J nat Rubb Res **8(2)**, 137 – 145

Ultrastructure of Hevea *Bark on Tapping: Parenchyma Cells in Secondary Phloem*

JI-LIN WU* AND BING-ZHONG HAO*

The parenchyma cells of secondary phloem in the trunk of Hevea brasiliensis *were observed using transmission electron microscopy In an untapped tree, the axial parenchyma cells and ray parenchyma cells rather than the companion cells are mainly storage cells which contain varied reserves and show less activity in metabolism In addition to the cells with vacuolar proteins in a fibrous form reported before other axial parenchyma cells were found containing thicker diameter fibrils in the central vacuoles In a tapped tree, the parenchyma cells near the tapping cut underwent many ultrastructural changes decrease of reserves which include starch grains vacuolar proteins in the protein storing cells and phytoferritin in plastids formation of plasmatubules and related structures and the myelin-like structures occurrence of electron dense materials outside the protoplast, increased accumulation of tannins These changes were found in axial parenchyma cells and ray parenchyma cells, and not in the companion cells except for the formation of plasmatubules and related structures All the changes suggest the activation of metabolism in the parenchyma cells*

Hevea bark consists of different cells which are interrelated in a complex way With regards to latex production, the laticifers must be the most important component in the bark However, to understand the structure and function of the laticifers we should study not only the laticifers themselves but also the other components of the bark There is sufficient information on the structure of *Hevea* bark at light microscopical level[1-4] but not at ultrastructural level, except on the laticifers The ultrastructure of a type of parenchyma cells rich in proteins in secondary phloem was described[5,6] The P proteins of sieve elements and phytoferritin of plastids in secondary phloem were also studied[7,8] using electron microscopy This paper reports the ultrastructure of parenchyma cells in the secondary phloem in *Hevea* trunk and the effects of tapping on the cells

MATERIALS AND METHODS

The ten- to eighteen-year-old rubber trees of clones RRIM 600 and GT 1 with or without regular tapping, grown in the experimental fields of the South China Academy of Tropical Crops on Hainan Island were used in this study The bark samples were collected with a 1 5 cm punch 2 cm below the tapping cut in tapped trees In untapped trees, the samples were obtained at the position corresponding to that in tapped trees

For electron microscopy, the samples were immediately immersed in chilled 6% glutaraldehyde in 0 1 mol/litre phosphate buffer at pH 7 2 The samples were sub-sectioned into a smaller size after 20 min, and fixed in the glutaraldehyde solution at 4°C for 24 h before post fixation in 2% O_sO_4 in the same buffer for 6 h at room temperature They were dehydrated in an ethanol series and embedded in Epon 812 resin Ultra-thin sections were cut on a LKB V ultra-microtome, stained in uranyl acetate and lead citrate, and examined in a JEM100CX-II electron microscope

For light microscopic histochemistry, IKI reaction was used to show starch, and ferric sulphate reaction to show tannins[9] The vacuolar proteins in protein-storing cells were shown with mercury-bromophenol blue reaction on ethanol-fixed paraffin sections[10]

* Laboratory of Tropical Crop Biology South China Academy of Tropical Crops Daxian Hainan 571737 People's Republic of China

RESULTS

Ultrastructure of Parenchyma Cells

The main part of the bark to be tapped is the secondary phloem where the various kinds of parenchyma cells are present (*Figure 1*). As in other dicotyledonous woody plant[11], the parenchyma cells in *Hevea* can be broadly classified on the basis of their structure and function into axial parenchyma cells, companion cells and ray parenchyma cells.

Axial Parenchyma Cells

In *Hevea,* as in the other woody seed plants, the axial parenchyma cells may have specialised contents and it is from these contents that some of the cells had their names derived in literature. Tannin cells, for example, refer to the cells containing tannin materials[2] and the protein-storing cells describe the cells rich in vacuolar proteins[5,6]. We propose to categorise axial parenchyma cells as follows.

General axial parenchyma cells. These cells (*Figure 1*) are characterised by having large central vacuoles and no specialised contents. The cells contain the typical organelles of plant cells, such as nucleus, endoplasmic reticulum, ribosomes, dictyosomes, mitochondria, plastids, microbodies, spherosomes, *etc.* in the thin layer of peripheral cytoplasm. The plastid and microbody are shown in *Figures 2* and *3.*

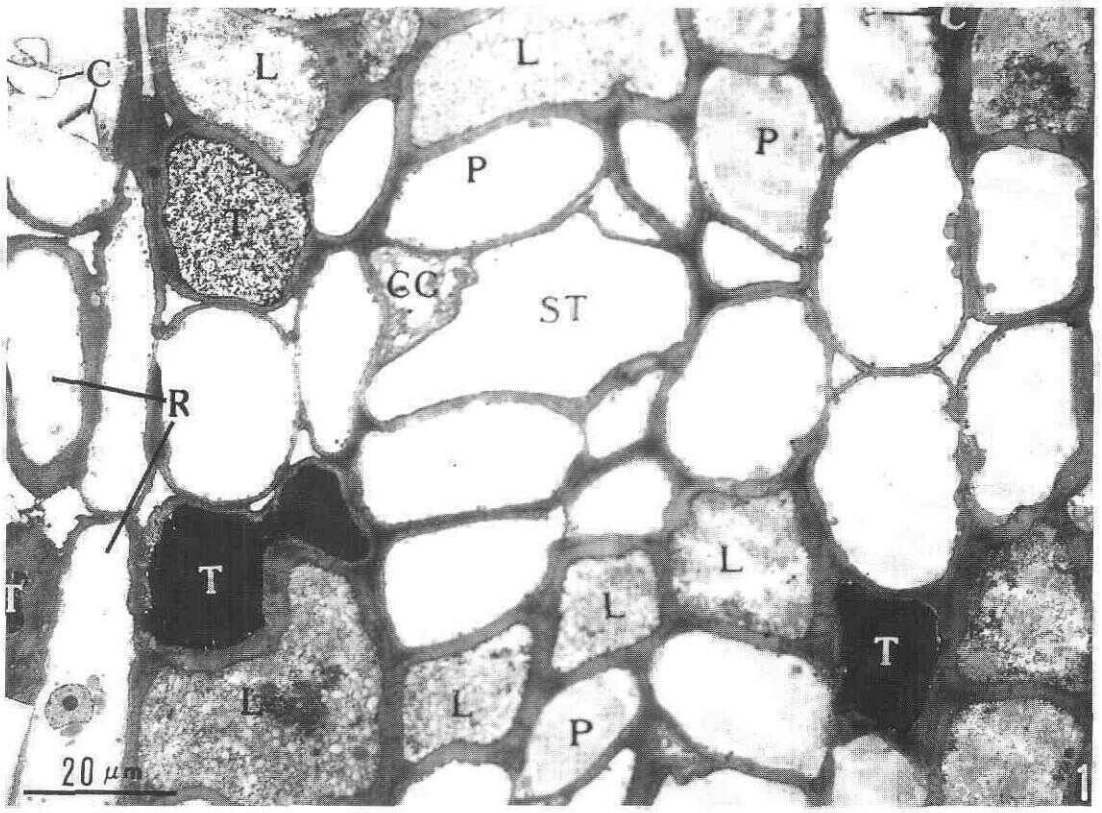

Figure 1. Untapped tree. Transection of the inner bark showing various kinds of parenchyma cells. R, ray parenchyma cell; P, protein-storing cell; T, tannin cell; CC, companion cell; no label for general axial parenchyma cell. C, crystal; L, laticifer; ST, sieve tube.

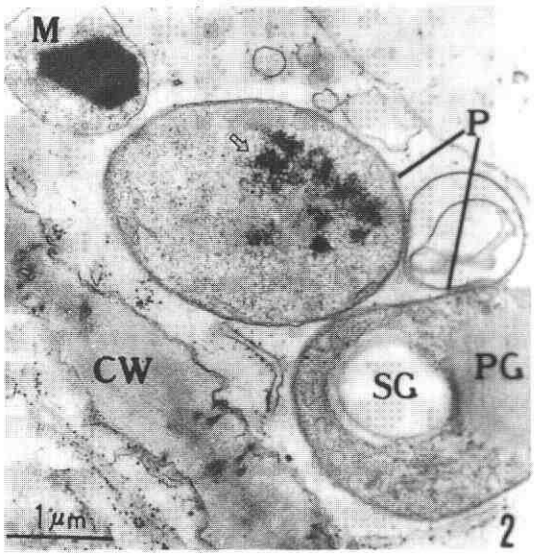

Figure 2. Untapped tree. General axial parenchyma cell. P, plastid; PG, plastoglobulus; SG, starch grain; arrow, phytoferritin; M, microbody; CW, cell wall.

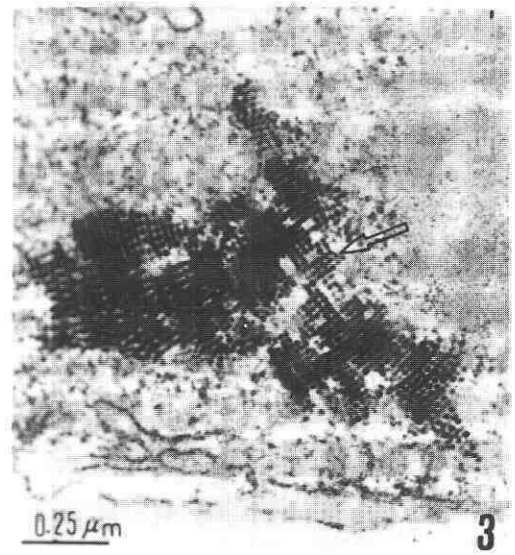

Figure 3. Untapped tree. Magnified phytoferritin (arrow).

The plastids usually possess poorly developed internal membrane systems and no grana-fretwork system is formed. Starch grains, plastoglobuli and phytoferritin are usually found in the plastids and all these three components of plant cells are considered to be reserves[12]. In addition, the spherosomes are often present in the cells and this organelle of plant cells is also considered as a storage organelle in which the reserve is lipid[12].

Protein-storing cells. These cells (*Figure 1*) are distributed in the functional and inner non-functional phloem of the tree trunk. The unique feature of the cells is the proteins in a fibrous form present in the central vacuoles[5,6]. Except for the vacuolar proteins, no obvious difference was found between the protein-storing cells and general axial parenchyma cells. Besides the protein-storing cells, there are a few cells containing vacuole inclusions which appear in thicker fibrils (*Figure 4*) and are believed to be proteinaceous substances.

Crystal cells. Bobilioff[1] described the crystals of calcium oxalate present in the cells of almost all tissues of *Hevea*. The crystals were observed in some axial parenchyma cells in this study (*Figure 1*). The crystal cells possess the same structural features as the general axial parenchyma cells except that the crystals are in the central vacuoles.

Tannin cells. All parenchyma cells in secondary phloem with the exception of companion cells may contain tannins. The tannins are often accumulated in the parenchyma cells next to the laticifers (*Figure 1*). In the samples with glutaraldehyde and osmium tetroxide fixation, electron-dense materials were observed under the electron microscope in the cytoplasmic vesicles and central vacuoles of some cells. These cells correspond to the cells which were demonstrated to be tannin cells by the ferric sulphate test under the light microscope and the electron-dense materials are believed to be tannins. The tannin cells usually exhibit a denser osmiophilic cytoplasm. The cytoplasm even becomes dark and thick in some cells, indicating that a great amount of tannins are accumulated in the cytoplasm (*Figure 5*).

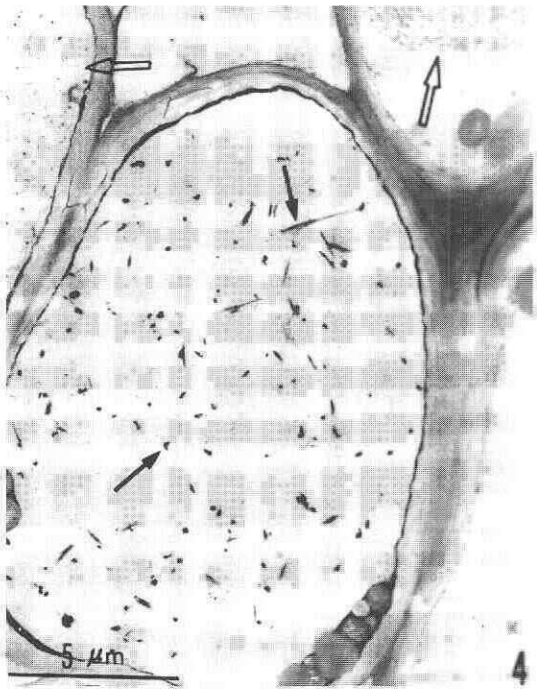

Figure 4. Untapped tree. Protein-storing cell (middle) with thicker fibrils (black arrow) and protein-storing cell with fine fibrils (white arrow).

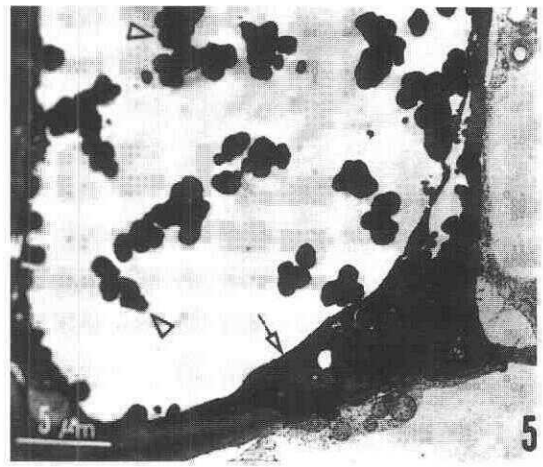

Figure 5. Untapped tree. Tannin cell in axial parenchyma cells. Note electron-dense material (arrow head) in central vacuole and dark cytoplasm (arrow). CW, cell wall.

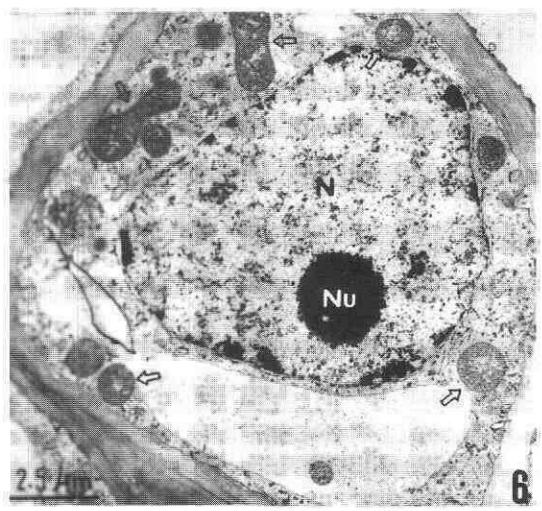

Figure 6. Untapped tree. Companion cell. N, nucleus; Nu, nucleolus. Note numerous mitochondria (arrow).

Companion Cells

The outstanding features of the companion cells (*Figures 1* and *6*) are the smaller vacuoles, dense protoplasts and close association with sieve elements. In transection, the companion cells are much narrower than the sieve elements and each companion cell is in contact with a sieve element (*Figure 1*). Many mitochondria were observed in the cells (*Figure 6*). There are less plastids and they have neither starch grains nor phytoferritin. The companion cells and sieve elements are interconnected typically by numerous branched plasmodesmata.

Ray Parenchyma Cells

These cells (*Figures 1, 7* and *8*) resemble the axial parenchyma cells in their general structure. The cells have reserves, starch grains, plastoglobuli and phytoferritin in plastids, and lipid in spherosomes, but no vacuolar proteins like those found in the protein-storing cells are present in the cells. As

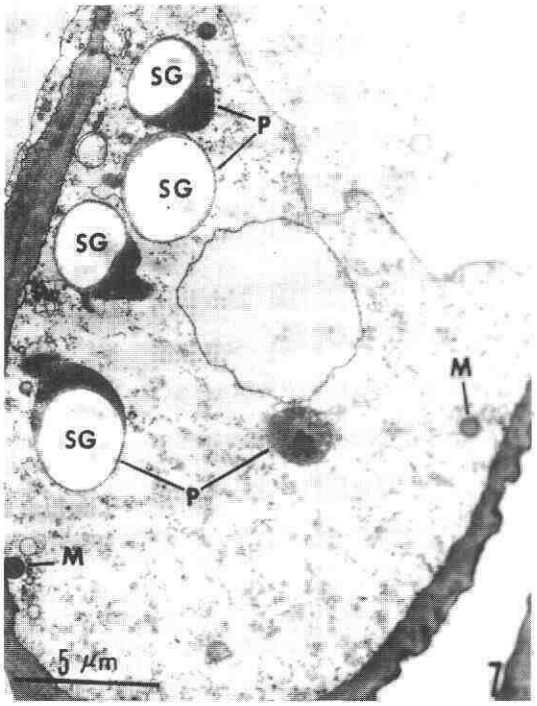

Figure 7. Untapped tree. Ray parenchyma cell. Note plastid (P) with starch grain (SG), plastoglobulus (PG) and phytoferritin (arrow head), spherosome (Sp) and mitochondrion (M).

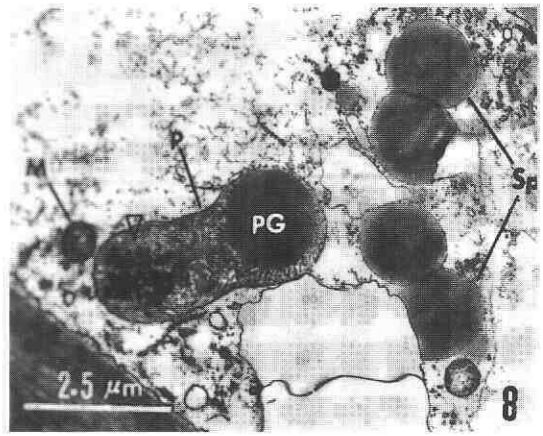

Figure 8. Untapped tree. Ray parenchyma cell. Note plastid (P) with starch grain (SG), plastoglobulus (PG) and phytoferritin (arrow head), spherosome (Sp) and mitochondrion (M).

in the axial parenchyma cells, there are crystal cells and tannin cells in the ray parenchyma cells (*Figure 1*).

Effects of Tapping

Compared with the parenchyma cells in an untapped tree, the cells near the tapping cut in a tapped tree showed some changes in their ultrastructure.

There were less reserves in the parenchyma cells near the tapping cut. Fewer and smaller starch grains were observed in the parenchyma cells, especially in the ray parenchyma cells. The decreased starch is clearly shown in the light micrographs (*Figures 9* and *10*). It has been indicated[8] that phytoferritin was rarely found in plastids of parenchyma cells near the tapping cut. The reduced amount of vacuolar proteins in protein-storing cells near the tapping cut has also been described[5] and this is shown in the light micrographs (*Figures 11* and *12*). *Figure 13* shows a ray parenchyma cell which contains no reserves but many mitochondria.

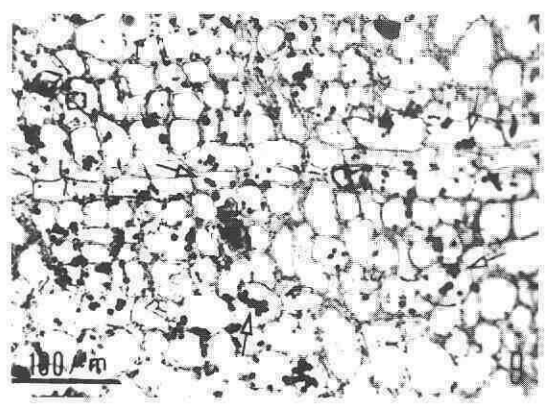

Figure 9. Transection light micrographs of bark, IKI test showing starch grains. Note numerous starch grains (arrow) in untapped tree.

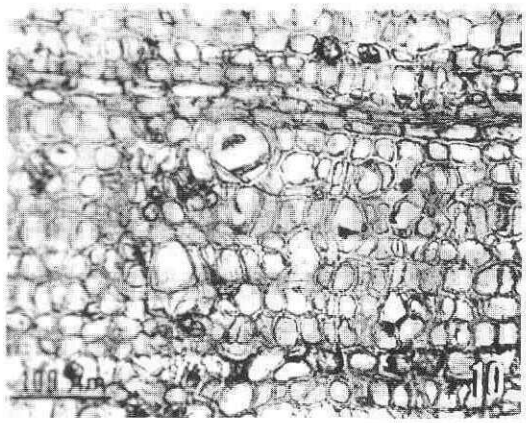

Figure 10. Transection light micrographs of bark, IKI test showing starch grains. Note numerous starch grains (arrow) in untapped tree.

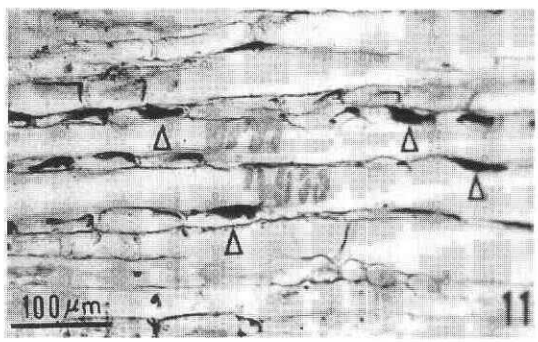

Figure 11. Longitudinal section light micrographs of bark. Mercury-bromophenol blue test showing proteins (arrows) in protein-storing cells. Note a great mass of proteins in the cells in the untapped tree.

Tubules of about 30 nm in diameter and vesicles of varied sizes were often found between the plasma membrane and cell wall. The tubules may be identified as plasmatubules[13], the tubular evaginations of the plasma membrane in many plants, and the vesicles may be the structures related to the plasmatubules. *Figure 14* shows the plasmatubules in transection.

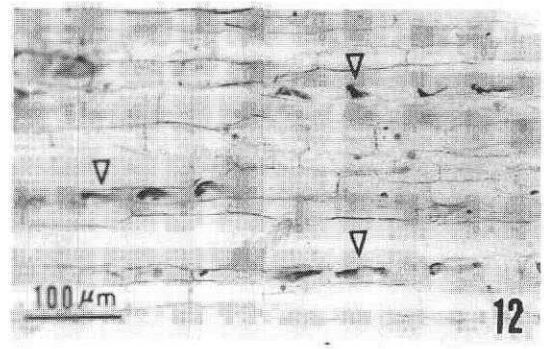

Figure 12. Longitudinal section light micrographs of bark. Mercury-bromophenol blue test showing less proteins in the tapped tree.

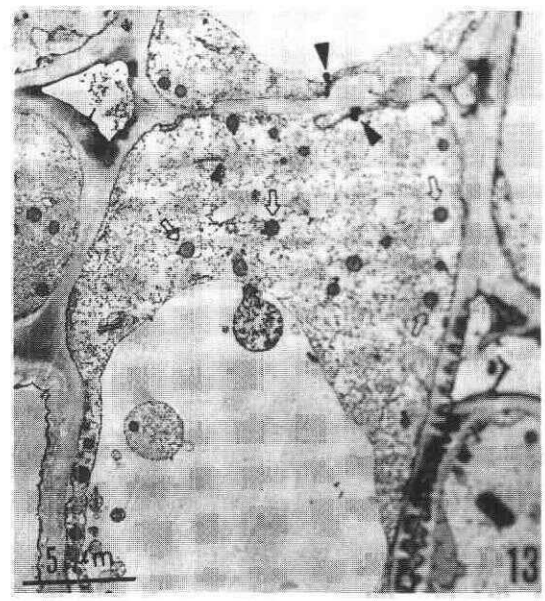

Figure 13. Tapped tree. Ray parenchyma cell. Note mitochondria (arrow) and myelin-like structures (arrow head).

The myelin-like structures (*Figures 13* and *15*) were often found in the parenchyma cells in association with the varied membrane structures of the cells.

The electron-dense materials in amorphous and grainy forms were found between the

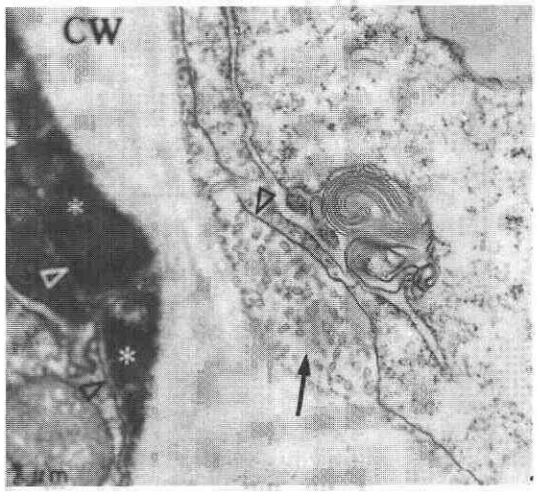

Figure 14. Tapped tree. Axial parenchyma cell showing plasmotubules (arrow) in transection and electron-dense materials (star) between cell wall (CW) and plasmolemma (arrow head).

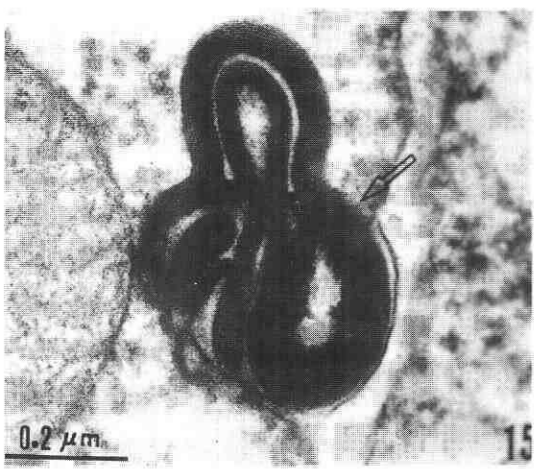

Figure 15. Tapped tree. Myelin-like structures (arrow) in axial parenchyma cell.

plasma membrane and cell wall (*Figures 14* and *16*). The distribution of the grainy materials suggests that these were incorporated into the cell wall and accumulated in the inter-

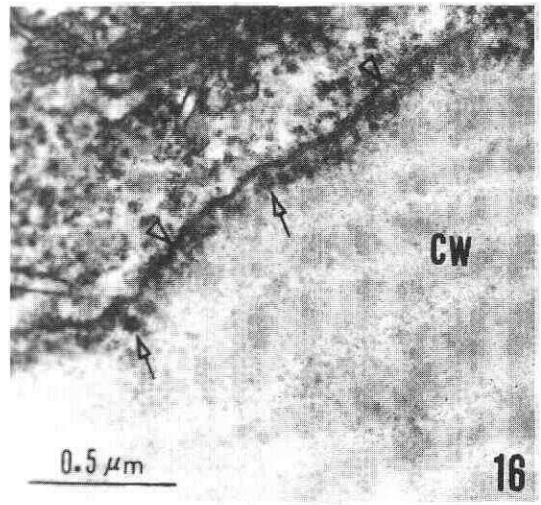

Figure 16. Tapped tree. Electron-dense grains between cell wall (CW) and plasmolemma (arrow) in axial parenchyma cell.

cellular layer. It is unknown whether the amorphous materials are also incorporated into the cell wall.

One of the most obvious changes of the parenchyma cells caused by tapping is the accumulation of tannins in the cells. This can be clearly seen in the light micrographs (*Figures 17* and *18*).

The changes caused by tapping described above occurred mainly in the axial parenchyma cells and ray parenchyma cells. No obvious changes except the formation of plasmatubules and related structures were found in the companion cells.

DISCUSSION

A general survey was carried out on the ultrastructure of parenchyma cells in the secondary phloem in *Hevea* trunk. The companion cells have many organelles especially mitochondria but lack reserves. These facts show that the cells are active in metabolism. In contrast with the companion cells, the other parenchyma cells are mainly storage cells, which contain varied reserves and appear to be less active in metabolism.

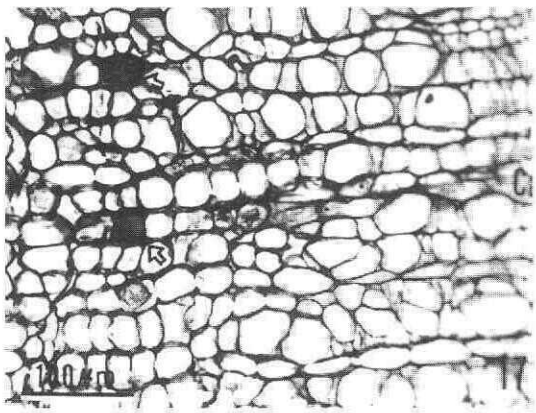

Figure 17. Transection light micrographs of bark. Ferric sulphate test showing tannin cell (arrow) in untapped tree Ca, cambium.

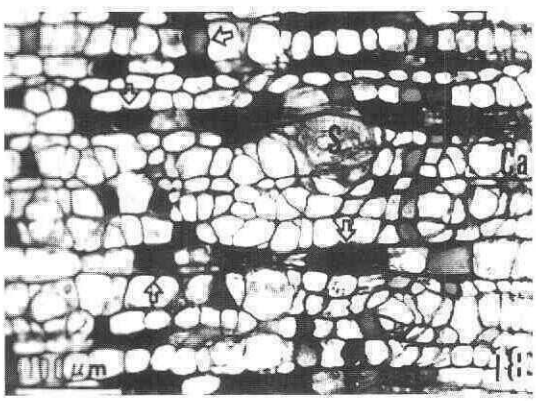

Figure 18. Transection light micrographs of bark. Ferric sulphate test showing tannin cell (arrow) in tapped tree. Ca, cambium.

The presence of protein-storing cells is a specific characteristic of the parenchyma cells in *Hevea*. In addition to the protein-storing cells reported before[5,6], other cells containing thicker fibrils in the central vacuoles were also found in this study. These cells might be another type of protein-storing cell. The biochemistry of the vacuolar proteins in both types of protein-storing cells remains obscure. The function of the proteins also needs further study although it was suggested that the proteins were used as reserves[5].

The axial phloem parenchyma cells in contact with laticifers have been described as the parenchymatous sheath for the laticifers[4,14]. However, these cells appear to have the same ultrastructure as the parenchyma cells found away from the laticifers. All kinds of axial parenchyma cells including general axial parenchyma cells, protein-storing cells and tannin cells are seen in contact with laticifers although the tannin cells are mainly arranged around the laticifers (*Figure 1*). The laticifers are also located next to ray parenchyma cells (*Figure 1*). These cells cannot be distinguished from the ray parenchyma cells found away from the laticifers.

A series of changes in ultrastructure were found in the parenchyma cells near the tapping cut. All the changes suggest the activation of metabolism in the cells. First, the decreased reserves including starch grains, phytoferritin and vacuolar proteins in the protein-storing cells show that they were consumed by activated metabolism. Second, the many plasmatubules found in the companion cells and other parenchyma cells might indicate enhanced phloem (sieve element) and intercellular translocation since the plasmatubules have often been observed in plant cells that are active in the translocation of solutes[13]. Third, the myelin-like structures which are membranes of a specific form occurring extensively in the cells might represent an excessively active biogenesis of the membrane. Finally, the accumulation of tannins in the cells and electron-dense materials outside the protoplasts must be the result of the activation of the metabolic pathways related to these substances.

The latex biosynthesis in laticifers caused by tapping probably account in part for the activation of metabolism in the parenchyma cells. The consumption of the reserves and enhanced translocation appear to be essential for the latex biosynthesis. In this context, Hebant and Fay[14] suggested that since the majority of the laticifers are located outside the conducting phloem, the ray parenchyma

cells might play an important role in radial transport of nutrients. The authors also showed that higher respiratory and phosphatase activities were frequently detectable within ray cells although no comparison was made between tapped and untapped trees.

However, some changes caused by tapping may have no direct relationship to latex formation. The tannins might have been induced by wounding as the formation of these substances (phenolic compounds) is often stimulated in the cells of wounded plant tissues[15]. In addition, activation of metabolism is also a general characteristic of wound response of plant tissues[15]. Thus many of the ultrastructural changes of the cells observed near the tapping cut may be considered a wound response.

ACKNOWLEDGEMENTS

This study was supported by the National Science Foundation of China. We thank Tan Haiyan for his assistance. Thanks are also due to Ao Ningjian, Zhou Huilin, and Zhou Gang (South China Tropical Crops Testing Center) for assistance on electron microscopy

Date of receipt: November 1992
Date of acceptance: March 1993

REFERENCES

1 BOBILIOFF W (1923) *Anatomy and Physiology of* Hevea brasiliensis *Part I Anatomy of* Hevea brasiliensis Art Institut Orell Fussli Zurich

2 GOMEZ J B (1982) *Anatomy of* Hevea *and its Influence on Latex Production Malaysian Rubber Research and Development Board Monograph No 7*

3 HAO BING ZHONG AND WU, JI LIN (1986) Some Aspects of Structure and Development of *Hevea brasiliensis* in Relation to Latex Production *Proc IRRDB Rubb Physiol Exploitation Meeting 1986 Hainan, China*, 85

4 FAY, E AND JACOB J L (1989) Anatomical Organization of the Laticiferous System in the Bark *Physiology of Rubber Tree Latex, (d'Auzac J, Jacob J L, and Chrestin H eds)*, Ch 1 Boca Raton, Florida CRC Press

5 WU, JI-LIN AND HAO, BING ZHONG (1987) Protein storing Cells in Secondary Phloem of *Hevea brasiliensis Kexue Tongbao*, **32,** 118

6 WU, JI-LIN AND HAO, BING ZHONG (1987) Ultrastructure and Differentiation of Protein-storing Cells in Secondary Phloem of *Hevea brasiliensis* Stem *Ann Bot*, **60,** 505

7 WU, JI LIN AND HAO, BING ZHONG (1990) Ultrastructure of P protein in *Hevea brasiliensis* during Sieve-tube Development and after Wounding *Protoplasma* **153,** 186

8 WU, JI-LIN AND HAO, BING-ZHONG (1990) Phytoferritin in Plastids of Secondary Phloem of *Hevea* Stem *Chin J Trop Crops* **11(2),** 27

9 JENSEN, W A (1962) *Botanical Histochemistry* Ch 9 San Francisco and London W H Freeman & Co

10 PEARSE A G E (1960) *Histochemistry Theoretical and Applied*, 792 London J &A Churchill Ltd

11 ESAU K (1977) *Anatomy of Seed Plants*, 2nd edition, Ch 11 New York John Wiley and Sons

12 HALL, J L (1983) Cells and their Organization Current Concepts *Plant Physiology (Steward, F C ed)*, Vol VII, 3 New York Academic Press

13 HARRIS, N, OPARKA, K J AND WALKER-SMITH, D J (1982) Plasmatubules an Alternative to Transfer Cells? *Planta*, **156,** 461

14 HEBANT, C AND DE FAY E (1980) Functional Organization of the Bark of *Hevea brasiliensis* (Rubber Tree) a Structural and Histoenzymological Study *Z pflanzenphysiol*, **97,** 391

15 LIPETZ, J (1970) Wound-healing in Higher Plants *Int Rev Cytol* **27,** 1