46)
Total cyclitols (0.3-0.8)
Sucrose (0.1-0.3)
Glucose (0.01)
Glutathione (0.01)
Free amino acids (0.08)
Ascorbic acid (0.02)
Other organic acids
Nitrogenous bases (0.04)
Ribonucleic acids, Deoxyribonucleic acids (0.02)
Mononucleotides (0.02)

Bottom Fraction

Proteins (0.28)
Total cyclitols (0.2-0.3)
Sucrose (0.06-0.08)
Glucose (0.03)
Phospholipids (0.04-0.05)
Glycolipids, Pigments, Sterol esters, *Fatty acid esters*, Waxes (0.02)
Triglycerides (0.02)
Sterols (0.04)
Free fatty acids, Tocotrienols (trace) (0.05)
Phenolic compounds (0.01)
Diglycerides, Monoglycerides, Alcohols (0.01)
Trigoneline (0.007)
Ergothioneine (0-0.05)
Plantoquinone
Ubiquinone

Figures within brackets indicate their approximate concentration in g/100 g latex.

discrepancies in the distribution of the proteins between the major phases of latex exist. Archer and McMullen (1961) reported that 20% of the total proteins was absorbed on the rubber surface, 66% in the C-serum and 14% in the bottom fraction. Later, reports variously described the distribution as 20%, 60% and 20% (Archer et al. 1963) and 27.2%, 47.5% and 25.3% (Tata, 1980) for the rubber phase, C-serum and bottom fraction respectively.

Proteins on the surface of the rubber particles: The existence of proteins in association with phospholipids on the surface of rubber particles was recognised as early as 1953 by Bowler (1953). He attributed that this protein-phospholipid layer imparted a net negative charge to the rubber particle, thereby contributing to the colloidal stability of these particles (Bowler, 1953). By measuring the iso-electric points of various latex samples, he concluded that there was more than one protein adsorbed on the rubber surface and that the relative proportions of the adsorbed proteins varied with clones.

Apart from the estimation that the protein adsorbed on rubber surface accounted for about 1% of the weight of rubber (Cockbain and Philpott, 1963) the proteins on the rubber surface remained unknown until recently. The major protein on the rubber surface has been shown to be negatively charged and has a molecular weight of approx. 65,000 (RRIM, 1982). It migrates towards the anode at a higher rate than the major C-serum protein α-globulin and contrary to the earlier suggestion, is therefore not identical with the latter.

Of the numerous enzymes reported in Hevea latex, only two have been found to be associated with the rubber surface. These isopentenyl pyrophosphate polymerase (Lynen, 1967; Archer et al. 1963a) and rubber transferase (Lynen, 1967; Archer et al. 1963; Archer and Cockbain, 1969; McMullen and McSweeney, 1966; Archer et al. 1966). Their presence on the rubber surface is not surprising, since they are involved in rubber biosynthesis. What is perhaps more surprising is that so few have been detected on the rubber surface.

Proteins in the serum: Nearly half the enzymes examined in Hevea latex appeared to be located in the C-serum of latex. These include enzymes for the glycolytic pathway (Bealing, 1969; d'Auzac and Jacob, 1969) as well as many of the enzymes for rubber biosynthesis (Archer and Audley, 1967). Recently, twenty-seven enzymes were separated by electrophoresis by Jacob and co-workers, of which, seventeen were shown to exist in multiple forms (Jacob et al. 1978).

Until the development of the ultracentrifugal separation of fresh latex (Cook and Sekhar, 1953; Moir, 1959) the proteins investigated in the serum

of Hevea were mainly those which remained in the serum after acid coagulation or other treatments of latex (Bishop, 1927; Kemp and Twiss, 1936; Bondy and Freundlich, 1938; Kemp and Straitiff, 1940; Roe and Ewart, 1942). However, with the development of the ultracentrifugation techniques, seven protein components were demonstrated in the C-serum by Archer and Sekhar (1955) using paper electrophoresis. The same workers also confirmed the presence of seven protein components in the B-serum, ie. serum which is obtained by prolonged freezing and thawing of latex.

The first protein to be isolated from Hevea latex was from C-serum. It was named α-globulin by Archer and Cockbain (1955). This protein is the major protein component of C-serum. It is readily adsorbed at a water-air or oil-water interface with a resulting fall in the interfacial tension. This led to the suggestion that α-globulin was one of the proteins on the surface of rubber particles and that it contributed to the colloidal stability of fresh latex (Archer and Cockbain, 1955). However, as mentioned earlier, α-globulin was later found not to be present on the surface of the rubber particles (RRIM, 1982).

With the introduction of more sensitive techniques, further discoveries on the proteins of C-serum were made. Using starch gel electrophoresis, Tata and Moir (1964) reported the presence of twenty-two protein bands in C-serum. Seventeen of these were anionic at pH 8.2, whilst five were cationic and existed in much lower concentrations. A comparative study on the proteins in the C sera from four clones viz. RRIM 501, GT 1, Tjir 1 and Pil A44, revealed very little differences between their general electrophoretic patterns (RRIM, 1963). There was also no significant difference in the proteins with seasonal variation within a single clone. Later, the list of proteins in C-serum was enlarged to twenty-four (Tata and Edwin, 1970), using the same starch gel electrophoretic technique. Using polyacrylamide gel electrophoresis, Yeang et al. (1977) reported 26 protein bands from C-serum at alkaline pH and 15 bands at acid pH. These workers also did not observe significant differences in the protein patterns of C sera between clones (Tjir 1, PR 107, GT 1, PB 86 and BR 2), in agreement with the earlier conclusions from starch gel electrophoresis (RRIM, 1963).

Proteins in the bottom fraction: Proteins in the bottom fraction are essentially studied as the soluble proteins in B-serum. These have been examined with various techniques, including paper electrophoresis (Moir and Tata, 1960), starch gel electrophoresis (Tata, 1975; Tata and Edwin, 1969) and polyacrylamide gel electrophoresis (Yeang et al. 1977). Irrespective of the technique used, the proteins of B-serum were found

to be markedly different from those of C-serum. Upon electrophoresis, the B-serum proteins were usually separated into two major protein bands at the extreme anionic and cationic ends, with several minor bands in between.

Hevein: The major protein in B-serum is hevein, which accounts for about 70% of the water soluble proteins in the bottom fraction (Archer et al. 1969). Hevein is a low molecular weight anionic protein (Approx. 5,000 daltons) with a higher (5%) sulphur content (Tata, 1975; Archer, 1960; Tata, 1976). All the sulphur in hevein exists as eight disulphide (S-S) bridges of cystine (Archer, 1960; Tata, 1976). Because of its low molecular weight and the large number of S-S bridges, hevein is heat stable, and is not precipitated by the common reagents for precipitating proteins eg. trichloroacetic acid (Tata, 1975; Tata, 1976). The molecular weight of hevein was first estimated to be about 10,000 ± 500 daltons by Archer (1960). Subsequent analysis showed that earlier preparations (Archer, 1960; Karunakaran et al. 1961) of hevein were mixtures containing hevein, traces of esterase and a protein with slightly less anionic mobility than hevein, termed pseudo-hevein (Tata, 1975; Tata, 1976). When pure hevein (free of pesudo-hevein) was isolated and characterised, it was found to be a single peptide chain with glutamic acid as the N-terminus and a molecular weight of approximately 5,000 daltons (Tata, 1975; Tata, 1976). (The molecular weight of pseudo-hevein was also 5,000 daltons). Later, an almost complete amino acid sequence of hevein was reported (Walujono et al. 1976). It contained 43 amino acid residues in a single polypeptide chain and an estimated molecular weight of 4729 daltons. A comparison of the three-dimensional structure of hevein with that of wheat germ agglutinin and ragweed pollen allergen (Drenth et al. 1980) showed that there were similarities in the position of the disulphide bridges (Walujono et al. 1976). The biological functions of hevein and pseudo-hevein are unknown. Although hevein resembles other proteinase inhibitors in general structural characteristics, it did not exhibit inhibitory activity against trypsin, chymotrypsin or carboxypeptidase (Walujono et al. 1976).

The microfibrillar protein: Dickenson (Dickenson, 1965; 1969; 1963) in his ultrastructural studies and electron microscopic investigations of lutoids, first described some fibrillar components having a tightly coiled helical structure, which he named microfibrils. These structures were observed within lutoids of young latex vessels but were absent from mature vessels. These microfibrils were later shown to be proteins containing upto 4% carbohydrate, and having an isoelectric pH of about 4 (Audley, 1965; 1966). At ambient temperature (20°C), the microfibrils break up into smaller segments which reassemble on freezing (Audley, 1965; 1966).

The microhelices: These structures were first observed by Dickenson (1963) in lutoids from mature trees. However, Dickenson described them as stretched microfibrils. Later, Southorn and Yip (1968) and Gomez and Yip (1974; 1975; 1976) carried out detailed investigations and reported that these zig-zag structures differed from microfibrils in that they were larger in dimensions and were open helcies (not lightly coiled helices of the microfibrils). They were called 'Microhelices' by Gomez and Yip (1975). Lowering of the ionic concentration of B-serum by dialysis against water or by dilution with water resulted in the formation of microhelices (Tata, 1975; Gomez and Yip, 1974, 1975, 1976). Furthermore, their formation required the combination of two glycoproteins in a certain ratio. These are an acidic 'assembly factor' (molecular weight 160,000) and a slightly basic 'pro-helical protein' (molecular weight 22,000) (Tata, 1975). A third glycoprotein termed the 'bundling factor' (molecular weight 5,000) appeared to promote the combination of single microhelices into bundles (Tata, 1980). The 'pro-helical protein' has some flocculating activity on suspensions of rubber particles in vitro. Microhelices are rarely seen in lutoids from young trees.

The basic proteins: The presence of basic proteins in B-serum was first demonstrated when B-serum or an aqueous extract of freeze-dried bottom fraction was electrophoresed (Tata and Edwin, 1970; Moir and Tata, 1960; Karunakaran et al. 1961). Two basic proteins - a major and a minor basic protein - which account for about 4% of the total proteins in latex were found to have lysozyme and chitinase activities (Tata, 1980; Tata et al. 1983). The Hevea lysozymes were found to have similar pH optima and molecular weight as lysozymes from papaya and fig, but their activities were different from avian lysozymes (Tata et al. 1983). The major basic protein has been crystallised and its molecular weight (approx. 26,000) determined. Its first 21 amino acid residues were elucidated, and found to differ significantly from those of hen egg, duck egg, baboon milk and T4 phage lysozymes (Tata et al. 1983). The major basic protein, also referred to earlier as 'band (i) first peak protein' (Tata, 1980) was found to be identical with heveamine A, a cationic protein described by Archer (Archer, 1976), another basic protein in B-serum.

Carbohydrates: The major soluble carbohydrates in Hevea latex are the total cyclitols, sucrose and glucose in that order (Low, 1978). It had been reported earlier that latex contained mainly sucrose and a smaller amount of raffinose (Tupy and Resing, 1969). However, Bealing (1969) later found that glucose, fructose and sucrose were the only free sugars present in latex in significant quantity and that other unidentified substrates

(probably pentoses) sometimes detected in paper chromatograms had no quantitative significance. The particularly low fructose concentration in latex sera is a result of rapid metabolism of this sugar in preference to glucose (Bealing, 1969; d'Auzac and Jacob, 1967) by a specific hexokinase present in latex (d'Auzac and Jacob, 1969; Jacob and d'Auzac, 1967).

Quebrachitol is the predominant cyclitol in latex with smaller amounts of ℓ-and myo-inositols (Bealing, 1969). The distribution and concentration of the major soluble carbohydrates in latex have been described (Low, 1978). The concentration of total cyclitols ie. quebrachitol, ℓ-and m-inositols appear to vary with clones and are in the range of 13.0-32.0 mg per ml of C-serum. Like total cyclitols, the concentration of sucrose in C-serum also varies with clones, and is usually between 4.0-10.5 mg per ml serum in the five clones examined. Total cyclitols and sucrose are confined mainly to C-serum whilst glucose is located mainly in the lutoids. The finding that sucrose and total cyclitols are located mainly in C-serum is hardly surprising since the enzymes for carbohydrate metabolism are present in C-serum (Bealing, 1969; d'Auzac and Jacob, 1969).

Lipids and phospholipids: Lipids and phospholipids associated with the rubber and non-rubber particles in latex play a vital role in the stability and colloidal behaviour of latex. Earlier studies (Cockbain and Philpott, 1963; Blackley, 1966) demonstrated that the rubber particles are strongly protected by a complex film of protein and lipid material. It is believed that some of the lipids are present within the rubber particles. The concentration and distribution of lipids between the rubber cream and the bottom fraction had been studied (Ho et al. 1976). These lipids were isolated and divided into neutral lipids and phospholipids for further analysis. There appeared to be distinct clonal variation in the total amount of neutral lipids extractable from rubber cream and from bottom fraction. Colloidal stability of latex was found related to the natural lipid content of rubber particles (Sheriff and Sethuraj, 1978). Lipids from different clones, however, were qualitatively similar. Triglycerides and sterols were the main components of the neutral lipids of rubber particles, whilst sterols and long-chain free fatty acids mainly made up the neutral lipids of the bottom fraction. More recently, a furanoid fatty acid containing a methylfuran group was found mainly in the triglyceride fraction of the neutral lipids (Hasma and Subramaniam, 1978). It constituted about 90% of the total esterified acids. It was suggested that the main triglyceride in Hevea latex contained three furanoid fatty acids, hence making it a rare triglyceride known in nature. The phospholipid content of the rubber particles (approx. 1% on the dry weight of rubber) was similar between

different clones. The total phospholipid content of the bottom fraction was much less (only about 10%) than that in the rubber cream. It was suggested that the amount of neutral lipid (especially triglycerides) associated with the rubber particles was inversely related to the plugging index of the clone which the latex originated from (Ho et al. 1976). Lutoid stability, as indicated by bursting index, was found to be negatively correlated with the phospholipid content of the bottom fraction of latex (Sheriff and Sethuraj, 1978).

A systematic study of the glycolipids from natural rubber was reported (RRIM, 1980). The glycolipid fraction was found to consist mainly of esterified sterol glucoside (ESG), monogalactosyldiglyceride (MGDG), sterol glucoside (SG) and digalactosyldiglyceride (DGDG). The sterol attached to ESG and SG was mainly β-sitosterol, while the acid components of ESG, MGDG and DGDG were of 14:0; 16:1; 18:0; 18:1; 18:2; 18:3 and furanoic acids. The constituents of the phospholipids are mainly phosphatidyl ethanolamine (PE), phosphatidyl choline (PC) and phosphatidyl inositol (PI).

Nucleic acids and polysomes

The presence of nucleic acids in latex was discovered by McMullen (McMullen, 1962) and confirmed by Tupy (1969a). According to Tupy, Hevea latex contains both ribosomal RNA and soluble RNA, DNA and messenger RNA. These are all present in the serum fraction of latex. Later, functional polysomes were also discovered in the serum phase of latex (Coupe and d'Auzac, 1972). More recently, ribonucleic acid (Marin and Trouslot, 1975) and ribosomes (Marin, 1978) have been found to be located in lutoids. The lutoid ribosomes represent 11.9% of the total ribosomal content of Hevea latex. Two high molecular weight RNA components have also been identified and their nucleotide base composition determined (Tupy, 1969a). The presence of these membrane-bound ribosomes in lutoids led to the speculation that these ribosomes were transported from the groundplasm to the lutoids (which are also lysosomes) where they are rapidly destroyed (Marin, 1978).

PHYSIOLOGY OF LATEX FLOW

Response to tapping: When a tree is brought into tapping for the first time, the latex extruded from the vessels is viscous and contains a high density of rubber particles. The flow of latex is curtailed after a short time. Subsequent tappings at regular intervals result in increased yield due to longer duration of flow and more dilute latex until it reaches more or less a steady equilibrium. The increase in yield before reaching a state of equilibrium was termed by early workers (Pakianathan, 1967;

Pakianathan and Milford, 1977) as wound response. Thus, regular and controlled tapping not only increases the time of flow but also enhances the biosynthesis of rubber in the drained vessels below the tapping cut.

Water relations of latex vessels: Turgor pressures within latex vessels in the early morning hours range from 7.9 to 15 atmospheres. Pakianathan and Milford (1977) using a vapour pressure osmometer, obtained values of 10-12 atmospheres on drop samples of latex. Diurnal turgor and osmotic pressure measurements taken at various intervals from 0530 to 1900 h showed maximum turgor values at 0530 h whereas, maximum osmotic pressure values were recorded between 1300 to 1600 h (Buttery and Boatman, 1966). The extent of dilutions, five minutes after the tapping had commenced, were 24.7, 18.8 and 12.1%, for trees tapped at 0400 h, 0800 h and 1230 h respectively. The diffusion pressure deficit was highest in trees tapped at 1230 h. Trees tapped at 0400 h yielded more latex than those tapped at 0830 h or 1230 h (Buttery and Boatman, 1966). Thus, it appeared that latex production was largely influenced by the internal water relations of the tapping panel. These observations showed that latex vessels behaved as a relatively simple osmotic system. Turgor pressure falls during the day as a result of withdrawal of water under transpirational stress (Pakianathan, 1967; Buttery and Boatman, 1964).

Seasonal variations in yield, yield components and components of water relations were studied in some Hevea clones by Gururaja Rao et al. (1988) and Devakumar et al. (1988). Summer yield drops were found to be low in clones like RRII 105 and Gl 1. High latex vessel turgor and low solute potentials in the dry season in clone RRII 105 indicate the presence of osmotic adjustment. Higher plant water status and lowered transpiration rates in this clone might help in maintaining better turgor. Their studies also indicate that low transpiration coefficients are associated with high yields and drought tolerance in RRII 105 and Gl 1.

Events following tapping: On tapping, release of pressure occurs to a greater extent in the latex vessels than in the surrounding tissues. This results in a rapid elastic expulsion of latex flow through the vessels along the pressure gradient. The gradient is highest near the cut and becomes smaller with increasing distance away from the tapping cut. Frey-Wyssling (1952) and Riches and Gooding (1952) made extensive studies on the mechanism of latex flow and cessation of flow. Further work by Boatman (1966) and Buttery and Boatman (1967) demonstrated that flow is rapidly restricted by plugging of the vessels at or near the cut surface and this was usually the major factor causing a decline in the flow rate.

Plugging index

Paardekooper and Samosorn (1969) showed that the latex flow characteristics can be empirically defined by the expression $y = b.e^{-a.t}$ in which the flow rate y at a given time t is a function of the initial flow rate b (at zero time) and a time flow constant *a*. The time flow constant may be regarded as an index of plugging (Milford et al. 1969). This was expressed as the ratio of the flow for the initial five minutes to the total yield. For ease of handling, this ratio was multiplied by 100 (Paardekooper and Samosorn, 1969).

Mechanism of vessel plugging: It is clear that latex contains destabilising factors normally located in the lutoid particles. Consequently, any physiological or biochemical factor which affects the stability of the lutoids would undoubtedly affect the latex flow and plugging of the vessels. By repeated reopening of the tapping cut, Boatman (1966) demonstrated that flow was restricted rather rapidly by some process occuring at or near the surface of the cut. Pakianathan et al. (1966) observed flocs of damaged lutoids in tapped latex and suggested that dilution of latex during flow might damage the osmotically sensitive lutoids and provide a possible mechanism of latex vessel plugging. Electron microscopical observation of the ends of the tapping cut revealed both a cap of coagulum on the surface of the cut and internal plugs within the latex vessels (Southorn, 1968a). Lutoid counts taken before tapping and at various intervals during flow showed a rapid loss during the initial thirty minutes of flow indicating that lutoids were trapped on the cut surface and initial cap formation during the early stages of flow. Shear may play an important part in lutoid damage. Internal plugging occurs mainly during the fast initial flow whereas coagulation on the surface of the cut is effective when the flow is slow. It seems that there is no substantial reason to suppose that the two types of sealing processes are separated in time (Southorn, 1968b). (See Chapter 7. Eds.).

TAPPING PANEL DRYNESS (BROWN BAST)

Brown bast or Tapping Panel Dryness (TPD) is a syndrome encountered in rubber plantations, characterised by spontaneous drying up of the tapping cut resulting in abnormally low yield or stoppage of latex production. The disease was reported for the first time in Brazil in 1887 in Hevea in the Amazon forest and at the beginning of the century in plantations in Asia (Rutgers and Dammerman, 1914).

Symptoms

The symptoms range from partial dryness with no browning of the tapping cut, browning and thickening of the bark and cracking and deformation of the bark in some instances. The syndrome is characterised by the appearance of tylosoids and the coagulation of latex in situ (de Fay, 1981; de Fay and Hebant, 1980; Paranjothy et al. 1976). abnormal behaviour of the parenchyma cells adjoining the laticifers and general increase in synthesis of polyphenols (Rands, 1921). A detailed review of the histological, histochemical and cytological study of the diseased bark was presented by de Fay and Jacob (1989).

Investigations on causative organisms

The involvement of a causative organism was doubted by early workers (Keuchenius, 1924; Rands, 1921; Sharples, 1922). But these workers were unable to demonstrate the existence of an agent responsible for causing tapping panel dryness. Later the possibility of certain types of cortical necrosis which leads to stopping of flow through some pathogenic causes was reported by Nandris et al. (1984), Peries and Brohier (1965) and Zheng Guanbiao et al. (1982). Though rickettsia-like organisms (RLO) was implicated by Zheng Guanbiao et al. (1988), no confirmatory evidence could so far be made available.

Soil, climatic and clonal characters in relation to TPD

Influence of climate and growth period on the incidence of brown bast disease was reported by early workers (Harmsen, 1919; Vollema, 1949; Compagnon et al. 1953; Bealing and Chua, 1972). Through the analysis of soil, leaves and latex, the effect of unbalanced nutrition favouring the incidence of disease was reported by Pushpadas et al. (1975). Clonal sensitivity to tapping panel dryness was observed by many workers (Bangham and d'Agremond, 1939; Dijkman, 1951; Heusser and Holder, 1930; Ostendorf, 1941; Vollema and Dijkman, 1939).

Biochemical and physiological studies

The biochemical and biophysical changes take place at the later stage of this syndrome. The most common symptoms include a phase of excessive late dripping of latex and a simultaneous fall in the rubber content and after a period of time, the volume per tapping gradually declines. The colloidal stability of the latex will also be reduced resulting in particle damage, flocculation of rubber particles in situ, and early plugging of latex vessels (Chrestin et al. 1985). A reduction in turgor pressure

(Sethuraj et al. 1977), change in latex flow pattern (Sethuraj, 1968) and a sharp increase in bursting index (Eschbach et al. 1983) were also reported.

According to Chua (1967) the reserves of starch and other soluble carbohydrates are not depleted. Recent investigations by de Fay (1981) reported abundance of starch grains in the wood of affected trees and the vascular rays were reported to function normally.

The existence of an endogenous NAD(P)H oxidase in lutoids which generates toxic forms of oxygen ($O_2^{\cdot}$, H_2O_2, $OH^{\cdot}$) responsible for the peroxidase degradation of organelle membranes in the latex from diseased trees was reported by many workers (Chrestin, 1984; Chrestin et al. 1984; Chrestin, 1985; Cretin and Bangratz, 1983). Simultaneously, decrease in concentrations of latex cytosol scavengers (reduced thiols and ascrobate) (Chrestin, 1984) as well as virtual disappearance of scavenging enzyme activities (SOD and catalase) (Chrestin, 1984, 1985) was reported. The combination of increased peroxidative activities and considerably diminished quantities of scavengers in latex from affected trees result in destabilisation and lysis of lutoids leading to coagulation (Chrestin, 1989). The possible damage to all the membrane structures in latex cells and resulting impairment of nutrient supply and water exchange at plasmalemma was suggested by Chai Kim Chun et al. (1969) and Pushpadas et al. (1975).

High intensity of exploitation is known to promote incidence of tapping panel dryness in plantations; the proportion of dry trees increases with tapping intensity and particularly with tapping frequency (Bealing and Chua, 1972; Chua, 1967; Paranjothy et al. 1977). The intensive exploitation is reported to result in excessive outflow of latex and consequent nutritional stress (Chua, 1967; Schweizer, 1949; Sharples and Lambourne, 1924; Taylor, 1926), inadequate organic resources (Chua, 1966; Tupy, 1984), and Cu and K deficiency (Compagnon et al. 1953). Changes in mineral ratios, especially of K_2O/CaO and Mg/P was reported by Beaufils (1957). Also, an increase in K content and K/Ca, K/P ratios in latex was observed by Pushpadas et al. (1975).

Certain forms of bark dryness are transitory and do not display the characteristic symptoms of the formation of tylosoids or activation of the phenolic metabolism (de Fay and Jacob, 1989). Numerous traumatism (mechanical such as tapping, chemical or pathological infection) cause formation of ethylene (Yang and Pratt, 1978) and its influence in biochemical, anatomical and histological phenomena is proved (Liebermann, 1973). Over stimulation (dose and frequency) or over tapping can lead to excessive endogenous ethylene production and deleterious effect on cellular

systems (Chrestin, 1984, 1985). Induction of bark dryness through deliberate over stimulation with ethrel results in imbalance in peroxidase activities and consequently disorganisation of the membrane structures. This may lead to the onset of bark dryness.

According to Eschbach et al. (1986) a reduction in sucrose, thiol and Mg contents and increase in redox potential (RP) are connected with a higher rate of bark dryness. The reduced availability of assimilates and the essential enzyme systems may be the principal cause of more frequent occurrence of the disease.

Incidence of TPD can be reduced by reducing the exploitation intensity. Tapping rest imposed for varying periods may revive certain trees, but in majority of cases reoccurrance of the syndrome is encountered.

Recent thinking centres round the question why only certain percentage of trees in a monoclonal population get affected. The involvement of the genetics of root stock has been implicated and this aspect will receive adequate attention in the international network research programme envisaged by the International Rubber Research and Development Board (Sethuraj, 1989).

PROMOTION OF LATEX FLOW WITH CHEMICALS

A wide variety of chemicals ranging from simple inorganic salts to complex organic compounds bring about stimulation of latex yield in Hevea (Blackman, 1961). Periodic scraping of the outer layers of bark caused stimulation resulting in higher yields of latex (Kamerun, 1912). Synthetic auxins such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) applied to scraped areas of bark below the tapping cut resulted in marked increase of latex flow (Chapman, 1951; Baptiste and de Jong, 1953). Copper sulphate, when injected into trees, also caused stimulation (Mainstone and Tan, 1964). A number of workers have tested a variety of chemicals which included growth regulators, herbicides, carbonates, nitrides, bactericides, cyclic and acyclic olefines and halogenoparaffins to study their effects (Pakianathan, 1970; Taysum, 1961).

Increase in yields have also been obtained by the application of gases to the trunk of the tree (Banchi, 1968; Taysum, 1961). How is it that such a wide range of chemicals with completely different molecular structures could bring about yield stimulation activity? The common feature of all these chemicals which induce yield stimulation activity is that they produce ethylene either directly, or through injury of tissues. Some other chemicals have the ability to induce the production of ethylene in the

tissues. The ethylene-releasing compound commercially known as 'Ethrel' or 'Ethephon' has been found to be very effective in prolonging flow which resulted in marked increases in yields.

Mechanism of action of yield stimulants

Boatman (1966) and Buttery and Boatman (1967) showed that 2,4,5-T delayed the plugging. Pakianathan et al. 1966) observed that the proportion of damaged lutoids in latex samples collected during flow increased rather than decreased following 2,4,5-T treatment. In trees treated with ethephon or 2,4,5-T the loss of lutoids did not commence until 45 minutes after tapping, but thereafter increased until by the end of flow nearly 50% of the lutoids were lost compared with unstimulated trees. These observations in some way indicate that yield stimulants tend to delay the retention of lutoids by the cut vessel ends and hence delay plugging and prolong flow (Pakianathan and Milford, 1977).

Application of 2,4,5-T and ethephon result in an increase in yield and decrease in plugging index. The initial flow rates were not affected, and the yield difference was a consequence of extension of flow time (Abraham et al. 1968). Treatment of Hevea bark with 2,4-D or Naphthalene acetic acid (NAA) increased sucrose level, invertase activity, and sucrose utilisation in the latex.

Stimulation results in an increase in the displacement area (Lustinec and Resing, 1965; Pakianathan et al. 1975). This suggests a localised reduction in the vicinity of the tapping cut to form plugs.

Osborne and Sargent (1974) suggested a probable mechanism of promotion of flow by ethylene. Ethylene treatment possibly results in wider latex vessels with thicker, more rigid walls. This in turn leads to less constriction of severed vessels after tapping, reduced shearing forces at the orifice, a lower proportion of damaged lutoid particles, and less enzymic coagulation of rubber particles. The delayed occlusion of the vessel orifice results in prolonged flow.

THE ROLE OF CARBOHYDRATES IN LATEX FLOW

Relatively little is known about the role of carbohydrates in latex flow. Because of its high concentration in latex (1-2%) (Rhodes and Wiltshire, 1931) quebrachitol has been suggested to contribute as much as over 30% of the total osmotic pressure of latex serum (Sheldrake, 1973). The osmotic role of quebrachitol was further emphasised when quebrachitol (measured as total cyclitols, together with lesser amounts of ℓ- and m-inositols) was found to be present, in high concentrations, in a high plugging clone viz.

Tjir 1 and in low concentrations in a low plugging clone viz. RRIM 501 (Low, 1978). A high total cyclitols concentration would lead to a faster and higher dilution reaction during latex flow, resulting in possibly greater lutoid damage, a faster plugging reaction and an earlier cessation of flow. Similarly, clones with low total cyclitols content probably experience a slower dilution reaction, less lutoid damage, a slower plugging reaction and consequently, a more prolonged flow. The concentration of total cyclitols in latex was shown to decrease as the flow proceeded (Low, 1981). This lowering of total cyclitols concentration was similar to the decrease in total solids content in latex in the same latex samples, implying the dilution of total cyclitols during latex flow. Since the osmotic concentration of latex decreases in the progressively collected drop fractions (Pakianathan, 1967) after tapping (and hence flow time), the fall in total cyclitols concentration with flow must be a reflection of the osmotic role of total cyclitols in latex, as suggested earlier (Low, 1978; Sheldrake, 1973).

Sucrose is the next major soluble carbohydrate in latex, after quebrachitol (Low, 1978) and it is plausible that sucrose also contributes to the osmolarity of latex. However, the concentration of sucrose is small compared to that of total cyclitols. Therefore, the osmotic role of sucrose must be minor compared to that of total cyclitols in general and quebrachitol in particular (Low, 1978).

Recently, cell sap has been shown to exhibit strong destabilisation activity (Gomez, 1977; Yip and Gomez, 1978, 1984) on latex particles. This may be important in the cap coagulum formation over the vessel ends, during the later stages of flow (Yip and Gomez, 1984) resulting in the final cessation of flow. There is experimental evidence to suggest that simple sugars such as monosaccharides may delay the destabilisation effect of the cell sap (RRIM, 1980). Though the role of monosaccharides in the destabilisation activity of cell sap towards latex particles is not clearly understood at present, it may nonetheless be important in the final cessation of latex flow after tapping.

CARBOHYDRATES AND RUBBER BIOSYNTHESIS

Carbohydrates, particularly the hexoses in latex, are probably the primary source of acetate and acetyl-CoA, essential for the biosynthesis of rubber in Hevea. Hence, a discussion on the role of carbohydrates in rubber production is included in this chapter. (For detailed account on biosynthesis, please see Chapter 6. Eds.).

The importance of carbohydrates in rubber production had been studied in relation to various aspects. These include changes in the levels of

carbohydrates in latex (Low, 1968; Low and Gomez, 1982) and bark (Low and Gomez, 1984) in response to yield stimulation by ethephon, the effects of stimulation and exploitation on invertase activity in latex and the correlation of latex invertase activity with latex vessel plugging in Hevea (Yeang et al. 1984) Ethephon stimulation was shown to result in a decrease in sucrose and total cyclitols concentrations in latex (Low, 1968; Low and Gomez, 1982). The decline in sucrose concentration in latex after ethephon stimulation usually preceeded a similar decline in total cyclitols concentration in latex (Low and Gomez, 1982). In the unexploited tree, gentle gradients of starch and total sugars appeared to exist in opposite directions in the bark (Low and Gomez, 1984). Initiation of exploitation on previously unexploited trees resulted in a significant lowering of bark starch at the tapping panel, creating a 'source-sink' situation in the tree. The depression of bark starch with continued exploitation was however, not continuous. After a certain period, a lowered level of bark starch was maintained (Low and Gomez, 1984). Ethephon stimulation enhanced this depression of bark starch. The depletion of bark starch was confined to the tapped panel and did not extend to the opposite untapped panel or to an untapped region above the tapped panel (Low and Gomez, 1984). Hence, the benefits of panel changing, in relation to the exhaustion of bark carbohydrates on the tapped panel, are immediately obvious.

The importance of invertase in latex physiology and production has been widely studied (Yeang et al. 1984; Tupy, 1969b, 1973b, 1973c). Invertase, by virtue of its extremely low activity (d'Auzac and Jacob, 1969), has been suggested as a possible pacemaker enzyme in the glycolytic pathway of Hevea latex. Since the utilisation of sucrose, the primary carbohydrate substrate in latex, is controlled by invertase (Tupy, 1969b), it is plausible that invertase also controls the overall metabolic activity of latex (Tupy, 1973b). Ethephon stimulation on previously unstimulated trees, resulted in an increased invertase activity in latex. However, repeated ethephon stimulation caused an eventual decline in the invertase activity, which at times, was lower than that of the unstimulated controls, even though the yield response was positive (Yeang et al. 1984). Contrary to the findings of Tupy (1973) invertase activity was not always correlated with latex production (Yeang et al. 1984). Invertase activity was, however, found to be negatively correlated with the inclination to latex vessel plugging (Yeang et al. 1984).

The feasibility of using latex sucrose levels as an early warning signal to indicate overexploitation and subsequent dryness of the Hevea trees was examined. This investigation was based on the equivocal assumption that latex with a higher sucrose content was a reflection of the better

physiological status of the tree (Leong and Tan, 1978). Based on this criterion, the superiority of microtapping as compared to conventional tapping was assumed since microtapped latex was reported to contain a higher sucrose content than the latex from conventional tapping (Leong and Tan, 1978; Tupy, 1973a; Gener et al. 1977; Tupy and Primot, 1974; Primot and Tupy, 1976; Leong et al. 1976; Ramachandran, 1978; Ramachandran and Lee, 1980). However, other workers had demonstrated that the microtapped latex did not contain more sucrose than in the latex latex from conventional tapping (Samosorn et al. 1978; Low et al. 1983). Possible reasons for this discrepancy had been discussed (Low et al. 1983) and in the present uncertainty, it may not be prudent to evaluate the physiological health of the Hevea tree solely on the basis of its sucrose levels in latex.

Many non-rubber compounds emerge along with rubber in the latex when a tree is tapped, and the tree has to replace these compounds by synthesis. Among these compounds the very important ones are the proteins (enzymes) and the nucleic acids. The latex vessels have the machinery for synthesizing specific proteins. The synthesis is dependent upon the presence of the nucleic acids in the cells: DNA, transfer RNA (t-RNA), ribosomal RNA (r-RNA) and messenger RNA (m-RNA). Except DNA, all these nucleic acids have been found in latex (McMullen, 1959, 1962; Dikenson, 1965; Tupy, 1969). Most of the nuclei are left behind and do not come out with latex on tapping and this may be the reason for the lack of DNA in tapped latex.

Some correlation between the yield of rubber and the amount of nucleic acid in latex has been reported (Tupy, 1969) and it has been claimed that latex from high yielding trees synthesises nucleic acids much faster in vitro than latex from low yielding trees of the same clone. Furthermore, when a tree is intensively tapped, the nucleic acids at first decline in amount but are then resynthesised and quickly rise to a concentration well above that in trees tapped at normal frequencies. Nucleic acids and proteins, therefore, play an important role in the production of rubber and thus in the final yield.

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