4

Discussion
4.1 General discussion

Secretion in broad sense, takes place in all plant cells. The formation of cell wall and cuticle, suberization, wax deposition, and the migration of specific substances from the cytoplasm into the vacuole, all represent process of secretion (Fahn, 1979). There are also distinct cells, tissues or complex structures which are specialized to secrete specific substances. They may be present in various organs of the plant, and their presence may be characteristic of the species. Cells and tissues producing gums and resins can be categorized under this specialized group of secretory structures.
In spite of the vast literature now available on gums and resins, so far there is no conclusive answer as to what exact function they accomplish for the plant's metabolism. Conventionally the gums and resins are grouped under secondary plant products (Stephen, 1980; Wiermann, 1981). The secondary plant products are considered as those compounds that have no recognizable role in the maintenance of fundamental life process in the organism that synthesizes them (Bell, E.A., 1981).

Conn (1981) is critical towards the usage of the adjective "secondary" as it implies that they are "non-primary" or unimportant. Today the abundant evidence supports the belief that many secondary plant products play an indispensable role in maintaining a species during the course of evolution (Conn, 1981). Although plants seemingly accumulate their products in specialized cells or tissues without further metabolism, the sophisticated analytical techniques and the use of radioactive tracers today permit the measurement of the dynamics of secondary products' metabolism. The recent systematic investigations have repeatedly shown that these plant constituents are not metabolically inactive and inert end products, but rather they are subject to turn over and degradation (Barz and Köster, 1981).

However, to date there is no evidence to show that the gum or resin secreted into the ducts or cavities is absorbed
and utilized by the plant for its metabolism. Nevertheless, the close relation between certain resin terpenoids and gibberellins poses an emphatic question regarding the metabolic status of resins (Dell and McComb, 1978a). The resin component, Kaurenoic acid from Ricinocarpys styllosus causes the effect of gibberellin treatment on the dwarf maize (Katsumi et al., 1964). It clearly indicates that the resins need not necessarily be terminal products, incapable of further modification by plant metabolism.

As it is very difficult to experiment upon the effects of their presence or absence in the plant body, many of the functions attributed to internal resins are rather speculative (Dell and McComb, 1978a). The fact that, the gums and resins ooze out upon an injury and seal the wound, tends many workers to believe that the exudates have some function in wound healing and to provide antiseptic protection (Schery, 1954; Dell and McComb, 1978a). They are also believed to help in chemical or physical retention of water, thus enabling the plant to resist dessication in dry habitats (Schery, 1954; Kramer and Kozlosvki, 1979).

Evidence for a role of resins in the prevention of insect attack is very strong (see Dell and McComb, 1978a). The attack by pine needle miner is correlated with the number and size of resin ducts in the host. Pine weevils feeding in the cortex avoid resin ducts and cease feeding when they
cannot bypass them; and western white pine, relatively resistant to attack has more outer cortical resin ducts than the more susceptible eastern white pine.

Another belief is that, they store "waste products" of metabolism providing a mechanism for removing toxic substances. The frequent association of secretory ducts with vascular bundles led to believe that they serve for the reception of useless waste products which travel towards the vascular tissue. However, chemical character of the contents of secretory ducts hardly accords with this notion (Haberlandt, 1914). Moreover, the resins are synthesized by duct cells, and their rate of synthesis is not related to the general metabolic turnover (Fahn, 1979). Unless and until the movement of secondary metabolites into the duct or into the secretory cells, from the various tissues that are engaged in active metabolism is established, their role in excretion is questionable.

The present investigation, however, supports the concept of biochemical defence as the major function of gums and gum-resins. Bombax ceiba and Ailanthus excelsa do not have normal gum/gum-resin producing system in their wood or bark, but they develop traumatic cavities in secondary xylem in response to infection. The gum or gum-resin cavities develop de novo in the traumatic tissue (barrier zone) and ramify in tangential directions giving maximum
outlay in the periphery of secondary xylem. It is significant to note that the plant resins are a mixture of compounds typically including flavonoids, terpenoids and fatty acids. The antifungal property of flavonoids is well established (Harborne, 1980). Even though, the exudate of *B. ceiba* is a pure gum, it contains a considerable proportion of phenolic compounds (Anonymous, 1972). The exudate in the cavities may provide biochemical defense, while the traumatic tissue along with the vascular occlusions and tyloses provide a physical barrier against the pathogens.

The characteristic tangential anastamosis of ducts or cavities, observed in a large number of plants, strongly support their possible involvement with protective function. The five species investigated by me show a unique character—their ducts or cavities anastamose only in tangential direction. Tangential anastamosis is characteristic of gum/gum-resin containing structures in *Anogeissus latifolia* (Ghosh and Purkayastha, 1959); *Eucalyptus obliqua* (Skene, 1965); *Rhus glabra* (Fahn and Evert, 1974); *Commiphora wightii* (Setia, 1976); *Garuga pinnata, Foringa oiefera* (Subrahmanyam, 1981); *Lannea coromandelica* (Verkaiah and Shah, 1984); *Azadirachta indica* (Nair *et al*., 1985); *Citrus* sps. (Gedalovich and Fahn, 1985); *Acacia catechu, Melia azedarach* and *Terminalia bellirica* (Pandya, personal communication). Even when the cavities are present in two or more adjacent tangential bands, as
in *A. excelsa* and *Sterculia urens*, anastamosis occurs only in a tangential plane between the cavities of a single row.

The three hypothetical diagrams (Fig. 226 A-C) demonstrate how tangential anastamosis increases the efficiency of the defense mechanism. The diagrams represent three possible ways of anastamosis between the ducts arranged in two tangential rows in the outer secondary xylem. It clearly shows that while models A and B give easy access to the invading pathogens, model C gives maximum outlay of the ducts in the periphery and gives the best possible protection to the internal tissue.

4.2 Distribution of ducts and cavities

In *Vateria* stem, gum-resin ducts are present in the pith, while ducts and cavities are present in the secondary xylem. In *Garcinia*, ducts are present in pith and phloem, and ducts and cavities are present in the cortex. While the ducts or cavities in the phloem or xylem of *Vateria* and *Garcinia* anastamose tangentially sometimes forming a network, their pith ducts do not anastamose and they run parallel to each other. In *Terminalia crenulata* (Setia, 1976) and *Boswellia serrata* (Subrahmanyam, 1981), the pith ducts remain unbranched and parallel to each other while ducts in secondary phloem anastamose. The distribution pattern of ducts varies in different tissues. As already mentioned, the main function of ducts or cavities in the outer secondary...
xylem or bark could be to provide peripheral protection. But pith being an internal tissue, the aspect of peripheral protection does not arise and hence their anastamosis is absent. In *V. indica* when the tip of the seedling is cut off, the gum-resin oozes out and covers the exposed surface of the stem. In the young stem, thus pith ducts provide a sealing and/or protective mechanism.

Pith ducts in *Garcinia* are discontinuous, whereas, in *Vateria* they are continuous. But it could not be ascertained whether the same duct is continued from the base of the stem upto the apex. Pith ducts in *Vateria* and pith and cortical ducts in *Garcinia* have their continuations in the leaves.

Continuity of ducts from the stem into the leaf is also observed in many other plants, viz. *Boswellia serrata*, *Garuga pinnata* (Subrahmanyan, 1981); *Ailanthus excelsa* and *Lannea coromandelica* (Venkaiah, 1982). In *Vateria* leaf there are several ducts in the mid-rib and one duct each in the lateral veins. The duct of the lateral vein joins with a duct of the mid-rib, and all ducts of the mid-rib merge together at the base of the petiole to continue with the pith duct. Thus a continuous duct system is maintained with the stem and the whole leaf. When we consider that the gum-resin is produced by the epithelial cells surrounding the duct, the significance of such an elaborate continuous system is not
obvious. Such a continuous system may facilitate the exchange of gum-resin or its precursors between the photosynthesising leaf and the stem.

4.3 Development of ducts and cavities

The gum-resin ducts in the pith and leaf of *U. indica* and in stem, leaf and root of *G. cambogia* initiate schizogenously (by splitting apart of the cells at middle lamella). Schizogenous development of duct is common in members of the family, Anacardiaceae (Fahn and Evert, 1974; Nair, Venkaiah and Shah, 1983; Venkaiah and Shah, 1984). Similar initiation of duct is also observed in *Commiphora wightii* (Setia et al., 1977), *Boswellia serrata*, *Garuga pinnata* (Subrahmanyan, 1981) and *Ailanthus excelsa* (Venkaiah, 1982).

Cavities in *Vateria* and *Garcinia*, and ducts in secondary xylem of stem and root in *Vateria* develop lysigenously (by actual break down or lysis of some of the cells). Lysigenous development of duct is reported in *Sterculia urens* (Shah and Setia, 1976) and *Mangifera indica* (Joel and Fahn, 1980a).

Even though the initiation of duct is schizogenous, lysis of some epithelial cells is always observed during the continued duct development in *U. indica*. Hence their total development is schizo-lysigenous. There is no consistency in the manner of duct initiation even in different tissues of the same organ of the plant. Ducts are initiated
schizogenously in the pith and lysigenously in the secondary xylem of *Vateria*. A similar situation is observed in *Lannea coromandelica* (Venkaiah and Shah, 1984), where ducts develop schizogenously in the primary phloem, pith and xylem rays, and lysigenously in the secondary phloem and phelloderm.

4.4 Ethephon induced exudation

Ethephon (etherel, CEPA) is an ethylene releasing chemical which is stable in acidic form but breaks down in the plant tissue or in the presence of a base with the release of ethylene (Warner and Leopold, 1969). Ethylene is known to promote various types of exudations like guttation, gummosis and latex flow (Abeles, 1973). Ethephon induced gummosis or gum-resinosis has been recently reported in several plants (Bradely et al., 1969; Hillis, 1975; Greenwood and Morey, 1979; Nair et al., 1980, 1985; Olien and Bukovac, 1982; Bhatt and Shah, 1985).

Administration of ethephon induced copious exudation of gum/gum-resin in *B. ceiba, S. urena* and *A. excelsa*. The optimum concentration of ethephon for maximum yield is not uniform in all the plants.

The ethephon releases ethylene at a minimum pH of 3.5 and the rate of ethylene formation increases with the increasing pH (Abeles, 1973). Plant tissues of different acidity may show different capacities for ethylene evolution.
(Warner and Leopold, 1969), and this may explain the varying degree of response of the plants in the rate of exudation upon ethephon treatment.

As it has become abundantly clear that ethylene is involved in the induction or enhancement of exudation, it will be helpful to examine the various factors concerning the synthesis and control of ethylene in plant body.

Plant tissues produce ethylene and its synthesis is greatly enhanced due to various stresses (Abeles, 1973). The trauma which promotes the ethylene synthesis may be chemicals, insect damage, temperature extremes, drought, radiation, disease and mechanical wounding. It is interesting to note that most of the above factors are responsible for enhanced gum or resin exudation in plants.

Adams and Yang (1979) have shown that ethylene biosynthesis proceeds in the following sequence:

\[ \text{S-adenosylmethionine (SAM)} \rightarrow 1\text{-amino-cyclopropane-1-carboxylic acid (ACC)} \rightarrow \text{ethylene}. \]

Several workers have confirmed that ACC serves as an intermediate also in the synthesis of stress ethylene, and the increased production of stress ethylene is the result of an increase in the activity of the enzyme, ACC synthase, which catalyzes the conversion of SAM to ACC (Yu and Yang, 1980; Hoffmann et al., 1982;
Hoffmann and Yang, 1982). In *Cucumis melo* (Hoffmann and Yang, 1982) excision of fruits induced an increase in both ACC synthase and the enzyme converting ACC to ethylene (EFE, ethylene forming enzyme). It can be presumed that the stress-enhanced exudation in plants is mediated by accelerated production of ethylene synthesised through a similar pathway.

The exogenously provided ethylene can also promote autocatalytic formation of ethylene in plant body (Leopold, 1972). The role of ethylene in autocatalytic ethylene production is supposed to be in promoting the synthesis of ACC synthase and the enzyme catalyzing the conversion of ACC to ethylene (Hoffmann and Yang, 1982). So ethephon in addition to providing exogenous ethylene, also serves to accelerate the ethylene producing system of the plant.

Previous works in our laboratory showed that paraquat, a herbicide induced or accelerated copious exudation of gum/gum-resin in some trees (Subrahmanyam, 1981; Venkaiah, 1982; Nair et al., 1985). Here it is significant to note that ethylene has been shown to act as the intermediate in a number of effects attributed to several herbicides (Abeles, 1973). Herbicides promote ethylene production, but their action is probably due to their ability to form chemical stress in the tissue. Actually, non-specific wound or stress-ethylene can be expected from the application of high concentration of almost any compound (Morgan, 1976).
In *A. ceiba* gum exudation was considered as a disease symptom (Dymock *et al.*, 1890; Anonymous, 1972). Ethephon treatment induced the exudation of a large amount of gum, the physical nature of which agrees with the description of mocharas. Similarly in *A. excelsa* copious exudation of gum-resin was induced by fungal attack and ethephon treatment.

Ethephon administered to apricot also induced the exudation of a large amount of gum similar to that caused by severe bacterial infection injury (Bradely *et al.*, 1969). Recently Gedalovich and Fahn (1985) reported development of gum cavities in the secondary xylem of *Citrus* upon artificial inoculation with the fungus, *Phytophthora citrophthora*. Attack by microorganisms is enlisted as one of the factors inducing or enhancing gummosis or resinosis in plants (Agrios, 1978; Mehrotra, 1980). The evidences strongly indicate the involvement of ethylene in the infection-induced exudation. A large number of microorganisms are known to produce ethylene in culture (Mahadevan, 1984). It is also possible that the stress-ethylene produced by the plant due to infection is involved in the process. Mahadevan (1984) lists a number of plants in which ethylene evolution has been reported in host-parasite interaction. The increased synthesis of ethylene upon microbial attack may help the host plant to resist the infection. Differences have been observed in the rate of ethylene synthesis in the case of plant tissues that
are either resistant or susceptible to specific pathogens (Sequeira, 1973). In the resistant reaction, rapid ethylene evolution stimulates the synthesis of phytoalexins and other antifungal or antibacterial compounds, via increased activity of the enzyme, phenylalanine ammonia lyase. Further, ethylene treatment results in increased peroxidase activity in plant tissues, and such increase has been frequently correlated with the plant's resistance to microbial invasion (Stahmann et al., 1966; Sequeira, 1973). It is also reported that application of ethylene influences disease symptoms characteristic of those induced by parasites (Mahadevan, 1984). So when we take into account all these information, and if we consider that the increased exudation in infected plant is intermediate by ethylene, then the question whether the increased ethylene is produced by the pathogen, or by the host plant or by the interaction of both, remains to be answered. As Dell and McComb (1978a) point out, it is unfortunate that modern works on plant pathology scarcely mention the occurrence of resins, and much remains to be known on the pathological and biochemical aspects of gum and resin synthesis.

4.5 Induced gum/gum-resin producing tissue systems

Ethephon administration caused the development of traumatic gum/gum-resin cavities in the secondary xylem of B. ceiba, S. urens and A. excelsa. Traumatic development of gum ducts or cavities in the secondary xylem, due to mechanical injury
or ethephon treatment is reported in several other plants like *Anogeissus latifolia* (Ghosh and Purkayastha, 1959); *Sterculia urens* (Purkayastha, 1959); *Eucalyptus obliqua* (Skene, 1965); *Prosopis glandulosa* (Greenwood and Morey, 1975); *Azadirachta indica* (Nair et al., 1985) and *Citrus* spp. (Gedalovich and Fahn, 1985). In *B. ceiba*, *A. excelsa* and *S. urens* the cavities are developed in the newly formed traumatic parenchyma in the xylem. In many trees anatomical response to wounding is characterized by the formation of anomalous parenchyma cells by uninjured cambium around the wound (Shigo and Hillis, 1973; Rademacher et al., 1984). The nature of the traumatic parenchyma fits to the definition of a barrier zone, which is a protective tissue formed in response to infection as well as to mechanical wounding and serves to isolate the necrotic sapwood from the living cambium (Tippett and Shigo, 1981). Trees once invaded by vascular pathogens initiate various resistance mechanisms such as tyloses or gum formation to impede the vertical spread of the fungus propagules. The barrier zone limits the lateral spread of pathogens and favours the survival of the cambium. The absence of vessels in the traumatic tissue agrees with the observation of Mulhern et al., (1979) and Rademacher et al., (1984) that the barrier zone is a non-conducting tissue. In the barrier zone, the protective features develop at the expense of reduced water transport and strength. In *B. ceiba*, cells in traumatic parenchyma
accumulate phenolics which may provide a further biochemical protection against pathogens. Hillis (1975) also observed enhanced formation of polyphenols upon ethephon treatment in the sapwood of Rhus species.

Secretion is holocrine in B. ceiba, S. urens and A. excelsa. The gum/gum-resin is formed from the disintegration products of the lysing cells. In B. ceiba and A. excelsa the cambiform cells bordering the cavities cut off derivatives into the cavity which in turn undergo lysis, augmenting the exudation. The process is continued for some time which accounts for the prolonged exudation in the plants. A similar mode of gum secretion is reported in Eucalyptus obliqua (Skene, 1955). In E. obliqua a 'peripheral cambium' is formed around the kino veins, the derivatives of which enlarge and accumulate polyphenols, and then disintegrate increasing the quantity of kino. However, B. ceiba is distinct in its characteristic pattern of cell lysis and in the development of specific tissue complexes in the traumatic parenchyma.

One common feature observed in the distribution of cavities in the secondary xylem of S. urens, A. excelsa and Vateria indica is that, the cavities do not traverse the ray cells. In S. urens, however, a few ray cells near the site of ethephon treatment showed signs of lysis, but the ray cells were the last to disintegrate. Curiously
enough, the intact multiseriate rays remain like islands amidst the ramifying system of cavities. Similar observation has been made in several other plants like *Anogeissus latifolia* (Ghosh and Purkayastha, 1959), *Eucalyptus obliqua* (Skene, 1965); *Azadirachta indica* (Nair et al., 1985); *Acacia catechu*, *Melia azedarach* and *Terminalia bellirica* (Pandya, personal communication). The significance of this observation is so far not clearly understood.

At least in ethephon treated plants, a reasonable explanation can be sought by correlating this feature with the functional status of axial and ray parenchyma cells. Axial parenchyma is primarily a storage tissue, whereas, the ray cells are actively engaged in the radial transport of water (Kramer and Kozlowski, 1979; Zimmermann, 1983). Hence it is probable that from the ray cells, the ethylene is rapidly removed and the required minimum retention of ethylene is not achieved in the ray cells so as to induce any appreciable histological response.

In *A. excelsa*, the traumatic parenchyma remains unli- gnified for some time. The cells of traumatic tissue show marked differences in its wall composition. The multiseriate rays have unliignified cells in the traumatic tissue and lignified cells in the secondary xylem. Evidently, lignification has been suppressed during the development of traumatic tissue. An explanation becomes difficult as we consider that
ethylene is involved in the formation of traumatic gum-resin cavities. Ethylene (Gross, 1977; Grisebach, 1981; Higuchi, 1981; Miller, 1985) and infection (Friend, 1976; Bell, A.A., 1981; Collendavelloo et al., 1982, 1983) are supposed to promote lignification in the plant. Lignin represents an undergradable barrier for most of the fungi and its increased production in infected plants has often been proposed as a mechanism of resistance. However, there are also isolated reports indicating inhibition of lignification by ethylene. In etiolated Pisum, ethylene inhibits xylogenesis and fiber lignification, and lignification is resumed once the ethylene is withdrawn (Goodwin, 1978). In wheat leaves, lignin was rapidly synthesised around the areas infected by non-pathogenic fungi, whereas, lignification was slower in response to pathogenic fungi (Grisebach, 1981). It was suggested that virulent pathogens inhibit the development of lignin barriers, by diverting lignin precursors into other metabolic pathways.

A diversion in the biosynthetic pathway of lignin is possible in A. excelsa. The gum-resin cavities are formed in traumatic parenchyma soon after its differentiation and the synthesis of gum-resin occurs. Flavonoids, one of the three major components of resin, and lignin have common precursors, intermediates and enzymes up to the synthesis of p-coumaric acid (Dougall, 1981). A shift in the biosynthetic
pathway of phenolics, diverting the precursors of lignin in the direction of resin synthesis can also be a plausible explanation for the suppression of lignification in traumatic parenchyma.

4.6 Ultrastructure of gum-resin ducts

Early differentiation of gum-resin ducts in *V. indica* is discussed in part 4.3.

*V. indica* and *A. excelsa* produce gum-resin, a mixture of carbohydrates and lipophilic material. As two chemically different compounds are secreted simultaneously in the duct, two different systems must be operating in the epithelial cells for their synthesis (Setia et al., 1977). *V. indica* and *A. excelsa*, however, show a marked difference in the mode of synthesis of the material and their secretion.

In the epithelial cells of *V. indica* the osmiophilic material is not observed in the plastids, mitochondria, ER or in dictyosome cisternae. The osmiophilic substance (resin) appears in the cytoplasm as globules with or without a limiting membrane. Vesicles and multivesicular bodies transport the osmiophilic material towards the plasmalemma and release the substance into the apoplast in granulocrine manner. The amount of osmiophilic substance stored in the extracytoplasmic space is prominent.
It becomes apparent that the synthesis of resinous material takes place in the cytoplasm of the epithelial cells. In Origanum dictamnus (Bosabalidis and Tsekos, 1982a), the secretory cells of the glandular scales are rich in organelles but they are not directly associated with the synthesis of essential oil, and the oil droplets arise in the cytoplasm. Synthesis of lipophilic substance in the cytoplasm without direct involvement of cell organelles is also observed in Papaver somnifera (Dickenson and Fairbairn, 1975), Plumbago capensis (Rachmilevitz and Joel, 1976), Commiphora wightii (C. mukul) (Shah et al., 1982), and Citrus deliciosa (Bosabalidis and Tsekos, 1982b, c). Experiments with labelled precursors also have proved that terpenoids may be synthesized in the cytoplasm (Dell and McComb, 1978b). But the epithelial cells are distinct from the other cells by their dense cytoplasm, large nucleus and abundance of cell organelles which are indicative of their elevated metabolic status. Histochemical and histoenzymological studies also have shown that the epithelial cells of gum or gum-resin producing ducts of many plants have higher metabolic activity (Setia and Shah, 1979; Shah et al., 1980). Thus, an indirect implication of some organelles in the secretory process cannot be ruled out.
In *A. excelsa*, the epithelial cells are characterized by abundance of cell organelles. Most of the organelles are directly or indirectly involved in secretion. The role of each organelle in the process may be examined separately.

Plastids are profusely distributed in the epithelial cells. They lack well developed membrane structures and many of them contain osmiophilic globules and/or starch grains. According to Dell and McComb (1974, 1977), plastids lacking well defined membrane structures may well prove to be a general feature of resin producing cells. The presence of osmiophilic material in the plastid matrix, similar to that found in the cytoplasm and extracytoplasmic space involves them as a possible site of resin synthesis. Plastids are indicated as a site of resin formation in a large number of plants secreting lipophilic substances (Fahn and Evert, 1974; Fahn and Benayoun, 1976; Dell and McComb, 1977; Werker and Fahn, 1981; Bosabalidis and Tsakos, 1982b, c; Pridgeon and Stern, 1983). The conspicuous sheathing of plastids by ER suggests the association of ER with the transport of the resin synthesized in the plastid to the plasmamembrane. Similar sheathing of plastids by ER is frequently observed in many types of secretory cells synthesizing lipophilic substances (Fahn and Evert, 1974; Fahn and Benayoun, 1976; Dell and
McComb, 1977; Galatis and Apostolakos, 1977; Galatis et al., 1978b; Benayoun and Fahn, 1979; Joel and Fahn, 1980b; Heslop-Harrison and Heslop-Harrison, 1981; Bosabalidis and Tsekos, 1982a, c; Pridgeon and Stern, 1983; Nair, Venkaiah and Shah, 1983). But opinion differs among the workers in regard to the significance of plastid-ER association. According to Bosabalidis and Tsekos (1982a) and Galatis and Apostolakos (1977), the significance of plastid-ER association, which becomes prominent in the secretory cells at the stage before secretion lies either in the involvement of the ER element in transfer of soluble carbohydrates or other products to and from the plastids, or in that the ER cisternae are the site of synthesis of substances required for plastid differentiation. Some workers do not imply the plastids as a site of resin synthesis in spite of the presence of osmiophilic globules in plastid matrix (Galatis et al., 1978b; Durkee et al., 1981; Bosabalidis and Tsekos, 1982a). Gunning and Steer (1975) state that in plants most of the plastids contain osmiophilic droplets (plastoglobulai) which contain plastid quinones and they may function in electron transport during the light reactions of photosynthesis in thylakoid membranes. However, in A. excelsa the plastid seems to have a direct role in the synthesis of resin, because it is abundant in the epithelial cells, and the osmiophilic globule found in the cytoplasm
in association with the plastids, and those found inside their matrix have similar electron density. The presence of abundant plastids ensheathed by ER and the absence of distinct normal membraneous system in those plastids suggest the role of this ER in secretion rather than in plastid differentiation.

Epithelial cells of both *V. indica* and *A. excelsa* show many mitochondria. This is a common observation in plant secretory cells (Lüttge, 1971; Fahn and Evert, 1974; Schnepf, 1974; Fahn, 1979; Robins and Juniper, 1980a; Werker and Fahn, 1981; Bosabalidis and Tsekos, 1982a; Nair, Venkaiah and Shah, 1983; Pridgeon and Stern, 1983). The presence of a large number of mitochondria confirms the higher metabolism of the secretory cells in relation to other tissue (Bosabalidis and Tsekos, 1982a). The contorted shape of some mitochondria is significant as it increases their surface/volume ratio which may be essential to obtain a sufficiently rapid exchange of metabolites with the cytoplasm to maintain the high rate of ATP synthesis (Robins and Juniper, 1980a).

Osmiophilic material is encountered in the mitochondria, indicating their involvement in resin synthesis. Involvement of mitochondria in the synthesis of resinous material is reported in many other plants (Fahn and Evert, 1974;
Fahn and Benayoun, 1976; Setia et al., 1977; Birchem and Brown, 1979; Joel and Fahn, 1980a; Bhatt and Shah, 1985).

Rough endoplasmic reticulum is well represented in the epithelial cells. Their association with osmiophilic globules in the cytoplasm and with other organelles strongly suggests their role in the synthesis and/or transport of the resin. Ample evidence from the literature suggests the function of ER in the synthesis and/or transport of resinous material in secretory cells (Fahn and Evert, 1974; Dell and McComb, 1974, 1977; Gunning and Steer, 1975; Thomson et al., 1976; Fahn and Benayoun, 1976; Benayoun and Fahn, 1979; Robins and Juniper, 1980c; Bosabalidis and Tsekos, 1982b; Platt-Aloia et al., 1983; Durkee et al., 1984; Bhatt and Shah, 1985).

The association of osmiophilic material with different cell organelles may indicate that either all these organelles are capable of resin synthesis or that different resin components are synthesized by different organelles (Joel and Fahn, 1980b). In Rhus glabra (Fahn and Evert, 1974) all cell organelles are involved in the synthesis and/or transport of the lipophilic material.

The role of rough ER in protein synthesis is well known (Gunning and Steer, 1975; Chrispeels, 1976). The ribosomes in the cytoplasm and in association with ER
are in polysomal configurations. It suggests a high protein synthesis which is essential for the differentiation and high metabolism of the epithelial cells. ER is also indicated as a site of carbohydrate synthesis in mucilage secreting cells (Moore and McClean, 1983).

The frequent stacking of ER close to the plasmalemma is a feature commonly observed in plant cells involved in secretion or transport of material (Gunning and Steer, 1975; Chrispeel, 1976; Robins and Juniper, 1980c; Heslop-Harrison and Heslop-Harrison, 1981; Durkee, .. 1982; Platt-Aloia et al., 1983; Nair, Venkaiah and Shah, 1983; Bhatt and Shah, 1985). The cisternae may be strategically located to collect the incoming, or alternatively to supply the outgoing molecules to the plasmalemma (Gunning and Steer, 1975; Robins and Juniper, 1980c; Bhatt and Shah, 1985). But there is also another view that, the characteristic organization and distribution pattern of ER cisternae near the site of wall deposition are suggestive of their role in cell wall formation (Chrispeel, 1976; Juniper et al., 1981). They may be directing the passage of cell wall polysaccharides or wall proteins (Juniper et al., 1981).

Occasionally some of the ER cisternae form concentric rings delimiting cytoplasmic portions. Similar ER loops are found in the secretory cells of *Pinus elliotti*. 
(Birchem and Brown, 1979) and Mangifera indica (Joel and Fahn, 1980b, c). In Pinus, the cytoplasmic elements delimited by the ER loops turn into osmiophilic amorphous substance. In Mangifera fruit (Joel and Fahn, 1980c) the ER loops form pseudo-vacuoles and function as storage bodies for mucilage. However, in A. excelsa, the function and significance of the ER rings are not clearly understood.

The dictyosomes appear very active, producing large number of vesicles. But osmiophilic material is not encountered in the dictyosome vesicles. The dictyosome derived vesicles fuse together, form large vesicles and they fuse with the plasmalemma and release their contents into the apoplast. Many studies have manifested that the dictyosomes are involved in the synthesis of the polysaccharide component of various plant secretions (Morré and Jones, 1967; Gunning and Steer, 1975; Paull and Jones, 1975, 1976; Chrispeels, 1976; Joel and Fahn, 1980a; Trachtenberg and Fahn, 1981; Schnepf and Deichgraber, 1983; Moore and McClean, 1983).

Dictyosome derived vesicles are also implicated in the contribution of polysaccharides for wall synthesis (Gunning and Steer, 1975; Rachmilevitz and Fahn, 1975; Robins and Juniper, 1980a; Parker 1984). In Ailanthus, the inner tangential wall of the epithelial cell has a loose microfibrillar structure and sloughed off
appearance which suggest that wall material is continuously removed presumably as a component of the gum. Similar observations are also reported in *Rhus glabra* (Fahn and Evert, 1974); *Commiphora wightii* (Setia *et al.*, 1977); *Anacardium occidentale* (Nair, Venkaiah and Shah, 1983) and *Mangifera indica* (Bhatt and Shah, 1985). Thus, by replenishing the wall material, the dictyosomes may indirectly contribute to the gum synthesis in *A. excelsa*.

Dictyosome derived coated vesicles are frequently observed in the cytoplasm and in the plasmalemma invaginations. In secretory cells, the origin of coated vesicles from dictyosomes has been described also by Bennett and Newcombe (1966); Unzelman and Healay (1974); Galatis *et al.*, (1978a); Heslop-Harrison and Heslop-Harrison (1981) and Durkee *et al.* (1984). In *Pinus radiata* needles, the coated vesicles are seemingly formed from smooth vesicles (Singh, 1984). In *Marchantia paleacea* (Galatis *et al.*, 1978a), the coated dictyosome vesicles make a major contribution in the oil body development by providing material for the synthesis of their limiting membrane. It is reported that the dictyosome derived coated vesicles contribute to the formation of plasmalemma and may transfer carbohydrates to the cell wall as in cell plate formation (Cronshaw and Esau, 1968; Fouke *et al.*, 1975) or in the actively expanding
Apart from their contribution in membrane formation, the coated vesicles may also carry enzymes and/or carbohydrates from dictyosomes to the cell surface (Bennett and Newcombe, 1966; Galatis et al., 1978a). In *A. excelsa*, the epithelial cells show characteristic wall ingrowths obviously involving additional synthetic mechanism for wall formation.

Presence of parallel arrays of microtubules along the wall of epithelial cells furnish another feature of cells undergoing active wall thickening. Close association of microtubules with the deposition of secondary wall thickening is well manifested (Newcombe, 1969; Hapler and Palevitz, 1974; Juniper et al., 1981; Galatis et al., 1984). In the cytoplasm, the microtubules direct the vesicles to the area where the wall synthesis takes place (Robards, 1968; Pickett-Heaps, 1968). In *Marchantia paleacea* (Galatis et al., 1978a), however, the microtubules play a different role in the formation of oil bodies by facilitating the flow of dictyosome vesicles into them. In *Rhus glabra* (Fahn and Evert, 1974) and *Commiphora wightii* (Setia et al., 1977) the microtubules indirectly serve in the formation of gum by continuously replenishing the wall material of the epithelial cells while the outer wall layers gradually become part of the contents in the duct. A similar role of microtubules is suggested in *A. excelsa*. 
Further, they may also facilitate the enhanced cell wall synthesis required for the wall ingrowths of the epithelial cells.

In A. excelsa, the epithelial cells are characterized by wall ingrowths at radial and inner tangential walls. The wall ingrowths are reported to be frequent in cells involved in active transport or in the secretion of various polysaccharide exudates (Pate and Gunning, 1972; Gunning and Pate, 1974; Rachmilevitz and Fahn, 1975; Robins and Juniper, 1980a, b; Heslop-Harrison and Heslop-Harrison, 1981). However, in my knowledge, this is for the first time that wall ingrowths are observed in cells secreting a lipophilic substance. The wall ingrowths consist of specialized form of unlignified secondary wall deposited on the inner side of an ordinary unspecialized primary wall (Pate and Gunning, 1972). As the plasmalemma follows the contours of the wall ingrowths, this adaptation increases the surface area of the plasmamembrane and thus, enhances the efficiency of the cell in short distance transport of solutes. It is suggested that the wall ingrowths possess open channels which presumably reduce the restrictions to the passage of low molecular weight solutes (Pate and Gunning, 1972). In Ailanthus, the presence of osmiophilic globules in the matrix of wall
ingrowths (and not observed in the normal wall) supports the suggestion that the ingrowths have more access for the movement of substances through their matrix. Several genera of carnivorous plants appear to store the secretory protein within their labyrinthine wall ingrowths (Robins and Juniper, 1980b).

In both *V. indica* and *A. excelsa*, elimination of the substance from the protoplast of epithelial cells occurs by granulocrine secretion, which proceeds by the formation of secretory vesicles that later fuse with plasmalemma or are surrounded by invaginations of plasmalemma (Schnepf, 1974; Fahn, 1979). Granulocrine secretion is most often cited as the mode of secretion of lipophilic substances (Morré and Jones, 1967; Morré and Mollenhauer, 1974; Fahn and Benayoun, 1976; Benayoun and Fahn, 1979; Werker and Fahn, 1981; Pridgeon and Stern, 1983; Bhatt and Shah, 1985).

Once the osmiophilic and other materials reach the apoplastic, how they are released into the duct lumen is not very clear. The material may migrate through the microfibrillar layers of the cell wall, as it has been suggested for several other plants secreting lipophilic or polysaccharide exudates (Morré and Jones, 1967; Setia et al., 1977; Hammond and Mahlberg, 1978; Nair et al., 1981; Shah et al., 1982; Bosabalidis and Tsekos, 1982a,b; Nair,
Venkaiah and Shah, 1983; Pridgeon and Stern, 1983). By following special procedures for retaining the resinous material in the cell during the processing for electron microscopy, Nair et al. (1981) and Bhatt and Shah (1985) could locate osmiophilic material in the wall matrix of epithelial cells in Commiphora wightii and Mangifera indica respectively.

Myelin-like bodies are occasionally observed between the plasmalemma and inner tangential wall. The myelin-like bodies present along the wall may have some role in the apoplastic movement of the secretory substances (Bosabalidis and Tsekos, 1982b, c). These structures probably carry lytic enzymes for the dissolution of noncellulosic wall matrix resulting in the formation of a capillary system between the microfibrils (Bosabalidis and Tsekos, 1982b).

Morre and Jones (1967) state that while the synthesis of polysaccharide, segregation into vesicles and their discharge from protoplasts require active metabolism, the step involving their movement through the cell wall appears to be a passive process influenced by the degree of hydration of the material and cell turgor. However, in V. indica and A. excelsa, even if extrusion of material takes place through the wall, the process is not adequately potent enough to cope up with the high rate of secretion in the epithelial cells. Consequently, the amount of osmiophilia increases in the
apoplast and cytoplasm, ultimately leading to the lysis of epithelial cells. It is suggested that in oil cells, the oil produced by, and stored in the cytoplasm may be toxic, leading to the death of the cell (Amelunxen and Arbeiter, 1967 and Amelunxen and Gronau, 1969, as cited by Platt-Aloia et al., 1983). Similarly, the specialized wall surrounding the idioblast of avocado fruit (Platt-Aloia et al., 1983) and the direct movement of essential oil from the plastic into ER in citrus fruit (Bosabalidis and Tsekos, 1982c) are considered significant in avoiding the possible poisoning of the cytoplasm by the essential oil. Storage of osmiophilic material in the vacuoles is observed in A. excelsa. This is common in many plants secreting lipophilic materials (Dell and McComb, 1974; Setia et al., 1977; Robins and Juniper, 1980a; Platt-Aloia, et al., 1983 Durkee et al., 1984). Vacuoles are believed to play a major role in the vitality of a cell by removing the toxic secondary metabolites from the cytoplasm (Matile, 1982).

In Vateria and Ailanthus, the epithelial cells after a stage of secretion undergo autolysis and disintegration. The most prominent feature of a dying cell is the "darkening" of its cytoplasm which is mainly due to an increase in osmiophilia. This feature is reported as a development preceding the destruction or degeneration of the cell (Schulz and Jensen, 1968; Toth and Kuijt, 1976;
The senescing cells show heavy vacuolation, presence of autophagic vacuoles, membrane degeneration, loss of tonoplast, distortion of mitochondria and dilation of ER cisternae. These are cytological alterations indicating the loss of integrity of phytolysosomal system with a possible concomitant release of hydrolases and loss of compartmentation (Gahan, 1981, 1982).

Eventually the dead cell filled with osmiophilic contents is detached and disintegrate in the duct lumen. The wall material of the lysing cell may again contribute for the gum component of the gum-resin. The dictyosomes are believed to play a role in the lytic process in dissolving the cell remnants in the duct lumen (Joel and Fahn, 1980a). Occasional lysis of epithelial cells in the duct lumen is observed in many other plants during their continued secretion (Joel and Fahn, 1980a, b; Nair, Venkaiah and Shah, 1983; Nair, Shah and Subrahmanyan, 1983). However, in Vateria, lysis of epithelial cells is too frequent and it seems to contribute to the formation of gum-resin in the duct.
4.7 Development of tyloses and vascular occlusions associated with exudation

Development of gum/gum-resin ducts or cavities in the xylem is associated with the blockage of several vessels in their vicinity either with tyloses as in *V. indica*, *B. ceiba*, and *S. urens* or with gum-like substances as in *A. excelsa*. A detailed study on the development and histochemistry of vascular occlusions has been made in *A. excelsa* in which occlusions are developed due to fungal infection.

In the infected stem of *A. excelsa*, fungal bodies are consistently observed in the outer secondary xylem where the gum-resin cavities and occlusions are prevalent. Since simple mechanical injuries do not induce the gum-resin cavities or occlusions, it can be assumed that the pathogens have a role in inducing the occlusions and gum-resin cavities. But the plant exuded also through old infected blazes and the tissue collected from such exuding region showed gum-resin cavities and occlusions. It follows therefore, that the gum-resin cavities and occlusions at the old blazes are induced as a defense reaction of the plant against the invading pathogens.

Blockage of vessels by tyloses or gum-like material is very well manifested as a defense mechanism of host
plants in many bacterial and fungal diseases (Agrios, 1978; Bell, A.A., 1981). In many trees plugging of vessels is one of the first mechanical barriers to form in the tissue after wounding (Rier and Shigo, 1972). Oullette (1978) observed plugging of vessels in American elm shortly after inoculation with Dutch elm disease pathogen. The propagules and polluting products of the action of pathogen may be moved throughout the transpiration stream and in many host plants the resistance to vascular disease has been associated with the speed with which they respond in producing gels and tyloses (Beckman, 1964; Dimond, 1970). The occlusions can also give a biochemical defence by the phenolic material entrapped in them (Talboys, 1968). By electron microscopic studies Garner et al. (1983) showed that in citrus, the bacterial cells are digested and disintegrated in the vascular plugs.

In A. excelsa, the occlusions show the presence of lipid, protein, polysaccharide, phenolics, lignin and possibly pectin. The occlusions developed in cassava roots are composed of lipid, carbohydrates and lignin-like material (Rickard et al., 1979; Rickard and Gahan, 1983), while the rose stem occlusions are composed of carbohydrates, lipids, pectin and protein (Lineberger and Steponkus, 1976). In the occlusions of Ailanthus, the presence of pectin could not be confirmed, since the extraction in ammonium
oxalate did not retard the staining reaction. However, it is possible that in the occlusions the pectin in tightly complexed with other lignin-like polymers. Studies of Leppard et al. (1971) and Leppard and Colvin (1971) indicate that in the fibrous material formed in suspension culture cells of Daucus and Phaseolus, the polygalacturonate polymers are tightly complexed with other polymers, like lignin. Their chemical analysis shows that the fibrous material is mostly of polygalacturonic acid polymers, but it is resistant to pectinase and cellulase digestion. Pectin is indicated as one of the main constituents in vascular plugs in white elm infected by Ceratocystis ulmi (Gagnon, 1967) and in tomatoes during Fusarium wilt (Gothoskar et al., 1953; Dimond, 1955; Pierson et al., 1955; Badami, 1977).

Most of the occlusions show the presence of lignin. Gagnon (1967) got positive staining for lignin in vascular plugs of white elm but considered it as due to presence of vessel wall particles in the occlusion. Rickard and Gahan (1983) also did not confirm the presence of lignin in the occlusion of cassava because condensed tannins formed from the polymerization/condensation of catechins and leucoanthocyanidins may give lignin-like properties. In Ailanthus, however, removal of unbound phenolics by extraction does
not reduce the fluorescence of occlusions, and their fluorescence similar to that of vessel wall and fibers substantiate the presence of lignin in the occlusions.

The occlusion of the vessels are not chemically uniform in *A. excelsa*. But in cassava roots, all the occlusions, showed comparable response to cytochemical tests, even though patchy responses were obtained (Rickard and Gahan, 1983). Here, many occlusions are heterogeneous in composition and in many cases different components are seen distinctly in the lumen. The droplets of occluding substance migrating into the vessel lumen also found to be of dissimilar composition. These observations strongly indicate that different constituents migrate independently into the lumen where they mix to form the occlusion. The different components may be released into the lumen by different cells or by the same cell at different intervals. The heterogeneous condition represents the phase before mixing and polymerization. But it could not be ascertained whether all the occlusions have a similar composition at one stage. However, discharge of materials into the already filled vessels indicates that the chemical composition of the occlusions is subjected to variation after their early formation.

All the detected components of the occlusion except lignin and pectin are observed in the adjacent parenchyma
cells also. They migrate into the vessel lumen through the pits in the vessel wall. By TEM studies Rickard et al. (1979) observed a similar mode of initiation of occlusions in cassava root. In cassava, the occluding material is extruded from cells adjacent to the xylem vessels via breaks in the pit membrane. Some cells respond by the production of tyloses. Chattaway (1949) held that when the pit aperture of the vessel is small, the ray cells secrete gummy substances, and when these substances come in contact with the air in the vessel they solidify and form gums and gels of various types and composition. These observations contrast with that of Vander Molen et al. (1977). Their TEM observations indicate that the gel formed in the vessels of many host plants, induced by vascular pathogens originates from perforation plates, end walls and pit membranes by a process of distension of middle lamella constituents.

The mode of elimination of occluding substance into the vessel lumen and the presence of similar compounds in the adjacent parenchyma cells indicate that in A. excelsa much of the occluding material must be of host origin. In wilt diseases, the polysaccharides secreted by pathogens seem to play a significant role by passively interfering with the translocation of water (Dimond, 1970). By electron microscopic studies, Oullette (1978) concluded
that in American elm, the occluding material is mostly contributed by the pathogen. In citrus, the occlusions are of plant origin, but entirely derived from middle lamella, along the border pits (Gardner et al., 1983).

Even though in A. excelsa, a direct role of pathogen in the origin of deposit is unlikely, it may have indirect function through hormones or enzymes produced during their metabolism. Pectolytic and cellulolytic enzymes produced by many wilt inducing fungi act on the middle lamella and primary cell wall of cells surrounding the vessels and leads to the formation of occluding gels (Dimond, 1970; Robb et al., 1975; Cooper and Wood, 1980). In the occlusions of A. excelsa, the sources of pectin and lignin are not understood. Middle lamella of the cells surrounding the vessels may be the source of pectin in the occlusion. Lignin might be derived from the vessel wall. But the vessel wall looks undisturbed and it is not clear how the lignin component is released. It would be speculation to assign that role to the pathogen.

In my experiments, the histochemical response of the exudate differed from that of the vascular deposits. In the virus infected citrus stem the gum formed in the pockets is water soluble, whereas, the gum deposited in the vessels is water insoluble (Wallace, 1959). Olien and Bukovac (1982) reported that in sour cherry, gum
in the cavities migrates into vessels and both exuding and occluding gum have similar neutral sugar composition. But the migration of gum from cavities into vessel lumen is not conclusively proved by them. However, as an effective barrier against pathogens, and to withstand the hydraulic stress, the occluding material should be stable, impermeable and solid.