Discussion
IV. DISCUSSION

The architectural ingenuity of a tree is remarkable, and the dexterity with which the lateral branches are connected with the main axes is worth applauding. It is this region of joint of two axes, growing in two different directions, which has marvelled the structural design of tree. There is no parallel to such a structural design in the chronicles of engineering developed by man. Just imagine, the construction of a cement concrete loft on a straight pillar. It needs additional strong iron bars
connected right up to the basal-foot of the column. A specialized design of bends of iron bars is also needed. With a very strong anchorage spanning such a length can only make it remain steady. Moreover, such constructions remain steady, as they have no elastic property. If the joint of the structure is removed from its original position, the loft is knocked down. And, now look at the branch joint on a stem axis. The embedded basal part of the branch not only holds a very heavy load of several meters long branch, its next-rank branches and foliage, but also keeps on encountering innumerable mild to violent jolts caused by wind for the whole life span of the tree. This is simply amazing. This is possible only due to the presence of a conical structure of the knot in which the cell layers reduce their angle of inclination when traced from periphery of the branch to the central part.

To quench the vehement desire of knowing such a marvellous structure — popularly known as knot — many scholars, ranging from natural scientists and engineers to artists, have tried to view it with different angles and evaluated it in accordance to either the end-use to which they wanted to put the wood or their academic and
aesthetic interests. The knot has thus been a subject of investigation since very long time. It is generally maintained that knot is an artifact of wood developed during growth. Hence, it is an interesting situation that the structure which is important and very strong in light of tree architecture is simply discarded as a "natural defect".

In spite of the enormous work done on the physics and engineering of knot, very little is known about its biological aspects. Its gross structure has been described by many workers, but the details of its structure or histobiochemistry have not at all been worked out. This is really surprising. May be that the heterogeneity of wood elements in this region makes it extremely difficult to cut it into thin slices for microscopic studies. The aspects that have been worked out in this investigation have very little or no back ground data. Hence, my observations described earlier are discussed in light of various faces of investigation, and it has been my trial to correlate these with other relevant wood biological problems.

At the point of insertion of lateral branch on the stem axis, there is a vast deviation in cellular arrangement
in the main axis. Though the wood elements have identical arrangements in the internodes of the mother and the daughter stem axes, it is the point of knot which becomes a meeting point of wood elements oriented in different directions - the vertical and the leaning.

The angles formed by the branch with the mother axis on abaxial and adaxial sides of the former are different in most of the cases. It is acute on adaxial side and obtuse on abaxial side in the presently investigated species. This naturally brings about the changed cellular arrangements on the two sides of the branch base. The disturbed cellular arrangement causes the lateral distortion of grain in knot region, resulting into the formation of cross grain as mentioned by Panshin and DeZeeuw (1970).

The newly added layers of secondary wood tissue are laid down over the branch in a progressive order in centrifuged direction. With the addition of the new layers of secondary xylem in the mother axis its cambium is moved away from the original point of insertion of the branch into the mother axis. This makes more and more part of the branch base embedded into the vertical tissue. The embedded part of the branch base cannot grow in its girth.
due to the want of room. Hence, it becomes wider as traced away from the centre of the mother axis. All these activities together sum up in the formation of an inverted cone shaped knot. At the tip of the cone lies the pith of the mother axis (Eames and MacDaniels, 1947). This indicates that the knot experiences continued changes throughout its life. However, the knot tissue embedded deep into the mother axis undergoes aging caused changes along with its surrounding tissue, and becomes dead. And, the part of it towards the periphery remains living.

In a highly jumbled zone of the knot, many cells get distorted, while majority of them get themselves adjusted in the available room. This adjustment is possible only due to the changed pattern of cambial activity bringing about the reduction in the elements produced during secondary growth. It is also believed that the conducting tissues of the branch and mother axes remain distinct in the knot, and parenchyma tissue fills in the gap between the two (Eames and MacDaniels, 1947). This thinking led them to believe that the anchorage of the branch is not as strong as it appears to be. It is, therefore, not a question whether it appears to be strong or not. It is the reality that it holds the branch for life long period
under various stress conditions. I have not noticed any distinct filler tissue of parenchyma between the two conducting tissue of the two axes. Not only that but the distinction of the two is also not possible.

Variation in dimensions of wood elements within the tree is very common. The wood elements at various heights and girths of the stem differ in their dimensions due to different rates of growth of the axis at the time the cambial initials were formed (Philipson and Butterfield, 1967, Philipson, Ward and Butterfield, 1971). Even within a short segment of stem, the elements of node are shorter and wider than those of the internodes. This is largely correlated with the short length and congestion of the wood elements at the node (Cheadle, 1943a,b; Shah, Unnikrishnan and Poulose, 1967). In the present study the analysis of wood elements of knot indicated that their dimensions vary due to many reasons, the main amongst them being the changed orientation, plane and frequency of division in their initials. As shown earlier, after each increment in growth of the mother axis and the base of the branch, the mode of action of cambium gets changed, due to variation in the stress exerted on it by growing tissue at each level.
The dimensional changes in the wood elements of knot may be the manifestation of the pressure built up on the cambial tissue at the base of the knot (Joost, 1901). Such a pressure may induce high frequency of anticlinal division of the cambial cells and lack of distension in the resultant derivatives. This results into shorter mother cells of wood elements. The elements which are differentiated from them are also shorter than the normal elements. Instead of anticlinal divisions in the cambial initials, the mother cells of the wood elements may first differentiate into their respective elements, but pseudo-transverse divisions may take place in them to form short elements at the knot (Philipson et al., 1971). Another known concept for the occurrence of shorter wood elements at the junctions of stems or roots, or the nodal regions, explains its correlation with the growth readjustments occurring at these points (Bailey and Tupper, 1918).

In the node the vessels are shorter and narrower than those in the cleanwood of investigated species Chow (1946), Onaka (1949), Baresfoot (1965), Hoster (1972) and Beiguelman (1962) believed that the reduction in diameter of vessels might be due to the reduced distension growth of the vessel mother cells. At the node, knot region in
particular, the flow of auxins from the terminal parts of the main stem and the branch axes downwards merge into each other resulting into their higher concentration at the point of their merger. The high auxin concentration is known to increase the density of vessels and decrease their dimensions (Zimmermann, 1978).

While one kind of elements of vertical system of the wood, i.e., vessel elements, show drastic reduction in their dimensions, another constituent elements of the same system, i.e., fibres, show variable behavior in the three species investigated here. In KW of A. indica and S. saman they were found to be shorter, whereas in M. indica they were longer than those in the clean wood. Fahn (1982) attributed the growth of fibres to intrusive growth. The short fibres may be differentiated from the transversely dividing fusiform initials. Hence, the length of fibres depends on the proportion of apical intrusive growth and the transverse division taking place in their mother cells. Hence, the longer fibres as found in knot wood of M. indica may be the result of apical intrusive growth of their initials which might not have been accompanied by high frequency of transverse divisions in their mother cells.
The fibre-population was also found to be increased as in case of vessels. The increased auxin level at this point may result into either high division activity or the intrusive growth of fibre primordia. In knots, the third component of the vertical system of wood, the axial parenchyma, invariably had short and narrow cells. Their density was also recorded to be decreased. The decrease in axial parenchyma population could be attributed to the possibility that the fusiform cambial initials might have undergone differentiation into more number of fibres and vessels than the axial parenchyma cells.

Rays of the horizontal wood system experienced a very drastic reduction in their height and width within the knot wood. The narrowing of rays may be resulted either by the intrusive growth of the fusiform initials into broad rays splitting them into narrow segments as suggested by Evert (1961), Cheadle and Esau (1964), or due to their differentiation from the narrow and short segments of fusiform initials. When the stem axis is brought out from its vertical plane to inclined one, the wood shows growth eccentricity making the upper half of the axis broader.
than the lower one in angiosperm families (Browning, 1963; Scurfield and Wardrop, 1963; Timell, 1969, 1973; Wardrop, 1956, 1961, 1964, 1965; Robards, 1965, 1966; Mia, 1968; Brown, 1974). It is a general belief that the eccentric growth brings about the formation of reaction wood, known as tension wood in angiosperm, on the upper side of the inclined stems (Dadswell and Wardrop, 1949; Wardrop and Dadswell, 1955a, b; Hughes, 1965; Timell, 1969, 1973; Scurfield, 1964, 1967, 1972, 1973). However, it is not necessary that should occur only in the upper half of the leaning branches. Kaeiser (1955), Fisher and Stevenson (1981), Kucera and Philipson (1977), Reghu (1983), and Rao (1983) reported the reaction wood in the upper as well as the lower halves of the inclined branches in a number of angiosperm trees. However, the amount of tension wood was greater on the upper side than that on the lower one. Patel, Reghu and Menon (1984) demonstrated the occurrence of tension wood in the vertical stem axis of Polvalthia longifolia. In A. indica and M. indica Reghu (1983) reported the occurrence of larger quantity of tension wood in the upper half than that in the lower half of the inclined branches. This was found true even in our inquiry into the problem by studying the inclined branches. The
knot being the basal part of inclined branch should also be expected to have the identical situation as shown earlier. However, the observations showed that the entire knot had fibres with G-layer, and the tissue of the vertical axis around the knot region was full of G-fibres with distinctly thick G-layer. This is a very interesting situation where the reaction structures are so enormous in the mother axis around the knot. The formation of reaction wood is the result of many reasons, the main being the stress of various kinds (Metzger, 1908; Boyd, 1977; Dadswell and Wardrop, 1969). The branch has its own load of secondary and higher ordered branches and foliage, and it is under constant stress of its own load, wind and gravity. The branch has to tolerate all these and at the same counter them mainly at its base. The condition of tension and compressive stresses existing in the adaxial and abaxial regions of the knot may be the major causative factors to alter the tissue into reaction structures. In Silver wattle Dadswell and Wardrop (1949) demonstrated that at the base of the lateral branches more secondary growth takes place on the lower side, but surprisingly great number of gelatinous fibres occurred in the upper one. In addition to the stresses enlisted above, the secondary growth activity at the knot may also
be one of the causes to exert stress. Due to the secondary growth activity taking place in the basal tissue of the horizontally growing branches, the resultant new tissues of both the systems exert pressure on each other, bringing about the stress reactions in their biochemical and histological complexion.

The reaction changes occurring in the lower side of branch restore its orthotropic orientation, while those occurring in the upper side help the branch to prevent it from excessive bending in the geotropic direction (Tomlinson, 1978). This is occurring in the entire length of the leaning axis, but the anchoring region of it is more responsible for the performance of the said functions. Naturally, therefore, this region has the highest reaction-wood in its ownself as well as the surrounding tissues of the main axis.

As discussed earlier, the knot is an inverted cone which widens on its outer rim. The basal narrow part of it is the first one to experience stress of secondary growth of the two axes. Hence, that is the portion of knot which has the visible reaction changes in its entire body. But, as the cambium of the mother axis is shifted towards its
periphery, the mother axis encases more part of the branch base into it. The central tissue of the newly embedded part of the branch is dead and cannot undergo any additional reaction changes. So, its living peripheral part only shows the addition of reaction changes. This makes the heavy coat of reaction wood around the funnel shaped knot.

Any part of the plant body, which is under constant stresses of various kinds, is bound to be metabolically very active. Knot, by any measure, is such a part of the plant body. Therefore, it was essential to evaluate it in terms of major metabolites, histochemically and biochemically. I failed in frenzy of enthusiastic search of localing even a single study on histo-biochemical aspects of the knot. Hence, this is the first investigation of its own kind, where the problem has been taken up to elucidate some of the hitherto unexplored aspects.

In terms of space and time, the mother axis and the knot regions are different from each other. Furthermore, the knot originated from the mother axis. It is obvious, therefore, that the two systems are bound to be at different metabolic levels. Furthermore, the knot is the
region where the flows of metabolites from their sources at the upper foliageous parts of the mother and the branch axes meet. This makes their level in the knot very different from the rest of the parts of the two axes. The simple soluble carbohydrates produced during the process of photosynthesis are being utilized at the sites of either the new growth or the need of energy, and the surplus ones are converted into insoluble form of complex carbohydrate—the starch (Kramer and Kozlowski, 1979). In woody plants the reserve food material in the form of starch is abundant (Zeigler, 1964; Strafford, 1965; Freiss and Levi, 1980). Most of the metabolite reserves are accumulated in living parenchyma of wood (Stewart, 1966, 1969). In the three species, investigated here, the starch content in the knot wood was less than that in the clean wood. Whereas, the total carbohydrates were more in the former than in the latter. Moreover, the level of soluble sugars in knot wood was also higher than that in clean wood. These data indicate that the reserves in clean wood in the form of starch may be getting converted into a ready energy source, the soluble sugars, at a low pace as compared to that in the knotwood. This is an indirect evidence of high metabolic activity in the knot. Smith
and Goebel (1952) observed that starch rapidly gets converted into soluble carbohydrates during growth flushes.

Central part of the knotwood (CKR) is invariably poor in starch content, whereas the total carbohydrates in *M. indica* and *S. saman* are the maximum in this region, and in *A. indica* they are moderate. This shows that CKR is rich in soluble sugars. The region of knot just below CKR and contiguous to the central part of mother axis has the highest starch content in *M. indica* and *S. saman*, whereas in *A. indica* it occupies the third rank amongst the four regions of the knot. Amongst the remaining two regions adaxial knot region has either more, or less starch content than that the abaxial one has in *A. indica* and *M. indica*, respectively. However, in *S. saman* both of them have equal starch content. The above comparison shows that the central part of the knot has the maximum conversion of reserve metabolites into soluble sugars needed for the release of energy. Nonetheless, in *A. indica* this is applicable to AdKR, followed by CKR. When the structure of the four regions is compared, the tissues of CKR show the maximum disturbance in their dimension and orientation.
It is this region which undergoes the maximum stress, as a result of which it consumes high energy provided by the respiration of soluble sugars. In knot region, in addition to energy required to counter the stress, the secondary growth taking place in mother and branch axes too demands energy, for which high amount of starch is consumed as mentioned by Kramer and Kozlowski (1979).

High intensity of PAS reaction in AdKR is due to the presence of high population of G-fibres. G-layer of G-fibres is very thick in this region and it has a very high affinity for the stain due to its cellulosic nature (Norberg and Meier, 1966; Cote et al, 1969; Wilson, 1981; Reghu, 1983; Rao, 1983).

Another major metabolite which is stored in woody tissue is lipid (Ziegler, 1964; Stewart, 1966; 1969). Like starch, the lipids are also released as and when required for the energy (Strafford, 1965; Goodwin and Mercer, 1972). The lipids are synthesized both from the transported sugars (Ziegler, 1964) and starch (Higuchi, Fukazawa and Shimada, 1967). Furthermore, starch and lipids are interconvertible (Moggie and Fritz, 1976). The cleanwood of *M. indica* did not show appreciable change in its lipid content from
that in knotwood, whereas in *S. saman* it was fairly more in the former than in the latter. *A. indica* showed only a marginal variation between the two. The variability in lipid content of various zones in knotwood of different species did not show any particular pattern.

The analysis and comparison of starch and lipids amongst various cell types in CW and various zones of KW revealed certain interesting points. (1) The rays in all of them were rich in starch grains followed by APC, whereas fibres were the poorest elements in starch contents. (2) Lipids also fell into the same suit as that of the starch. (3) The regions and the cells rich in starch were poor in lipids. (4) The cell elements contiguous to vessels were rich in starch and lipids as compared to the noncontiguous elements. These relationships are indicative of a very close pattern of interconvertibility of the two metabolites. Bhat (1981) reported the occurrence of more metabolites in the contact parenchyma cells of the vessels, and thought that these cells were metabolically very active. High accumulation of the metabolites in such cells may be for an easy transport of soluble sugars, availed by the breakdown of starch and lipids, to the site of energy
requirement through the vessels. This is further supported by the fact that the pits on the common wall between the contact parenchyma and vessels have high accumulation of lipids, and the metabolic enzymes peroxidase and SDH.

The lipid contents were very high in G-fibres, especially those of AdKR, than simple-looking fibres. Rao and Patel (1983) and Reghu (1983) reported such a situation in angiosperm trees. It is possible that the crystalline cellulose of G-layer is synthesized from the lipids accumulated in these fibres. The fact that the occurrence of SDH and peroxidase in these fibres supports this possibility.

The protein contents in CW and KW of A. indica and S. saman are insignificantly different from each other, whereas in M. indica KW has higher value of cytoplasmic proteins. Thin film of protein was observed around the starch grains in A. indica and M. indica, which is indicative of surface digestion of starch grains as suggested by Bhat and Patel (1980). Some of the proteins may be enzyme proteins capable to digest the metabolites. The proteins that have been studied are metabolic proteins as well as enzyme proteins. Specific enzymes, viz. SDH and peroxidase were also localized, and it was noticed that they were more
in KW than in CW. Peroxidase did not exhibit any particular and common pattern of distribution amongst various regions of knot, but SDH was the highest in BKR of A. indica and S. saman. However, in M. indica SDH was the highest in AbKR. As in the case of metabolites, the pattern of distribution of enzyme SDH was in the descending order from rays to ARC to fibres, except a few exceptions. It appears from this that the rays are the major sites of metabolic activity. The fibres have very little metabolic activity which is restricted to the G-layer formation.

SDH is an important enzyme in a TCA cycle. The simultaneous presence of high starch and SDH content in knot region indicates that this region of the stem axis has a very high level of metabolic activities (Poovaiah and Rasmussen 1974; Bhatia and Malik 1977).

Peroxidase is the most versatile enzyme connected with many metabolic pathways. Its important role in the synthesis of lignin is well known (Siegel, 1956; Brown, 1961; Harkin, 1967; Helper, Rice and Terranova, 1972; Harkin and Obst, 1973; Katsuki and Higuchi, 1982; Ebermann and Stich, 1982). Lignification is comparatively very little in G-fibres with G-layer. The presence of
peroxidase in G-fibres and its link with lignin synthesis, therefore, need to be looked afresh. May be that it is also associated with G-layer formation. Intense peroxidase activity in G-layer has also been reported earlier by Wardrop and Scaife (1956), Scurfield and Wardrop (1963), Robards (1967) and Scurfield (1972). Lignin precursors cannot penetrate the G-layer, so the role of peroxidase in these cells for lignin synthesis is doubtful.

The conversion of metabolites into polyphenolic contents in wood takes place during the process of ageing. This results into heartwood formation (Higuchi and Fukazawa, 1966; Higuchi, Fukazawa and Nakashima, 1964; Higuchi, Fukazawa and Shimada, 1967; Higuchi, Onda and Fujimoto, 1969; Higuchi, Shimada and Watanabe, 1967). Under the stress condition of injury also the phenolics are produced in great quantity to compartmentalise the injured wood (Shortle, 1979, 1984; Shigo, 1985; Shigo and Shortle, 1983; Shigo, Shortle and Garrett, 1977; Eckstein, Leise and Shigo, 1979) from rest of the wood, so as to prevent the spread of microbial invasion and decay into a vast region of uninjured wood. The presence of high phenolic contents in KW of *M. indica* and *S. saman* suggests that quite a good amount of metabolites is
under pollution stress by Patel and Devi (1986). They believed that the increased lipid may be a result of increased lipid may be a result of conversion of starch into lipids under water stress. The physical properties of wood are lowered down by the presence of knot, because the machining of the two differently oriented tissue systems is extremely difficult. The "uselessness" of the knot, therefore, is only relevant to our selfish motive, and it has really no direct relationship with its usefulness to a tree as a whole. It is indeed a marvellous structure both in its design and chemical composition to withstand the years of hard blows of wind and continuous gravitational force enabling the tree maintain its beautiful canopy.