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CHAPTER 4

ANATOMY AND ULTRACYTOLOGY OF LATEX VESSELS

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Laticifers are present in a very large number of species belonging to about twenty different families (Metcalf, 1966), mostly dicotyledons. A few monocotyledons like Allium cepa (Hoffmann, 1933) and the genus Regenellidium of Marsiliaceae (Pteridophyta) are also reported to have laticifers (Gomez, 1982). The taxonomic importance of these structures is doubtful, although it has been a matter of fundamental interest.

The occurrence of laticifers in Hevea and their structure have been studied during the nineteenth century by Scott (1886) and Calvert (1887). Detailed investigations on the bark structure and the laticiferous tissue of the para rubber tree were carried out during the early twentieth century (Bryce and Campbell, 1917; Keuchenius, 1918; Arisz, 1919, 1921; La Rue, 1921; Heusser, 1921; Vischer and Tas, 1922; Bobilioff, 1919, 1920, 1923; Steinmann, 1923).

Nature of laticifers

The type of laticifers is characteristic of the plant species. The complex types of laticiferous systems in plants are classified as articulated and non-articulated according to the mode of origin (De Bary, 1877). Both are tubular structures and are described as latex vessels.

Articulated laticifers are compound in origin comprising a series of cells, either remaining blunt or becoming continuous tubular structures due to partial or complete dissolution of endwalls. Depending on the presence or absence of lateral connections they are further categorised as anastomosing and non-anastomosing respectively. The non-articulated laticifers are more simple in structure. They may remain as single unbranched cells or branched structures extending throughout the shoot and root system as in Euphorbia species. The groups of laticifers in plant kingdom can be classified as indicated below:

Laticifers: Specialised cells or tissues which contain latex.	Non-articulated: Develop from single cells, which elongate and their tips keep pace with growth of the cells of the surrounding meri- stem penetrating among the new cells.	Unbranched: More or less straight tubes developing into long structure	<u>Cannabis</u> <u>Urtica</u> <u>Catharanthus</u>
	Articulated: Originate from rows of cells by the partial or complete absorption of the separating walls in early ontogeny.	Branched: Each laticifer cell branches repeatedly forming an immense system of tubes.	<u>Asclepias</u> <u>Cryptostegia</u> <u>Euphorbia</u> <u>Ficus</u> <u>Nerium</u>
		Non-anastomosing: Long, compound tubes not connected with each other laterally.	<u>Achras</u> <u>Chelidonium</u> <u>Convolvulus</u> <u>Ipomoea</u>
		Anastomosing: Forms anastomoses laterally with cells or tubes of similar nature, all combine forming a reticulum.	<u>Argemone</u> <u>Carica</u> <u>Cichorium</u> <u>Hevea</u> <u>Manihot</u> <u>Taraxacum</u>

Classification of laticifers - A schematic representation
(Adapted from Panikkar, 1974)

In Hevea, the principal type of laticifers exploited commercially for its latex is the secondary laticifers, differentiated by the activity of vascular cambium as in the case of vessel elements and hence the term 'latex vessels' is appropriate. They are articulated and belong to the anastomosing, coenocytic type.

Aseptate thread-like laticifers, however, occur in the pith and leaves and also in young branches of Hevea tree. Presence of ray cells containing latex has also been reported (Bobliloff, 1923). Recently Xiuqian (1987) made detailed investigations on the nature of laticifers in Hevea brasiliensis and observed that the primary laticifers found in the seed (cotyledon), leaf, flower, root and young stem are non-articulated which show intrusive growth into the intercellular spaces of the primary phloem and cortex in contrast to the articulated anastomosing secondary laticifers.

Ontogeny

The entire laticifer system in a species may initiate from a few

initials present in the embryo, as in certain Euphorbia sp. (Scharffstein, 1932; Rosowski, 1968), which in the course of development, are found on the circumference of the central cylinder. In certain others the primary laticifers originate in the phloem or pericycle as in Taraxacum kok-saghyz or in the hypodermal region. In Allium, the laticifer initials are found in the third layer of leaf mesophyll or in the third layer below the abnormal epidermis of the bulb scale.

Ontogeny of laticifers in Hevea was outlined by Scott (1882) based on the observations on germinating seeds. He could identify the latex vessels as small elongated cells with characteristic granular contents devoid of the aleurone grains. According to him dissolution of cross walls takes place at a stage of root growth approaching 3-4 mm length in the seedlings. He considered the hypodermal system of laticifers as more advanced than the vascular laticifer system. Calvert (1887) identified three systems of laticifers in the stem of Hevea: hypodermal, principal and medullar. But Milanez (1946, 1948, 1951) later found that the primary laticiferous system is differentiated from the procambium in the vicinity of the phloem. More elaborate studies on the development of latex vessels, as reviewed by Gomez (1982), confirm that the principal system of primary laticifers is that observed in the procambial region belonging to the primary phloem proper.

Bobillioff (1919) described two modes of laticifer ontogeny in Hevea, one by the dissolution of cross walls of a row of cells and the other by extension of growth of certain cells. The first type of development in the formation of secondary laticifers was confirmed later (Panikkar, 1974; Xiuqian, 1987). In the course of development of the articulated laticifers in Hevea, before the dissolution of end walls of laticifer initials, small projections are formed on the lateral walls and the tubular projections of the adjacent laticifers come in contact and fuse to form the anastomoses.

Distribution

In Hevea laticifers are present in all organs except wood although occasional presence of plugged vessels having latex has been reported (Bobillioff, 1921, 1923). Non-articulated laticifers of primary origin are usually present in young organs in the primary state of growth. They are also observed in young leaves, flowers, cotyledons and pith.

Secondary laticifers are distributed in the bark, and the commercially exploited part of the tree is the main trunk. A comprehensive description of the structure of mature bark in Hevea (Fig. 1) was made by Bryce and Campbell (1917). In the bark, in addition to the outermost protective tissues consisting of layers of cork cells, there are two more distinguishable zones,

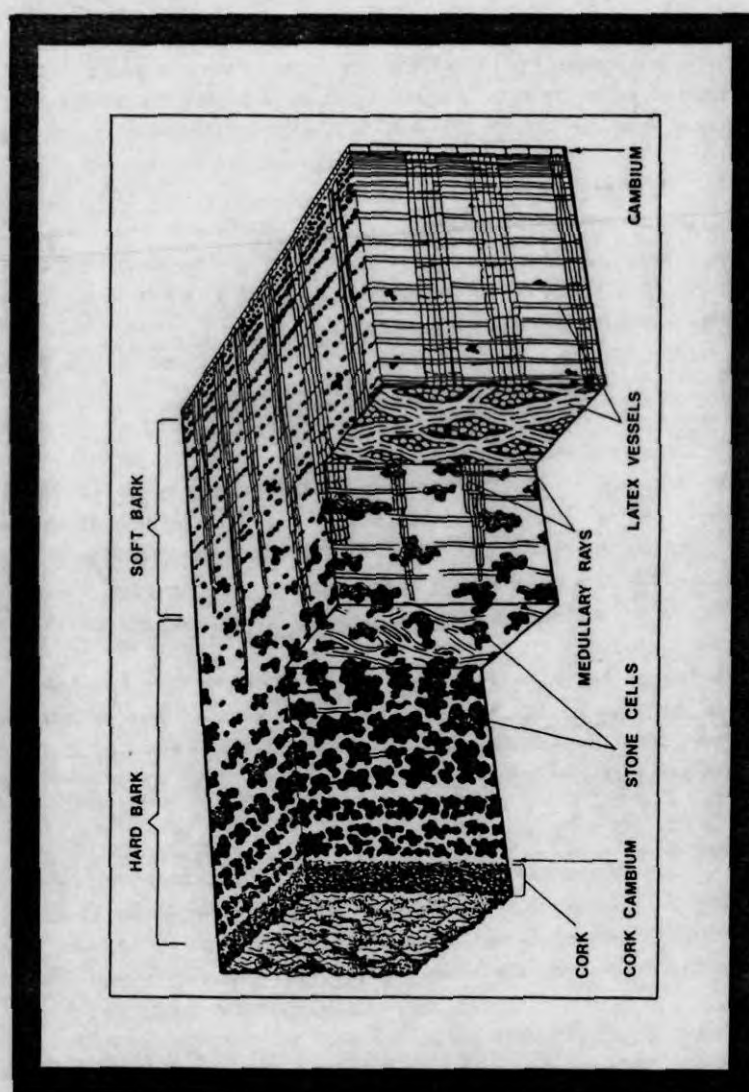


Fig. 1. Mature bark - a three dimensional view.

an inner soft zone and an outer hard zone. Hardness of the outer zone is due to the occurrence of sclerified cells in masses, known as stone cells. There are tannin cells also in the bark. The laticifer differentiation from the cambial derivatives is a rhythmic process and a ring of laticifers are produced each time. Hence the latex vessels in Hevea form concentric rings almost alternating with layers of other phloem tissue. The general structure and organization is the same in the main trunk and branches, though the bark thickness and number of latex vessel rows vary.

In a cross sectional view, latex vessels have a more or less circular shape. The concentric rows of latex vessels are seen almost parallel to the cambium, and are separated by layers of phloem elements.

In a longitudinal section in radial plane the latex vessels have tubular shape, and the vessels representing different rows look like straight tubes interrupted at the location of phloem rays. In the soft bast region the latex vessels are continuous, while most of them in the hard bast region are discontinuous and hence non-functional. In the hard bast region the latex vessels become crushed and broken due to the push and pull exerted by the surrounding tissue, especially the stone cells, during radial growth. The latex vessels between rows are not usually connected although rare instances of anastomosing are reported.

In a tangential longitudinal section of the bark through a ring of laticifers, the system resembles a meshwork. The cross sectional areas of phloic rays represent the sieves of the net. Weaving the phloic rays the tubular latex vessels run up, in a slightly inclined path to the long axis of the tree, in anti-clockwise direction. The degree of inclination varies from 2-7° depending on clone. The inclination of latex vessels was well known to earlier researchers (Petch, 1911; De Jonge, 1916, 1919; Gomez and Chen, 1967) and this trait was taken into account in deciding the slope of tapping cut.

The course of latex vessels from the base upwards interweaving the ray groups leads to a zig-zag pattern. The extent of waviness depends on the size and distribution of the phloic rays and hence is a clonal character (Premakumari et al., 1985). The degree of waviness of latex vessels within a tree varies according to the sampling distance from the cambium.

Premakumari et al. (1985) recorded a range of 6-9 connections, within a distance of 0.22 mm of anastomosing vessels among ten clones and observed that the intensity of anastomosing is a significant clonal character. Xiuqian (1987) observed 1-5 connections per laticifer cell arranged in a regular pattern.

QUANTITATIVE ASPECTS

The quantity of laticiferous tissue in a tree is determined by various factors such as the number of latex vessel rows, density of latex vessels within a ring, distance between vessel rings, distribution pattern of latex vessel rings, size of laticifers and the girth of the tree.

Number of latex vessel rings

The number of latex vessel rings is a clonal character (Bobilioff, 1923; Vischer, 1921, 1922; Sanderson and Sutcliffe, 1921) and the frequency of laticifer differentiation is genetically controlled. The number of latex vessel rows varies depending on the clone, age of the tree, growth rate and seasonal factors. During the active growth period, the rate of laticifer differentiation would be much higher than that during the rest of the year. Significant variation of cambial activity and number of vessel rings between different periods of the year have been noted in clone G1 1 (Premakumari et al., 1981). Higher number of latex vessel rows was associated with higher rate of cambial activity. Gomez et al. (1972) studied the influence of age of the tree on number of latex vessel rings and observed a linear relationship upto about 15 years.

In seedling trees, the number of latex vessel rows decreases with increasing height of the trunk owing to the positive relationship between latex vessel rows and bark thickness. Bark thickness decreases with increasing height due to the coniform nature of stems, compared to the cylindrical trunk in clones. This factor is given due consideration in determining the height and slope of tapping cut.

Gomez et al. (1972) studied 112 clones, aged eight and half, and recorded a mean of 25.6 rings. Gomez (1982) suggested that the increase in the number of laticifer rings, as seen from the reports of various studies over a period of fifty years, can be attributed to genetic improvement.

Concentration of latex vessel rings in the bark at positions distal to the cambium varies among clones. Nearly 10-35% of the vessel rings were observed in the second and third millimeter and the number diminished to zero over a distance of five to eight mm. Clonal differences in the distance between two consecutive latex vessel rings became prominent only beyond the third millimeter from the cambium.

The quantum of laticifers in the virgin bark is influenced by the age of the tree. Gomez et al. (1982) reported that in trees below five years, the laticifer rings were concentrated in the first 4-5 mm; only 40% being in the second. Between five and ten years, however, laticifer rings were more concentrated near the cambium tailing away to zero near to the

eighth millimeter and by the 25th year about 75% of the latex vessel rings were oriented at the innermost five mm of the bark.

Density of latex vessels within rings

The number of laticifers per unit circumference of the tree, usually referred to as density of latex vessels, indicates the quantity of laticiferous tissue in terms of laticifer area in the cross section of a tree. Gomez (1982) mentioned a difference in latex vessel density between two distant positions from the cambium. He observed an apparent clonal difference also. Highly significant clonal differences in this trait have been demonstrated later by Premakumari et al. (1985). Among ten clones they recorded a range of 24-31 latex vessels per row per 1.25 mm circumference. The density of latex vessels is also related to the width of phloic rays (Premakumari et al., 1984).

Diameter of latex vessels

The laticifer diameter is a known clonal character. Ashplant (1928) recorded high correlations (0.76) between yield and latex vessel diameter. Frey-Wyssling (1930) and Riches and Gooding (1952) related the diameter of latex vessels to rate of flow of latex, compared the relationship with Poiseuille's equation for viscous flow in a capillary and demonstrated that the volume of flow is proportional to the fourth power of the radius of the capillary.

Gomez et al. (1972) reported a variation of latex vessel diameter from 21.6 μm to 29.7 μm in eight RRIM clones. In a recent study Premakumari et al. (1985) recorded a range of 16.67 μm to 26.87 μm in nine year old plants of ten clones.

Laticifer area index

Laticifer area index is a tentative index proposed by Gomez et al. (1972) to approximate the quantity of laticiferous tissue of a tree in terms of cross sectional area. This index is believed to account all the main quantitative factors which are involved in latex production. The total number of laticifers in a tree is approximately assessed as nfG , where, n is the number of latex vessel rings, f , the density of latex vessels per ring and G , the girth in cm. Hence the total cross sectional area of latex vessel would be $nfG(\pi r^2)$, where r is the radius of the latex vessel.

Since the latex vessel rings are not interconnected, opening of more latex vessel rings on tapping gives proportionately higher yield. It is conventional to leave approximately one mm bark undisturbed near the

cambium to avoid injury to the cambium. About 40% of the latex vessel rings are distributed within the first millimeter from the wood and hence 'n' should be corrected as 0.6 n and again for half spiral cut it should be corrected as 0.3 n. For practical purposes the number of latex vessels cut on each tapping in half spiral cut should be calculated as 0.3 nfG and the cross sectional area as $0.3 \text{ nfG } (\pi r^2)$.

Variations at higher ploidy levels

Only limited studies have been made on the quantitative variations of laticiferous tissue of Hevea at different ploidy levels. A study on the comparative bark anatomy of the mature trees of induced polyploids of GT 1, Tjir 1 and RRII 105 showed considerable reduction in bark thickness, number of latex vessel rows and density of laticifers leading to reduced laticifer area for the tetraploids as compared to their respective diploids (Premakumari et al., 1988b). The effect was not uniform in clones studied but a tendency for higher size and reduced number of cells for the polyploids was indicated.

LATEX VESSELS AND YIELD

Correlations between structural features and yield in Hevea have been studied in detail by several researchers. Bobilioff (1920), La Rue (1921) and Taylor (1926) established highly significant correlations between yield and number of latex vessel rows in seedling materials. Later studies yielded still higher correlation of this structural trait with yield in clones and positive linear relationship of the number of latex vessel rows and bark thickness with yield of Hevea clones in 33 month old nursery plants was obtained (Narayanan et al., 1973). Elaborate studies and yield component analysis (Narayanan et al., 1973; Narayanan et al., 1974; Narayanan and Ho, 1973; Ho et al., 1973) proved that the number of latex vessel rows is the most important single factor related to yield. This character, combined with plugging index and girth, could account for 75% of the yield variations in young plants. The accountability was reduced to 40% at the mature phase which indicated a predominant role of genotype environment interaction. Sethuraj et al. (1974) demonstrated a significant positive correlation between number of latex vessel rows and initial rate of flow. Premakumari et al. (1984) proposed a negative relationship of phloic ray width and yield, mediated through the inverse relationship between laticifer density and phloic ray width.

In addition to the number of latex vessel rings, the density of latex vessels/ring, laticifer diameter and also the distance between laticifer

rows are factors determining the quantity of laticiferous tissue expressed in terms of laticifer area in cross section of the bark. Premakumari et al. (1988a) suggested laticifer area and orientation of laticifers as very important factors influencing yield of Hevea clones.

REGENERATION OF LATICIFERS

Hevea trees are exploited for a period of 20-23 years from the commencement of tapping. After the consumption of virgin bark, regenerated bark is exploited and hence regeneration of bark is of great significance. In tapping, only a thin slice of bark, 1.0-1.5 mm thick is shaved off to cut open the latex vessels. The cambium is not injured in this process. Moreover a layer of soft bast which is left intact along with the cambium during tapping gives protection to the cambial layer. Bark is regenerated due to continued activity of the vascular cambium, during which process new phloem tissues are produced and the normal process of laticifer differentiation continues. The protective tissue lost along with the bark is replaced by the formation and activity of a new phellogen below the cut surface (Bobilioff, 1923; Panikkar, 1974). Thus the process of bark regeneration is the function of two cambia, the continued activity of the vascular cambium and the formation and functioning of a new cork cambium.

The immediate effect of tapping in Hevea is a shrinkage of cells at the cut surface, which undergo necrosis. Immediately below the necrotic layer, cells enlarge and divide in irregular planes which later become oriented parallel to the cut surface. A wound phellogen differentiates at the hypodermal, or, occasionally the sub hypodermal, layer which cuts off phellem towards the periphery and phelloderm towards the inner side (Panikkar, 1974).

The extent of bark regeneration and differentiation of laticifer rows depends on the clone. Bobilioff (1923) found that the total number of laticifer rows, after one year of regeneration of bark will become equal to the number which was present in the virgin bark. It is also reported that the rate of regeneration of laticifer rows is determined by the inherent characteristic of the tree for laticifer differentiation in the virgin bark.

The extent of regeneration is controlled by various factors like depth of tapping and age of the tree. Gomez et al. (1972) observed that clonal differences appear to be more important only in the young trees. For very old trees the proportion of uncut vessel rings recorded were only 8-13% for the clones studied, whereas, it was 30-45% in younger trees. The effect of deeper tapping therefore would show only smaller response as the tree grows older.

Regeneration of laticifers in stimulated bark

Significant increase in bark thickness was recorded for stimulated trees by De Jonge (1955, 1957) in experiments using 2,4-D, and 2,4,5-T applied on renewing bark. However, no effect on the number of latex vessel rows was evident. Reduction in the number of latex vessel rows associated with increased periderm formation for oil/petroleum based treatment, has been reported by Gomez (1964) while no characteristic effect on the number of latex vessel rows could be noticed for Ethephon stimulation (Gomez, 1982). However, an increase of periderm tissue due to stimulant application was apparent.

Regeneration of laticifers after microtapping

Delayed formation of latex vessel ring just after microtapping was noticed in normal puncture tapped trees (Hamzah and Gomez, 1981). However, normal activity of the vascular cambium was not permanently affected as it continued normal production of latex vessels. The scar tissue or 'scab' formed on the surface of the bark under the flaky external bark, at the punctured portion, consists of the 'wound zone' and the periderm tissue formed after puncturing. The wound zone is a part of the bark at the punctured portion where the tissues show discolouration due to the presence of lignin, suberin, tannin, etc., which are supposed to aid protection of protoplast against desiccation and decay.

EFFECT OF MINERAL DEFICIENCIES ON LATEX VESSEL FORMATION

The relative importance of soil type on laticifer differentiation was studied by Keuchenius (1920) who recorded an annual average increment of 3.14 latex vessel rings in good soil, 2.14 rings in average soil and 1.74 rings in poor soil. Later studies (Mass, 1923; Hamzah et al., 1975) confirmed the effect of mineral deficiencies on laticifer formation. Deficiencies of nitrogen, phosphorus, potassium and magnesium have major detrimental effect on laticifer differentiation. Likewise calcium and sulphur deficiency also reduced the number of latex vessels. In addition to the major elements, certain minor elements such as manganese, iron and zinc were also found to influence formation of latex vessels.

LATEX VESSELS OF BROWN BAST AFFECTED TREES

Brown bast is considered as a physiological disorder very often related to over exploitation. The disorder mainly originates in the latex vessels and spreads along the vessels. However this does not usually spread from

one regenerated to another and from virgin bark to regenerated bark (Paranjothy et al., 1975). Brown bast often produces cancerous growths in the bark tissue leading to malformations of the tree trunk, and detailed investigations have been made on the bark anatomy of brown bast affected trees (Rands, 1919, 1921,a,b,c; Rhodes, 1930; Sanderson and Sutcliffe, 1921). Structural characteristics associated with brown bast incidence mainly involve depletion of starch in tissues, occurrence of a brown substance similar to tannin in abundance, frequent occurrence of oil globules and production of a large number of stone cells. Formation of a type of wound gum, resistant to acids and alkalies but reacting with tannins and lignin was observed in brown bast affected trees by Rands (1919, 1921a,b,c) and Rhodes (1930). Gomez (1982) observed formation of tyloses inside the latex vessels of brown bast affected tissue. The tyloses later became filled with tannins and their walls got lignified. The vessels, partially blocked with tyloses, check latex flow, leading to initiation of dryness. De Fay and Hebant (1980) have confirmed some of these findings. However, these histological reasons may only be secondary effects leading to reduced latex flow. The effect of diminished permeability of the latex vessels due to intensive tapping, as described by Bealing and Chua (1972), followed by coagulation of latex may be the major cause of critical reduction in latex flow.

ULTRACYTOLOGY

The anatomy and histology of Hevea and the ontogeny of latex vessels have been documented well. Hevea latex vessels being a difficult material for light microscopy due to the out flow of latex on sectioning and latex dissolution/coagulation during processing, only very little information could be gathered on the cytology of the latex vessel and its contents. According to Bobilioff (1923) the very early contention was that latex vessels are intraxylary spaces between tissues filled with sap which diffused in from neighbouring cells. The cytoplasmic nature of latex had been first proposed in 1886 by Berthold. However, this was still a debatable subject and the concept that latex is the cell sap bounded by a tonoplast gained more acceptance when published in standard works of Bobilioff (1923, 1930) and Frey-Wyssling (1952). Later high speed centrifugation of latex and its separation into four main fractions by Cook and Sekhar (1953) and subsequent research by Moir (1959) revived the cytoplasmic theory of latex vessels. Application of vital stains like neutral red improved the stainability of different zones and rendered support to earlier reports on the occurrence of luteoids and its identification as vacuoles (Wiersum, 1957). By this time there was considerable improvement in electron microscopy. This technique

provided clear micrographs of latex vessels in section which proved, beyond doubt, that latex in Hevea is a specialised type of cytoplasm (Fig. 2).

Cell wall

As in the case of sieve tubes the cell walls of laticifers are mostly made of cellulose. Photomicrographs (Gomez, 1976) reveal higher wall thickness for older vessels. A bulge is often characteristic of the laticifer wall, which can be attributed to the remnants of cross walls. Presence of partially dissolved cross walls at the initial stages of latex vessel ontogeny has been suggested by Dickenson (1969), who has also found the occurrence of microtubules apparently at the periphery of the walls with plasmodesmata between latex vessels as well as between latex vessels and neighbouring cells of very young vessels. In contrast to this, d'Auzac (1988) reported that laticifer cells do not have any pit fields connecting the neighbouring parenchyma. He found perforations connecting a laticifer with other laticifers.

Nucleus

Bobilioff (1923, 1930) reported coenocytic nature of latex vessels in Hevea. According to him the nucleus of a laticifer initial retains central position and during the course of ontogeny the cytoplasm forms a peripheral layer where numerous nuclei are embedded in mature latex vessels. Later observations with the aid of electron photomicrographs by Dickenson (1965) confirmed the coenocytic nature of the latex vessels and the parietal position of nuclei.

Mitochondria

The occurrence of mitochondria in young latex vessels of the tender parts of the tree was found by Dickenson (1965) and later by Gomez (1976). However, mitochondria were rare or absent in tapped latex which may be attributed to its parietal position after tapping. However, more detailed observations are needed to prove the nature and functions of such organs in latex vessels.

Endoplasmic reticulum, ribosomes and proplastids

Occurrence of cytoplasmic organelles like endoplasmic reticulum and ribosomes in young laticifers has been reported (Archer et al., 1963; Dickenson, 1965), and later confirmed by Gomez (1974, 1976). The presence of ribosomes in tapped latex has been reported by Coupe and d'Auzac (1972) after biochemical studies on latex. Occasional presence of plastids and



Fig. 2. Ultrastructure of a mature latex vessel from a tapping tree.

pro-plastids has also been noted (Gomez and Moir, 1979).

MAJOR CONSTITUENTS OF LATEX

Fresh Hevea latex is a polydisperse system in which negatively charged particles of various types are suspended in an ambient serum (C-serum). The two main particulate phases contained in Hevea latex are rubber particles constituting 30-45% and luteoid particles, 10-20%. The third type on a quantum basis is the Frey-Wyssling complexes.

Rubber particles

The rubber particles usually have a size ranging from 50 A° to about 30,000 A° (3 µm), although extreme cases having 5 or 6 µm are also found. They are spherical bodies in young trees and potted plants but in mature trees the particles are large, often having a pear shape. The shape in certain cases seems to be a clonal character. Pear shape is reported to be very frequent in clones such as Tjir 1 and PR 107 (Southorn, 1961).

Particle size of greater proportion is beyond the limit of the resolution power of light microscope and reliable information in this regard could be obtained only with electron microscopic observations. Tempel (1952) recorded 1000 A° size at maximum frequency. This was confirmed later by Gomez and Moir (1979). Schoon and van der Bie (1955), observed a multimodal distribution of rubber particles in latex of mature Hevea trees and proposed that larger particles are formed by the association of smaller particles. Gomez (1966) found a multimodal distribution in latices from young potted plants. In laticifers of very young plants small osmiophilic particles are seen freely in the cytoplasm. The structure and colloidal properties of Hevea latex has been well studied (Cockbain and Philpott, 1963; Ho et al., 1976).

A rubber particle of average size, about 1000 A°, contains hundreds of molecules of the hydrocarbon and is surrounded by a surface film of proteins and lipids. The rubber particles are also associated with triglycerides, sterols, sterol esters, tocotrienols and other lipids. Dupont et al. (1976) have confirmed the presence of phosphatidylcholine and small amounts of phosphatidyl ethanolamine in the lipids associated with rubber particles. The protein envelope of rubber particles is visible in sections of osmium stained rubber particles and is approximately 100 A° thick (Andrews and Dickenson, 1961). The envelope carries a negative charge and confers colloidal stability to the rubber particles.

According to Dickenson (1969) there are rubber particles with variously stained regions. An inner osmiophilic region surrounded by a weakly stained

periphery is attributed to lack of uniformity when rubber particles are deposited on existing particles during biosynthesis. He has also suggested that the inner particulate inclusion, having 50-80 A° thickness, might be molecules of rubber of molecular weight about 100,000 but further investigations are needed to confirm this.

Lutoids

Lutoids form the next major component of Hevea latex. They are membrane-bound bodies and are mostly larger in size than the rubber particles. They are 2-5 μ m in diameter bounded by a unit membrane of about 80 A° thickness. It was Wiersum (1957) who first suggested that the lutoids behave like vacuoles due to stainability with neutral red. Though controversy existed in this regard, the work of Ribailier et al. (1971) provided evidence for the vacuolar properties of lutoids.

The content of lutoids (B-serum) has a very rapid flocculating action on aqueous suspension of rubber particles in latex, resulting in the formation of microflocs (Southern and Edwin, 1968). This activity is apparently moderated by the ambient C-serum and is much reduced if B-serum is boiled. Southorn and Yip (1968) demonstrated that this fast initial flocculating action of B-serum is an electrostatic one involving the interaction between the cationic contents of B-serum and the anionic rubber particle surface.

By phase contrast microscopy and application of suitable staining procedures the structure of lutoid particles have been studied in detail. Mainly two types of fibrillar structures have been described. The first type, known as microfibrils, are characteristic of latex vessels in young tissues (Dickenson, 1965, 1969; Audley, 1965, 1966). As seen by phase contrast microscopy of tapped latex from young tissue, the microfibrils are freely suspended in the fluid content of the lutoid B-serum. The microfibrils are seen usually as grouped together in bundles. Each bundle has a diameter of 450-500 A°. Individual microfibrils are several micron long and 70-80 A° in diameter. The microfibrils can be isolated from the sediments of latex from young tissues which on negative staining with phosphotungstic acid shows further details. Each microfibril is a tightly coiled continuous helix with a hollow axis. The diameter of the helix is about 125 A° and that of the hollow axis 30 A°. The microfibrils consist of an acidic protein while nucleic acid seems to be absent. Microfibrils however are not present in tissue or latex collected from the mature bark. It is believed that they disintegrate as the particles mature or else the young lutoids containing microfibrils themselves disintegrate as the tissue

ages and are replaced by a population of lutoids without microfibrils. However, the microfibrils do not seem to have vital role in rubber biosynthesis.

The second type of fibrillar structures, observed in lutoids of latex from mature bark of stimulated trees, are known as 'microhelices', so named (Gomez and Yip, 1975) because of their spring like shape. These structures were first observed by Dickenson (1965, 1969). They are occasionally found in unstimulated trees and their number increases on dilution. However, microhelices are reported to be more frequent in lutoids of tapped latex than in situ latex (Southorn and Edwin, 1968; Gomez and Yip, 1975) and are occasionally observed in latex collected from young tissue also.

As reviewed by Gomez and Moir (1979) the microhelices are approximately 1 μm in length with a diameter of 200 \AA , having a fibre width of about 50 \AA and an open hollow helix having a 300 \AA wide pitch. Dickenson (1965) suggested the formation of microhelices from microfibrils but this has been questioned by Gomez and Yip (1975).

A third type of lutoid inclusion - minute spherical particles in Brownian movement - was observed by Schoon and Phoa (1956). Later Southorn (1960, 1961) found such particles in large numbers in the bottom fraction of ultra-centrifuged latex of long rested trees and this was confirmed by Dickenson (1969). The role of such particles in latex is unknown.

Frey-Wyssling complexes

Yellow globules, in clusters in tapped latex were first noted by Frey-Wyssling (1929). The existence of such particles in groups, associated with a vacuolar body was observed by Southorn (1969) in phase contrast microscopy and he found that the individual particles are covered by a membrane: this was confirmed by electron microscopy. Dickenson (1969) named these particles, enclosed as a single structure, as Frey-Wyssling complexes.

The Frey-Wyssling complexes are more or less spherical in shape in a size range of 3-6 μm (diameter) and are bounded by a double membrane. Within the membrane there are two types of particles - large osmiophilic globules in variable numbers and a system of rope-like tubules of about 750 \AA diameter, usually embeded in a membrane bound matrix of osmiophilic nature. The complex structure of Frey-Wyssling complexes has been elaborated by Dickenson (1969) who described a series of concentric lamellae of the double unit membrane and the system of tubules and also highly folded invaginations of the inner membrane.

The Frey-Wyssling complexes are considered to have vital role in metabolic activities. Though Dickenson (1969) opined that these structures may be possible sites of rubber biosynthesis, the double membrane and presence of carotene and polyphenoloxidase in the Frey-Wyssling complex led to a tentative suggestion that it is a type of plastid.

TYPES OF LATICIFERS

Gomez (1976) made a comparative study of latex vessels collected at different stages of development or from different positions of a tree and identified mainly five types. The first type was an embryonic vessel in leaf petiole at a stage prior to the fusion of laticifer initials. This resembled a normal living parenchyma cell in cell contents, except for the presence of numerous osmiophilic rubber particles.

A typical latex vessel from the secondary phloem of green stem had osmiophilic rubber particles, ranging from 100 A° to 5000 A° in diameter. Lutoids were prominent and they contained microfibrils. This type of vessel contained mitochondria and occasionally Frey-Wyssling complexes, golgi bodies and chloroplasts.

The third type was the latex vessel collected from the secondary phloem of the tree trunk at mature age, from the inner portion of the bark, i.e., within 1 mm from the wood. This contained numerous osmiophilic rubber particles of smaller size (50 A°-2 µm) in diameter. Lutoids were present but were devoid of microfibrils. Mitochondria were also present.

The fourth type was latex vessel under tapping and had rubber particles in very large numbers. Lutoids and Frey-Wyssling complexes were common and occasionally mitochondria and endoplasmic reticulum (at the periphery) were present. Rarely, nuclei were also detected.

The last type represented senescent vessels in the outer bark. In this type, the rubber particles were comparatively larger in size and the other organelles obscured.

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