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The Organization of the Cell Wall of the Conifer Tracheid

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III—The Organization of the Cell Wall of the Conifer Tracheid

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[Plates 17 and 18]

Introduction

The fundamental nature of the results which have been obtained during recent years by the application of physical methods to botanical research has demonstrated conclusively the advantages to be gained by the botanist from a close collaboration with the physicist. Especially has it become evident that only by the use of modern physical methods may we hope to obtain a clear idea of the structure and arrangement of the constituent units of the cellulose which forms the main bulk of the walls of such a tissue as the wood of the conifer. The present paper represents an attempt to elucidate certain problems connected with the growth of a softwood tree, using methods similar to those of crystal physics. It has been written in the hope of being of some interest and value to both botanists and physicists. To this end the development from the bud of the woody tissues of the shoot has been cut down to its essentials, and is illustrated by diagrams designed to convey to the physicist a mental picture of the relevant processes of growth. For the benefit of the botanist, on the other hand, reference to the physical principles involved is omitted wherever possible, and such mathematical conceptions as occur are discussed in a correspondingly simple manner.

For the investigations of cell wall structure described below the wood of the Conifer or softwood was found to be peculiarly suitable, because the arrangement of the cells in it is exceptionally regular and the botanical details of their development have been worked out in a fairly accurate manner. The wood consists mainly of considerably elongated, pointed cells (tracheids) lying parallel to the axis and arranged in radial rows. Between these rows of tracheids run shallow, narrow, rays consisting of comparatively short elements elongated in the radial direction. The regular arrangement of the tracheids arises from the repeated division of similarly elongated, delicate, living cells in the cambium ring immediately surrounding the woody cylinder. After being cut off from such a cambial initial, the future tracheid increases rapidly in radial dimension by intake of water ; its

wall becomes thicker, and finally the contents of the cell disappear. We are then left with a very fine, hollow, thread with thick, lignified walls and pointed ends. Maceration of such a tissue provides masses of these elements, which thus form very suitable material for the examination by physical methods of the finer details of the structure of the cellulose wall. The aim of the present research is to deduce the structure of the walls of the cambium cells, and finally of the walls of the meristematic cells of the shoot apex, from an interpretation of the structure of the walls of these lignified cells which compose the woody tissues of the shoot. At the same time, the whole process of deposition of successive layers of cellulose, the fundamental process by which wall deposition of most plants is occurring, may be profitably examined in the light of more information as to the organization of the cellulose in such a series of cells. This method of attack necessitates argument from the structure of adult wood elements to the structures present in the living cambium layer, since the latter cells have walls so very thin, and so intimately associated with protoplasm, that they cannot be examined directly by the application of physical methods at present available. Such knowledge, in turn, may ultimately prove essential to a thorough understanding of the properties of the adult tissues of the tree, so important to the tree during its life and to the forester and timber merchant in its industrial utilization.

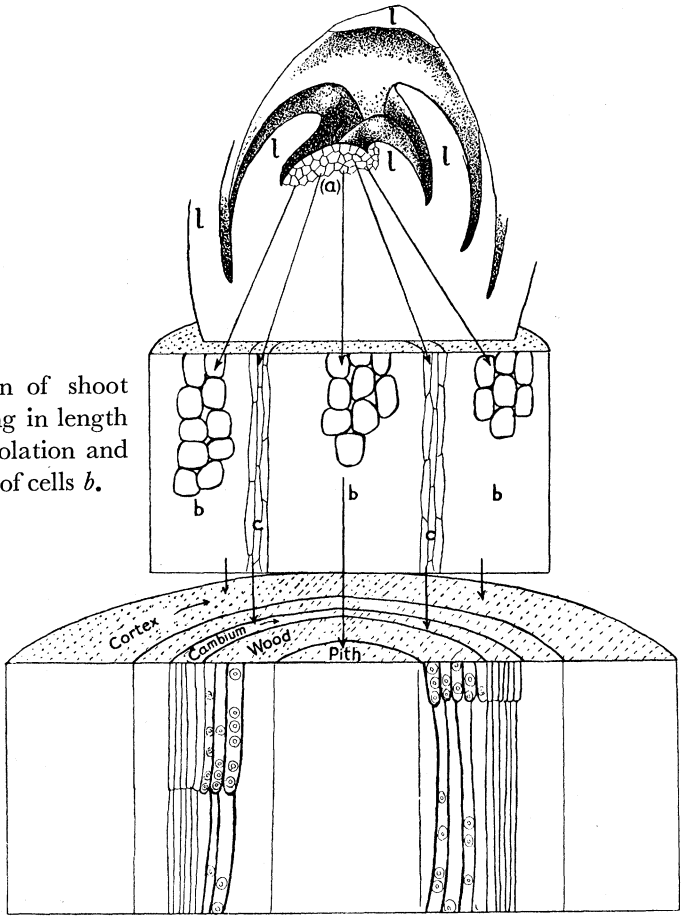
The elongated tracheids of the wood under consideration may be said to have an approximately square cross-section, with two walls in the longitudinal radial plane and two in the tangential plane. In the course of the work described in the following pages considerable difference has been found both in the structure of these pairs of walls and in their deformation as the length of the tracheid increases from one annual ring to the next ; yet, at the same time, indications have been obtained that their structures are in some respects closely connected, so that they cannot be treated as definitely independent. The work described in the present paper has led to the working hypothesis that both cambial initials and apical meristem cells have wall structures closely resembling those of adult wood elements. Yet while the models put forward towards the end of the paper explain in a very simple manner the known details of the structure of wood cell walls, and while the arguments used in their development in no way contradict the known botanical details of the differentiation of such walls, the conclusions in general and the models in particular are regarded only as suggestions. The results obtained are put forward merely as the first step towards the solution of a problem which is admittedly of a highly complex nature.

Scattered data are already available as to the behaviour of the walls of conifer tracheids, and the work of Jaccard and Frey (1928) will be quoted in its appropriate connection, but so far little effort has been made to link up the cellulose organization thus revealed with the processes of growth and development with which it must inevitably be connected. As it is from this standpoint that the organization of the cellulose wall is considered below, a brief section will first be devoted to a description of the sequence of events during radial growth ; the

general organization of the cellulose wall will then be examined in the light of recent chemical and physical investigations. Unnecessary detail will be excluded and the original papers must be consulted for further information. In order clearly to explain the significance of the results, the suggested correlation between cell length and wall structure will be briefly described before the supporting evidence is given, and will be discussed more fully in a later section.

Radial Growth in the Conifer

Before tracing in detail the process by which the softwood tree adds more wood to the axis in the course of radial growth, it will be well to take the enquiry back



Region of shoot extending in length by vacuolation and division of cells *b*.

Region of shoot where extension in length has ceased. Increase in girth has commenced by the formation of new wood elements.

Fig. 1—Shoot apex of Pine in median longitudinal section (very diagrammatic). The appearance of the cells is indicated in small segments at each level.

(*l*) Leaf primordia.
(*a*) Meristematic cells of the shoot apex : iso-diametric : thin walls.
(*b*) Vacuolating cells which increase in volume chiefly by intake of water : thicker walls.
(*c*) Procambium cells : isolated strands of cells elongated in the direction of the axis of the stem : thin walls.

to the shoot apex (bud) since the wood-forming cells are developed from this apex and recent studies (PRIESTLEY, 1932) have shown that the impetus to renewed radial growth is always initiated here. Some of the details of growth described below may be followed by reference to fig. 1 and to fig. 23, Plate 17. The descriptive lettering used refers to both figures.

The apex of the shoot is covered with small, isodiametric cells with fluid protoplasmic contents and very thin walls. These cells, *a*, are all meristematic; that is, they are all synthesizing new protoplasm and are dividing before the increase in volume exceeds certain limits. The range of cell size exhibited is therefore not wide. As the cells are all growing, they press upon one another so that their walls are in contact all over their surface. In section, the shoot seems clothed with a layer of tissue composed of angular cells usually 4, 5, or 6 sided with walls meeting at about 120°, with no intercellular spaces left between them, and resembling somewhat the bubbles in a froth. Presumably in the solid these living cells approximate to 12 or 14 sided polyhedra which would be capable of filling space under these conditions, but the shape of any individual cell is continually changing during growth and division. Just below this superficial layer, cells *b*, which were originally meristematic, begin to increase rapidly in volume mainly in a longitudinal direction (parallel to the axis of the stem). This increase is due chiefly to vacuolation (intake of water), and such vacuolating cells then proceed to develop the pith and cortex of the stem. But here and there among the vacuolating cells some elements remain meristematic, forming the procambial tissue, *c*. Unlike the tissue which surrounds them, these elements continue to increase in volume by increase in protoplasmic content. Conditions here, however, are somewhat different from those obtaining at the apex itself. These isolated bundles of meristematic cells are situated between two tissues which are extending mainly in length, and they become considerably elongated. This change takes place with relatively small increase in the proportion of mass to surface. The expansion of the cells, *b*, on either side of them may compress the cells somewhat in a radial direction, but, on the whole, their change in shape at this stage is due rather to an increase in length than to any great change in either radial or tangential dimension.

Now the vacuolating cells on the inside and outside of the young shoot finally reach a stage when they cease to divide, and intake of water is then solely the cause of further increase of volume. When the limit of this process is reached these cells, now the pith and cortex of the shoot, are no longer able to increase in dimensions. The shoot as a whole has then ceased to increase in length. On the other hand, while the young shoot is still increasing rapidly in length, the increase in protoplasm in the procambial cells seems to keep pace with the process; but as these cells continue to grow, when the vacuolating cells on either side have ceased to grow and divide, they begin to increase in mass both in a radial and a tangential direction, a change which is followed by division of the cells by longitudinal walls that are always in a plane tangential to the shoot. The cell numbers then proceed to increase in regular radial rows, the criterion of cambial activity, as shown diagrammatically in

fig. 2. Cambial activity has thus begun, in this region of the shoot, and is seen to be a natural development, marked by gradual stages, from the increase in mass of similar living, but iso-diametric, cells to be found at the shoot apex. The

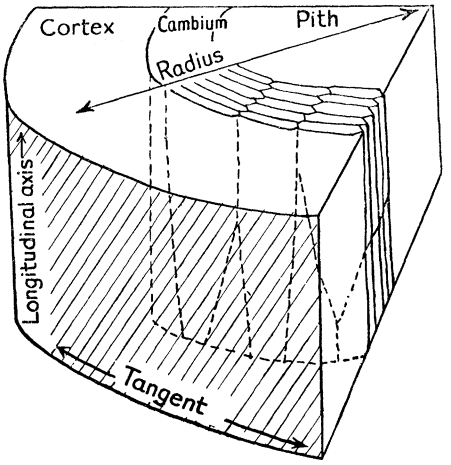


FIG. 2—Cross-section of the stem showing the regular radial rows of cells produced by repeated tangential division in the cambium. The cells are shown enormously enlarged, for clearness of figure.

elongated cells of this tissue now form part of a cylindrical sheet completely surrounding the inner tissues of the stem, and are referred to as the cambium.

The repeated tangential division in this cylindrical sheet of tissue, which occurs in the summer months during the whole life of the tree, cuts off similar elongated cells on either side. We are here simply concerned with those to the inside. These proceed to increase in volume in a radial direction as they take in water, after which their walls thicken and lignify whilst they lose their contents and are added to the woody elements of the shoot in the form of long, thin, hollow cylinders known as tracheids. Thus the process of radial growth has commenced which may continue for years and give rise finally to the trunk of the softwood tree. The enormous extension which the meristematic cells of the shoot apex undergo, as they differentiate into this vascular tissue, will be clear from the following figures which have been obtained during the course of the present research from

Pinus sylvestris :—

Average diameter of cells of apical meristem = 17μ
Average length of tracheids of first year shoot = 1,200μ
Average diameter of tracheids from first year shoot = 16μ

Thus the differentiation of these cells has involved an increase in length of some seventy times, *with practically no decrease in diameter*.

The differentiation of tracheids from cambial initials is rather complicated and we must examine the process in somewhat greater detail. As seen in perspective, a cambial initial divides longitudinally into two cells A and B, fig. 3 (*a*), by two walls

C, D, one to each daughter cell. The new walls are in a tangential plane, so that the radial dimension of each cell is approximately half of that of the original cell. For clearness of figure the walls C, D are shown widely separated. The cell A

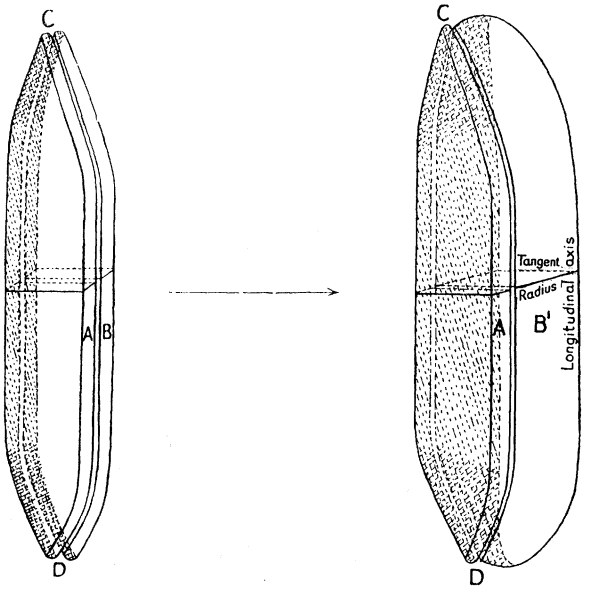


FIG. 3 (*a* and *b*)

remains meristematic while B proceeds to differentiate into a tracheid of the wood. This is evidenced by a pronounced radial swelling, its radial dimension increasing some four to eight times, while A swells only sufficiently to regain its original dimensions (fig. 3 (*b*)). Fig. 3 is, of course, very diagrammatic ; in any one row of cells we have a gradual transition from a fully developed tracheid to a cambium cell, whereas these two cells, for simplicity of figure, are shown in contact. Since the cambial initial itself is much wider tangentially than radially, the differentiating tracheid has now approximately the same radial and tangential dimensions. Finally the wall of the tracheid is thickened by the deposition of a secondary layer and the protoplasmic contents disappear. It is thus seen that while each tracheid has new tangential walls, the radial walls are all part of the same wall, and we might anticipate that this large radial extension would cause some difference in the structure of radial and tangential walls. This latter point will be dealt with more fully later on. We may simply note at present that while the tangential wall is of uniform thickness and generally has no special sculpturings, the radial walls always have thin circular or oval areas covered by a dome-shaped border—the bordered pits—which correspond exactly on adjacent walls and are presumed to be the sites of previous protoplasmic connections. Now while the stem as a whole has ceased to extend in length, the cells of the cambium continue to extend enormously. In order clearly to explain this apparently paradoxical statement perhaps we may arbitrarily divide the young shoot into three regions as in fig. 1. At the tip of the growing apex, the shoot is expanding more or less equally in all directions by random division of iso-diametric cells. Immediately below this we

have a region in which it is increasing chiefly in length, carrying the apex of the shoot vertically upwards ; any increase in diameter is accounted for mainly by the swelling of the vacuolating cells, a process which also allows for the simultaneous tangential increase. The conditions in the third and lowermost region are more complicated ; the increase in the diameter of the shoot here is due to the wood produced on the inside of the cambium sheet by the repeated tangential division, to which reference has already been made. In order to allow a simultaneous proportional increase in the circumference of the shoot it is obviously necessary and sufficient that the tangential dimension of the cambium sheet should increase. The most simple and obvious method whereby this could occur would be division of the cambial initials in longitudinal radial planes, which would cause an increase in the number of cells in the circumference of the sheet. Such divisions have never been observed. The increase in this tangential dimension is due solely to the increasing length of the cells in the cambium sheet and to their occasional transverse division.* Accommodation of this increase in length occurs, not by increase in length of the stem as a whole, but by interpenetration of the growing cells. Whether such accommodation is accounted for by an actual slipping of the cells past one another, or whether some other mechanism is involved, is at present a controversial point (Priestley, 1930). The cells may, however, be regarded *in effect* as sliding past each other. Thus, in any cross-section of the stem, we get more and more cells appearing in that part of the cambium sheet visible in the section, as the cells above and below it become more elongated. This explains the apparent paradox. The increase in length of the cambial initials is accommodated by the necessary increase in circumference of the cylindrical cambium sheet, and has no effect on the length either of the cambium sheet itself or of the stem as a whole.

Now since the cells of the cambium are continually cutting off replicas of themselves which do not increase much in length after differentiation, the increase in length of the cambium cells can be followed by observing the lengths of the cells cut off in succession. The most complete measurements in this connection appear to be those of I. W. Bailey (1915, 1920) and fig. 4 shows a curve drawn from his data giving the typical normal "length-on-age" curve for the cambium cells of conifers. During the first few years we have a rapid increase in length, followed by a period in which the elongation is less rapid, after which it becomes constant (actually fluctuating about a mean). All the cells of the cambium are growing and dividing at different times so that in any one annual ring of the wood we have a wide variation in length, the minimum usually being, as one would expect, just about half the maximum. The average, maximum and minimum, lengths of the cells are considerably less in young shoots than in old stems.

The continued growth of the fusiform initials, which has been emphasized above

* The existence of such transverse divisions is well recognized in the literature both for conifers and dicotyledons. They have been noted by Klinken (1914) in conifers and by Beijer (1927) for dicotyledons (both quoted from Priestley (1930)). From the results of Bailey (1920), Priestley (1930) has calculated that such division occurs, on an average, in each initial only once in fifteen years.

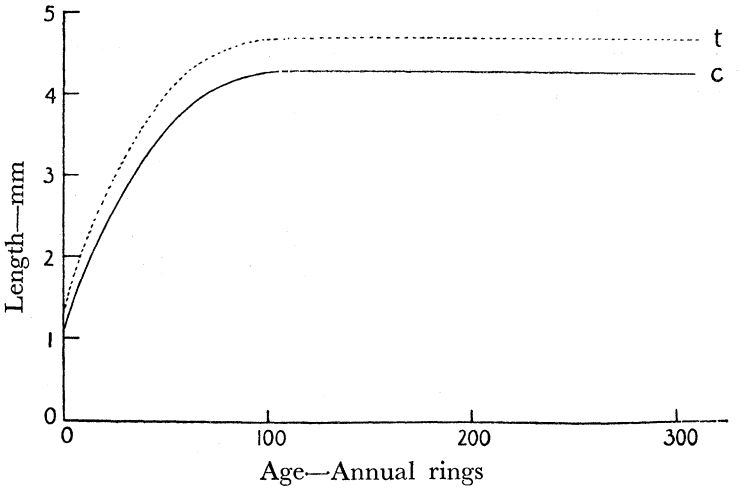


FIG. 4—Normal length on age curves, *c*, cambium, *t*, tracheids (after Bailey)

and which forms the basis of the present research, involves occasional transverse division and is further associated with an increasing obliquity of the wall thus formed. Immediately after such a division, the new walls C, D, fig. 5, dividing an initial

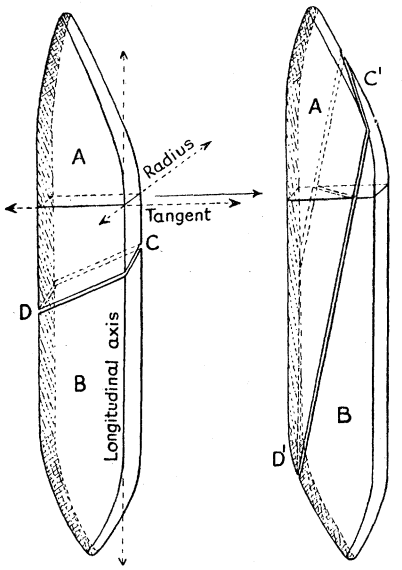


FIG. 5

A, B are fairly transverse. These walls now change position until they appear as at C′ D′. The change takes place so rapidly that observation of intermediate positions is extremely difficult. Thus the new transverse wall at such a division becomes almost immediately quite indistinguishable from a radial wall; in particular the secondary wall later deposited on it, in the differentiation of a tracheid, is indistinguishable from a longitudinal radial wall even in the polarizing microscope. This property of the so-called pseudo-transverse wall must be emphasized in formulating any theory of the structure of the walls of these cells. *The transverse wall must be such that with a suitable alteration in position it can become identical with a radial wall.* A further point upon which stress must be laid is that the cambial initials rapidly

regain their original dimensions after each *tangential* division, so that they are, in effect, *increasing in length at constant girth.*

Thus the wall of the tracheids, which is to furnish the bulk of the evidence for the suggestions put forward in the following pages, is seen to consist of a thick layer of lignified cellulose and to be developed by a somewhat complicated process from the walls of the meristematic cells of the shoot apex. It is obviously essential to examine now the structure of this tracheid wall, particularly as observed under the polarizing microscope since most of the data presented later refer to observations carried out with this instrument.

The Cellulose Wall and its Bearing on Cell Growth

The most important component of the cell wall of plants, from a structural point of view, is the polysaccharide cellulose. This forms, as it were, the skeleton framework around which the rest of the organic complexes are arranged, so that the inclusion of these other substances would seem to have little effect on the cellulose from the physical point of view developed below. Certainly the phenomena presented by the cell wall in polarized light are completely to be accounted for by the presence of cellulose.

For a long time it has been realized that a thick secondary wall such as we have under consideration here consists, not of one homogeneous layer, but rather of a series of layers differing in chemical composition to a greater or less degree (Balls, 1919, V. Wisselingh, 1924, Anderson, 1928). Swelling experiments have shown further that these layers themselves are not strictly homogeneous, but consist of numbers of long thin threads or fibrils which generally run in a spiral round the cell. From this point of view it is interesting to note that Seifriz (1931), using the Spierer lens, has shown that the unswollen wall presents just such an appearance as this structure would indicate ; but it would seem that further investigation of the utility of the Spierer lens is necessary before any reliable deductions can be made from its application.

Apart from the speculations of Nägeli and his contemporaries, based chiefly on swelling phenomena, it was not until comparatively recent times that any evidence was put forward as to the existence of still smaller units of structure. The idea of a crystalline " micelle " as put forward by Nägeli has been generalized to cover all lyophilic (gelatine) gels and has aroused much controversy in the field of colloid chemistry. On the cellulose side, evidence accumulated by X-ray, polarization, and other methods has placed the existence of some such smaller unit beyond all reasonable doubt, but there still remains the question of its precise form. By entirely independent methods Frey (1926, 1930), using the theory of Mixed Bodies as developed by Wiener (1912), and Meyer and Mark (1928, 1929), came to the conclusion that the wall did, in fact, consist of long, thin, rod-like aggregates whose diameter was small compared with the wavelength of light, *i.e.*, " micelles " almost in the sense of Nägeli. It is, however, highly probable that the cellulose micelle is not a definitely discrete unit separate from its immediate neighbours. The term

is now used merely as an expression for vaguely defined regions wherein the cellulose is more perfectly crystalline than that outside them. It necessarily follows that the term " fibril " is used in a similarly non-committal way.

The finer details in the structure of cellulose are known in a somewhat more concise way. X-ray methods (Sponsler, 1925, 1925-26, Sponsler and Dore, 1926, and Meyer and Mark, 1928, 1929) and chemical methods (Haworth, 1929) have combined to show that the cellulose in the cell wall is present in the form of molecular chains at least 500A long, each link of which consists of a β-glucose residue bound to its neighbours in the chain by primary valencies. Adjacent chains lie parallel to each other, and are bound together laterally by secondary valencies, forming a more or less well-defined lattice. Fig. 6 shows a small part of the lattice,

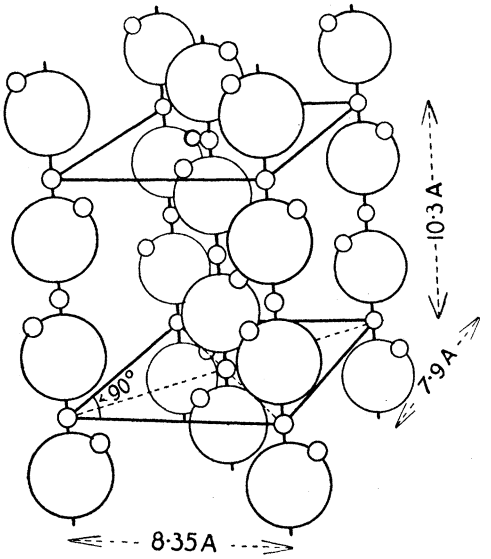


Fig. 6—Three-dimensional space lattice of cellulose. Large circles represent the glucose residues ; small circles represent oxygen (adapted from Meyer and Mark).

as a three-dimensional diagram adapted from Meyer and Mark. The micelles may be regarded simply as ill-defined bundles of these chains, the approximate thickness of a bundle being of the order of 50A (Hengstenberg and Mark, 1928). We may thus visualize a series of parallel cellulose chains, some 100 in number, united to form a micelle whose length is parallel to those of the chains composing it. These micelles in turn are aggregated to form fibrils. Moreover, in the fibril they are arranged parallel to each other and to the length of the fibril, probably in a fairly exact manner. We may therefore think of any one layer in the wall as composed of a series of cellulose chains forming a spiral round the cell, if we bear in mind that the chains are not necessarily continuous throughout the whole length of the cell. Now in many cells the cellulose chains in different layers of the wall have different inclinations to the cell axis. The tracheids under consideration, however, are more simple since in any small area of the wall the cellulose chains run parallel to each other throughout the whole wall thickness. This has been

demonstrated quite clearly during the course of the present research by an X-ray method, and receives some support from the results of other workers. In order to simplify the argument in the present section the homogeneity of the tracheid wall must be taken for granted pending further discussion below. Then any small area of the wall, consisting as it does of cellulose chains arranged in a parallel fashion, must have different properties in different directions. For instance, the swelling produced by water absorption will be much greater perpendicular to the fibres than parallel to them. Moreover, the wall will have different effects on light vibrations in these two directions, giving phenomena exactly similar to those presented by a doubly refractive crystal. The conception put forward by Robinson (1919), prior to the elucidation of the X-ray photograph of cellulose, that the doubly refractive properties of the cell wall of wood are to be explained by the development of large numbers of slip planes during cell extension, thus requires some modification. The double refraction is definitely related to the molecular structure of the wall, and mechanical strains can only affect it by causing more perfect alignment of anisotropic particles already in the wall. On the other hand, striations in the wall may well be due to displacement caused by mechanical stress, somewhat as suggested by Robinson. The direction of the chains, being defined by the so-called " major extinction position," can therefore be determined under the polarizing microscope in exactly the same way in which the corresponding details can be worked out for an ordinary crystal. It is not proposed to give here any description of the physical principles involved in these determinations. No useful purpose would be served by the discussion of a method of investigation based on principles which form the groundwork of physical optics, and whose reliability has been thoroughly tested both with actual crystals and in the realm of biology.

To recapitulate, then, the cellulose wall is now known to consist of chains of β-glucose residues linked together to form a space-lattice. Such a wall is certainly divided into vaguely defined regions to form " micelles " whose precise nature is not yet known. The micelles have therefore a three-dimensional crystal structure ; they show X-ray diffraction patterns from the breadth of whose spots Hengstenberg and Mark have deduced that in effect each micelle constitutes a lattice at least 500A long and about 50A wide. The micelles collect together to form micellar aggregates which, in elongated cells, take the form of fibrils running spirally round the cell. The fibrils in turn form the layers of the wall which, by inclusion of pectin, etc., can react chemically quite differently from each other. These layers finally form the whole wall.

We have thus a fairly accurate picture of the organization of the cell wall of the tracheid. Now the present research is based upon the investigation of the manner in which the continual elongation of the cambial initials affects the wall structure of the tracheids which they produce by repeated tangential division. It is the main argument of the present paper that cell expansion may occur in two distinct ways. These may briefly be mentioned here, pending a more rigorous discussion later. If the cell is expanding under the influence of its internal growth forces only, then we

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may expect that the insertion of fresh micelles (which must of necessity take place) will have no effect on the inclination of the micelles already in the wall. These may simply be displaced along the direction of growth. On the other hand, if the cell dimensions are changing under the influence of external forces then the arguments put forward in this paper indicate that the inclination of the micelles will also change, in a precise and definite way. The exact manner in which the former adjustment to expansion takes place is not yet clear, and little further discussion is as yet possible. The latter conception, however, demands some consideration in order to determine exactly how cell expansion under the influence of external forces will affect the micellar direction. We can consider only the extreme case in which the fibrils are regarded as continuous, rigid threads running the whole length of the cell. At the same time we must be prepared to expect changes in micellar direction ranging from the change thus calculated to the negligible one caused by a pure growth process. Before any discussion of this case is possible, it must be made quite clear that a further assumption is implied in the argument. The wall of the initial is considered to have a structure similar to that of the tracheid, so that it consists of cellulose chains running round the cell in a single spiral. This assumption in turn will be justified later both by the experimental results and by theoretical discussion of these results.

We must first consider the effect of the elongation of the cambial initial on the structure of its own wall. Let us investigate as a general case the conditions under which such a cell can increase in length or girth. We may think of the cambial initial as a long thin cell of rectangular cross-section, represented diagrammatically in fig. 7 (*a*), of which fig. 7 (*b*) is a segment cut open and pressed out flat. Let A, C, D, B be that part of a fibril which runs completely round a cell. Now consider two extreme cases, (1) in which the length only of the cell increases, (2) in which the girth alone increases.

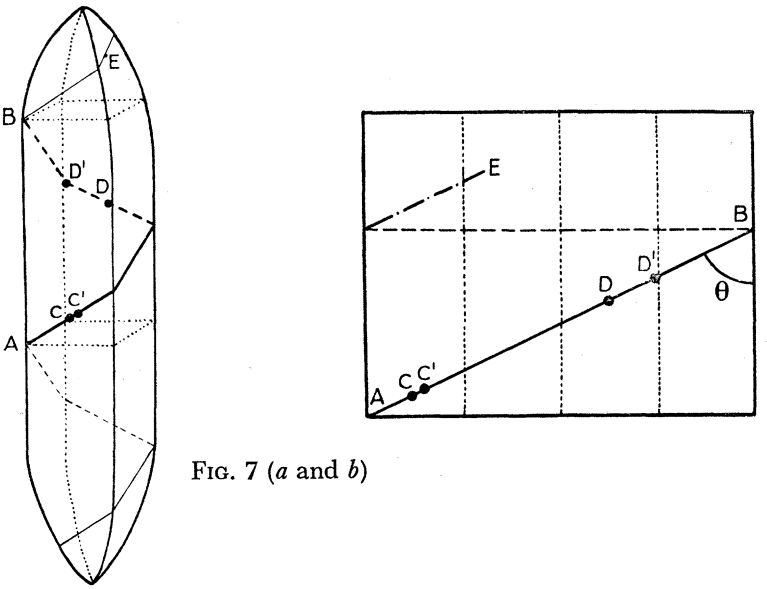

FIG. 7 (*a* and *b*)

(1) *Length Increase.*

During growth, the section of the initial between A and B becomes longer and wider. If we regard A as a fixed point, then the most obvious way for length accommodation to occur is by movement of B along the winding to E ; the original substance of the wall must then be pushed in front of E along the direction of the spiral winding. This has no effect on the steepness of the spiral, but it has the effect of displacing points in the wall. For instance, C, D, which were at the centres of two opposite walls, are now displaced to C′ and D′. Now any such displacement would be recorded in the wall of the tracheids cut off from the cambium. But structural features of this wall, such as the pits on radial walls and the trabeculæ, or bars of cellulose, which sometimes traverse the space of a tracheid from one tangential wall to the other, are extraordinarily constant in position. The pits are always on radial walls, their borders never overlapping the tangential walls, whilst the trabeculæ run in a very straight radial line through a radial file of tracheids. These facts are contrary to a method of growth of the wall which involves a continual spiral displacement of its substance.

The only alternative method whereby a cell can increase in length with no alteration in girth is that indicated in fig. 8. The inclination of the fibril alters so

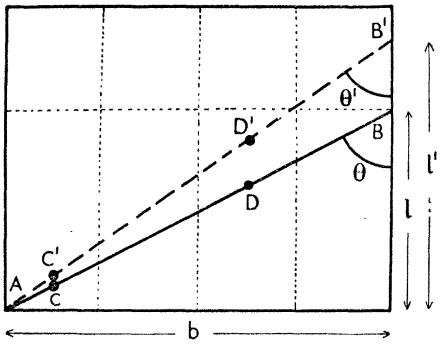

FIG. 8

as to accommodate the increased length of A B. Since now B moves vertically upwards to B′, therefore C moves vertically upwards to C′, and D to D′, and we get no lateral displacement. If now the inclination of the spiral to the cell axis be θ, the girth of the cell b, and the length corresponding to one turn l, then,

$$l = b \cot \theta.$$

If l', θ', be the corresponding quantities for the elongated cell, we have

$$l' = b \cot \theta'$$

and generally,

$$\mathrm{L} = \mathrm{K} \cot \theta, \quad \ldots \ldots \ldots \ldots \ldots \quad (1)$$

where L is the total length of the cell and K is a constant, *i.e.*, the length of a cell is directly proportional to the cotangent of the angle of inclination of its fibrils.

As to the increase in thickness of the fibrils, let AB, CD, EF, GH, IK, in fig. 9, be successive fibrils, and let ABIK be one continuous fibril. Then increase in thickness of CD moves AB vertically downwards, if no sideways displacement is allowed. EF, however, is moved upwards; this moves GH and, in turn, IK upwards.

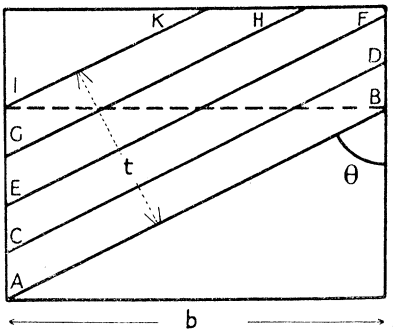

FIG. 9

Hence of the continuous fibril ABIK, AB moves downwards and IK upwards. Since I and B are the same point, the only solution seems to be a similar change as figured for increase in length of the fibril. The perpendicular distance between two windings of the same fibril is given by

$$t = b \cos \theta.$$

Hence, as the spiral becomes steeper θ decreases, $\cos \theta$ increases, and therefore t increases. This steepening of the spiral will therefore accommodate increased width of the fibrils.

(2) *Girth Increase.*

In a similar way it can be shown that increase in girth of a cell, taking place with no alteration in length, causes a change in the inclination of the fibrils as indicated in fig. 10.

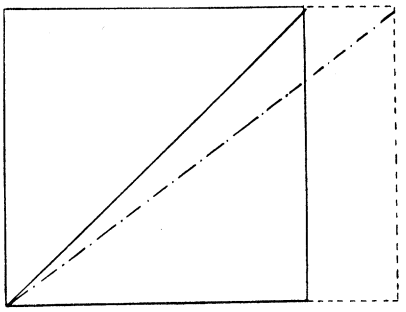

FIG. 10

Now it has already been emphasized that in the elongation of the cambial initial no alteration of girth is involved. Hence if the assumptions used in the above arguments are valid (in this instance), we should expect the cellulose chains in the initial to be so oriented that the length of the cell is proportional to the cotangent

of their inclination to the cell axis. Unfortunately we cannot test this relation directly. The only evidence we can as yet obtain concerns the tracheids cut off by the initials; this bears only indirectly on the initials themselves. Without making any further assumptions, however, we can work out a corresponding formula for the tracheids. This formula obviously refers to the radial walls only, since they alone undergo the full extension of the initial. The individual tangential walls formed at each division have undergone only a negligible part of this extension and so cannot be treated by argument along the present lines. It seems reasonable to suggest, however, that since these walls undergo such little extension, very little change in the direction of their fibrils will take place. This is, in fact, found to be so; there is no change in fibrillar direction on tangential walls of tracheids from year to year. Since the formula for the tracheid is here developed from that of the initial the test which is made later of this formula is necessarily a test of that applying to the initial. Certain details of the development of tracheids from cambial initials will be neglected in the development of these considerations. Their investigation, which would make the present argument unnecessarily lengthy, has been left to the final discussion.

Consider a cambial initial which has just divided by a tangential wall and one half of which is about to expand radially to form a differentiating tracheid. Let A, fig. 11(*a*), represent the cambial initial, the wall seen in surface view being the radial

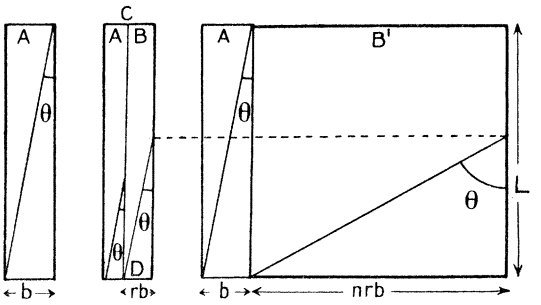

FIG. 11 (*a*, *b*, and *c*)

longitudinal. The cell is shown much shortened and square ended for convenience. Let the radial breadth be b, the length L, and the inclination of the fibrils to the cell axis θ. Then

$$L = K \cot \theta \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

where K is a constant for any initial and is a function of the number of turns of the spiral in the length of the cell, and of the circumference. After the division we have two cells, fig. 11(*b*), of almost the same breadth $\frac{1}{2}b$, the fibrils of which have the same inclination. Let us suppose in general that the radial breadth of the daughter cell, which is to form a tracheid, is rb, where r is a fractional number. The cell A, fig. 11(*b*), now expands radially until it is again identical with A, fig. 11(*a*), while B expands to a much greater extent and gives a tracheid B′, fig. 11(*c*). This process seems to be caused by rapid vacuolation so that the wall is stretched rapidly by a mechanical process and not merely by growth. No increase in length occurs whilst the tracheid

is thus being developed, so that we should expect a change in pitch to occur as in fig. 11(*c*). Let the breadth of the tracheid be $B = nrb$, and its inclination θ'. This change in fibrillar inclination has occurred with no change in length so that we have

$$\cot \theta = \frac{nrb}{rb} \cot \theta'$$
$$= n \cot \theta' \quad \ldots\ldots\ldots\ldots\ldots \quad (3)$$

Substituting (3) in (2) we have

$$L = Kn \cot \theta', \quad \ldots\ldots\ldots\ldots\ldots \quad (4)$$

which is the equation we require.

We must consider two cases :—

(1) Any increase in radial breadth of a tracheid is due to a proportional increase in that of the initial itself, *i.e.*, in *b*, or in the fraction of the initial which is cut off to form a tracheid, *i.e.*, in *r*, or in both. Here *n* is a constant and we have

$$L = K' \cot \theta', \quad \ldots\ldots\ldots\ldots\ldots \quad (5)$$

where K′ is a constant.

Therefore,

$$\frac{L}{\cot \theta'} = \text{constant}. \quad \ldots\ldots\ldots\ldots\ldots \quad (6)$$

(2) Both *r* and *b* remain constant and change in breadth is solely due to alteration in *n*. Here,

$$L = Kn \cot \theta'$$
$$= \frac{Knrb}{rb} \cot \theta'$$
$$= K''B \cot \theta'$$

i.e.,

$$\frac{L}{B \cot \theta'} = \text{constant}. \quad \ldots\ldots\ldots\ldots\ldots \quad (7)$$

The third possible supposition, that *n*, *r*, and *b* are all variable, cannot be considered at present. The solution of this would require a knowledge of *n* year by year for successive years. This possibility was therefore neglected in the hope that one of the other solutions would fit with fair approximation. In either case, the formula will be true for all radial files of tracheids so that on taking averages (6) and (7) become

$$\frac{L}{\mathbf{cot}\ \theta'} = \text{constant} \quad \ldots\ldots\ldots\ldots\ldots \quad (8)$$

and

$$\frac{L}{\mathbf{B\ cot}\ \theta'} = \text{constant}, \quad \ldots\ldots\ldots\ldots\ldots \quad (9)$$

where $\mathbf{L}^*$ is the average length ; **B** is the average breadth ; **cot** θ is the average cot θ.

* Throughout this paper heavy type is used to denote means.

We must now see which, if either, of these two formulæ holds good. It is to be noted that the above considerations only apply if there are few transverse divisions of the initials. As soon as any considerable number of these divisions takes place L is reduced with no corresponding change in cot θ'. Hence we should expect the formulæ to hold only in the first few annual rings produced by the cambium. Whichever formula applies, the change in direction of the fibrils on tangential walls is to some extent unpredictable, while that on radial walls should take place in a regular manner, if the radial breadths are fairly constant from year to year.

In trees so far examined, the inclination of the fibrils on radial walls is found to change exactly as predicted by the theory outlined above, while that on tangential walls is found to remain constant. This behaviour of the tangential walls, which has only been suggested in the theory as developed up to the present, will be discussed in more detail later.

Experimental Methods and Treatment of Data

(*a*) *Preparation of material*—The results to be presented consist of a series of readings of lengths and radial breadths of tracheids, and of the inclination of the fibrils in their walls, in the annual rings of various species of Softwood. Four specimens were used, a Cedar branch 27 years old, two seven-year-old seedlings of Japanese Larch, and a section of a twelve-year-old trunk of *Abies nobilis*. Only the spring wood of each specimen was used for two reasons: in the first place there is some evidence for assuming that spring tracheids reproduce more faithfully than the tracheids of summer wood the length of the corresponding cambial initials, and in the second place, the distinction between tangential and radial walls is made quite clear in spring wood by the fact that only the latter have bordered pits. This is not true for the last few layers of tracheids in summer wood. Other criteria which were sometimes used for distinguishing between tangential and radial walls were the more pointed ends of cells in tangential view and the deformation of the radial walls which marked the previous position of ray cells.

Present imperfections of technique made it quite impossible to isolate single radial files of tracheids, so that it was necessary to obtain statistical averages from samples containing many such rows. A cross-section of the wood was therefore obtained, some 2 or 3 cm thick, and a sector was cut from it whose sides were parallel to the radial files of tracheids. The angle at the apex of the sector was made sufficiently large for the first few annual rings of the wood to contain a number of cells great enough for sampling. In each instance quoted below the reading consists of an average of fifty random measurements. The length and breadth measurements were taken from straight, unbroken tracheids only, each reading being carefully checked. The most suitable method of preparation of the material was found to be as follows: wood shavings were macerated in 5% chromic acid at 30° C until disintegration just occurred on shaking with glass beads, when the acid was decanted and replaced by water. After vigorous shaking until no further separation seemed

to occur, the suspension of cells was centrifuged, the liquid poured off, water added, the whole vigorously shaken, and re-centrifuged. This washing process was repeated five or six times. A little of the suspension was then taken up in a pipette and allowed to flow over a glass slide previously covered with albumen fixative. The water was evaporated in an oven at 30° C, the glycerine removed by alcohol, and the slides stained with light green and mounted in canada balsam in the usual way. A micrometer eyepiece was used, calibrated from a stage micrometer.

The measurement of spiral inclination in the polarizing microscope presented more difficulty. In general no indication is given, visible under an ordinary microscope, of the direction of the cellulose fibrils in the wall. Now even a very thin section can, in general, contain only walls of a three-ply nature, the cellulose walls of two neighbouring cells being separated by some material possibly of a pectic nature. Thus the extinction positions already referred to lie along the bisectors of the angle between those of the individual walls, and no information can be obtained as to the extinction positions of the individual walls ; in fact, no true extinction is obtained in any position of the microscope stage. When the two cells have the same spiral sign the cellulose chains run in opposite directions in the two walls, and the extinction positions are approximately parallel and perpendicular to the length of the cell. Any such section does, in fact, show a few single walls as indicated by their more perfect extinction and by the inclination of their extinction positions, but the number is far too small for present purposes. Hence the wood must be macerated. For exactly the same reason the cells, when macerated, must be cut open in order to leave a single wall for observation. Several methods were tried for carrying out this operation, and finally the following was adopted. A suspension of macerated cells, obtained as described above, was allowed to flow over a slide previously thoroughly cleaned in alcohol and covered with a thin layer of albumen fixative, applied by means of a finger covered with a rubber fingerette. The water was evaporated at 30° C, the glycerine of the fixative removed by alcohol, and the slides replaced in the oven until the fixative was hard. The slides were then " shaved " with a sharp microtome knife. The majority of the tracheids were lying parallel to the surface of the slide, either on their tangential or their radial faces, so that this operation resulted in the partial or complete removal of one longitudinal wall, leaving the opposite wall for observation. In order to measure the spiral inclination of such a wall we require some co-ordinate axis to which to refer. This would be furnished by the long axis of the cell were it not for the fact that the wall is not, in general, bounded by parallel sides. It was therefore found most convenient to define the long axis of the cell at any point as the direction bisecting the acute angle between the direction of the two limiting side walls. Tips of cells, bordered pits, and parts containing obvious deformations were avoided. The inclination of the fibrils in any cell was then found to be fairly constant over its whole wall surface (radial or tangential) except perhaps at the tips, where some uncertainty occurred. Each individual reading was taken six times in all, the experimental error being not greater than $\pm 0{\cdot}75°$.

A Zeiss polarizing microscope was used, with a sensitive violet plate and an eyepiece analyser giving a large field of view. No account is given here of the method of use of such a microscope or of the physical principles involved. They can be obtained from any text-book on Physical Optics, including the book written by AMBRONN and FREY (1926) from the biological point of view which the author has found very useful. A brief account has already been given elsewhere (PRESTON 1931).

(*b*) *Statistical treatment of data*—In order to present some idea of the variation of the readings amongst themselves, the standard error of the mean is given for all of them. This is well recognized as representative of normal distributions and, although in most observations in the present paper the distribution is slightly skew, the approximation to normal is sufficiently close to warrant the use of this error. The standard error may be defined as follows :—

Let

$$x_1, x_2, x_3, \ldots . x_n,$$

be the individual readings, and $\mathbf{x}$ the mean defined as

$$\mathbf{x} = \frac{\Sigma x_r}{n}.$$

Then the standard deviation of the individual readings is given by

$$\sigma = \sqrt{\frac{\Sigma (x_r - \mathbf{x})^2}{n}}.$$

The corresponding standard error of the mean is then

$$\varepsilon = \frac{\sigma}{\sqrt{n}} = \sqrt{\frac{\Sigma (x_r - \mathbf{x})^2}{n^2}}.$$

To obtain the standard error of a quotient we use the formula

$$\varepsilon_C = \mathbf{C} \sqrt{\frac{\varepsilon_A^2}{\mathbf{A}^2} \quad \frac{\varepsilon_B^2}{\mathbf{B}^2}},$$

where ε_C = the standard error of the mean quotient **C**,

ε_A = the standard error of the mean numerator, **A** and

ε_B = the standard error of the mean denominator, **B**.

For present purposes we take

$$\mathbf{C} = \frac{\mathbf{A}}{\mathbf{B}},$$

and neglect any corrections in C for the standard errors and for correlation. For obvious reasons the correlation coefficients cannot in general be obtained, but it seems certain that this correction would be very much the same for any series of readings, and we are more concerned with the relative values of C than with its actual value.

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To test the significance of the difference between two means, where this difference is not greater than twice the sum of the respective standard errors, the method given by FISHER (1932) is used as follows. If

$$x_1, x_2, x_3, \ldots . x_{n1},$$

and

$$x'_1, x'_2, x'_3, \ldots . x'_{n2},$$

are two samples, the significance of the difference of whose means we wish to test, we calculate the following statistics :—

$$\mathbf{x} = \frac{\Sigma x_r}{n_1}, \qquad \mathbf{x}' = \frac{\Sigma' x_r}{n_2},$$

$$s^2 = \frac{1}{n_1 + n_2 - 2}\{\Sigma\,(x_r - \mathbf{x})^2 + \Sigma\,(x'_r - \mathbf{x}')^2\},$$

$$t = \frac{\mathbf{x} - \mathbf{x}'}{s}\sqrt{\frac{n_1 n_2}{n_1 + n_2}},$$

$$n = n_1 + n_2.$$

Having obtained t and n, Table IV (FISHER, *loc. cit.*, p. 151) is consulted to obtain the value of P, the probability that t is significant.

EXPERIMENTAL RESULTS

Investigations were carried out first with the 27 years old branch of Cedar, in order to obtain some idea of the variation of the steepness of the spiral on tracheid walls over a period which was probably too great for the rigorous application of the present elementary theory. Table I and fig. 12 give the results obtained for the inclination of the fibrils. The first point to note is the difference between the readings from radial and tangential walls for any year ; the spiral is always flatter on radial than on tangential walls. In this set of readings the individual standard errors are not given. They may be taken as 1° on an average, with a possible variation either way of not more than 0·1°. The difference between the inclinations on radial and tangential walls in the first few years is obviously real. For instance, the difference in the means of the first year is 12·8, while twice the sum of the standard errors is 4°. The closest approach between the two inclinations occurs at the fifteenth year. Here the difference is sufficiently small to necessitate the use of the method described above. We have

$$x = 41{\cdot}4^\circ \qquad x' = 42{\cdot}8^\circ$$

$$n_1 = n_2 = 50.$$

Hence we may calculate $t = 0{\cdot}98$, $n = 100$.

From the table we obtain the value for P between 0·3 and 0·4. This means that in about 35 times in 100 we should obtain this result by pure chance. Hence the difference is not significant. The limiting difference for significance is readily found to be 3·7°. Only in one instance in a hundred could this difference arise by pure chance. As will be seen from Table I, the difference is in general greater than

TABLE I—THE INCLINATION IN DEGREES ON RADIAL AND TANGENTIAL WALLS OF CEDAR BRANCH

STANDARD ERROR IN EACH IS 1° TO A CLOSE APPROXIMATION

	Inclination on Tangential Walls			Inclination on Radial Walls			
Annual Ring	Min	Max	Average	Min	Max	Average	Difference
1	30·0	56·0	44·4	42·0	76·0	57·2	12·8
2	31·0	57·8	42·8	36·0	67·0	56·0	13·2
3	27·2	55·2	42·5	39·8	71·0	51·9	9·4
4	33·0	57·6	44·1	36·6	70·0	49·3	5·2
5	34·6	60·2	46·1	36·4	69·2	50·9	4·8
6	32·0	54·0	46·5	37·0	62·2	50·4	3 9
7	27·0	51·4	41·1	28·8	58·4	43·9	2·8
8	30·0	49·8	40·9	33·4	59·4	47·7	6·8
9	32·8	55·2	42·2	36·2	59·2	47·0	4·8
10	34·2	52·6	41·3	28·2	66·0	48·1	6·8
11	27·0	53·2	39·8	34·4	62·4	46·5	6·7
12	30·2	47·0	38·8	39·0	61·5	46·1	7·3
13	25·2	51·0	40·7	33·2	58·2	45·4	4·7
14	35·0	48·6	41·7	32·6	55·0	44·7	3·0
15	30·0	50·0	41·4	30·0	53·4	42·8	1·4
16	30·2	55·2	42·1	30·4	61·8	45·9	3·8
17	28·2	54·4	42·2	30·2	59·8	46·6	4·4
18	32·2	47·2	39·0	35·2	57·0	43·0	4·0
19	31·2	50·2	40·8	34·2	59·0	44·7	3·9
20	31·5	54·5	40·9	32·4	64·8	45·9	5·0
21	30·2	49·6	41·4	32·0	60·4	46·4	5·0
22	25·0	48·0	39·2	32·4	66·0	45·8	6·6
23	34·4	49·2	42·6	38·4	64·2	50·6	8·0
24	31·4	49·0	42·8	38·0	62·8	49·6	6·8
25	29·0	52·4	42·7	39·2	64·2	50·8	8·1
26	29·0	50·8	41·1	35·6	60·0	48·7	7·6
27	30·4	56·2	42·2	35·4	66·0	50·0	7·8

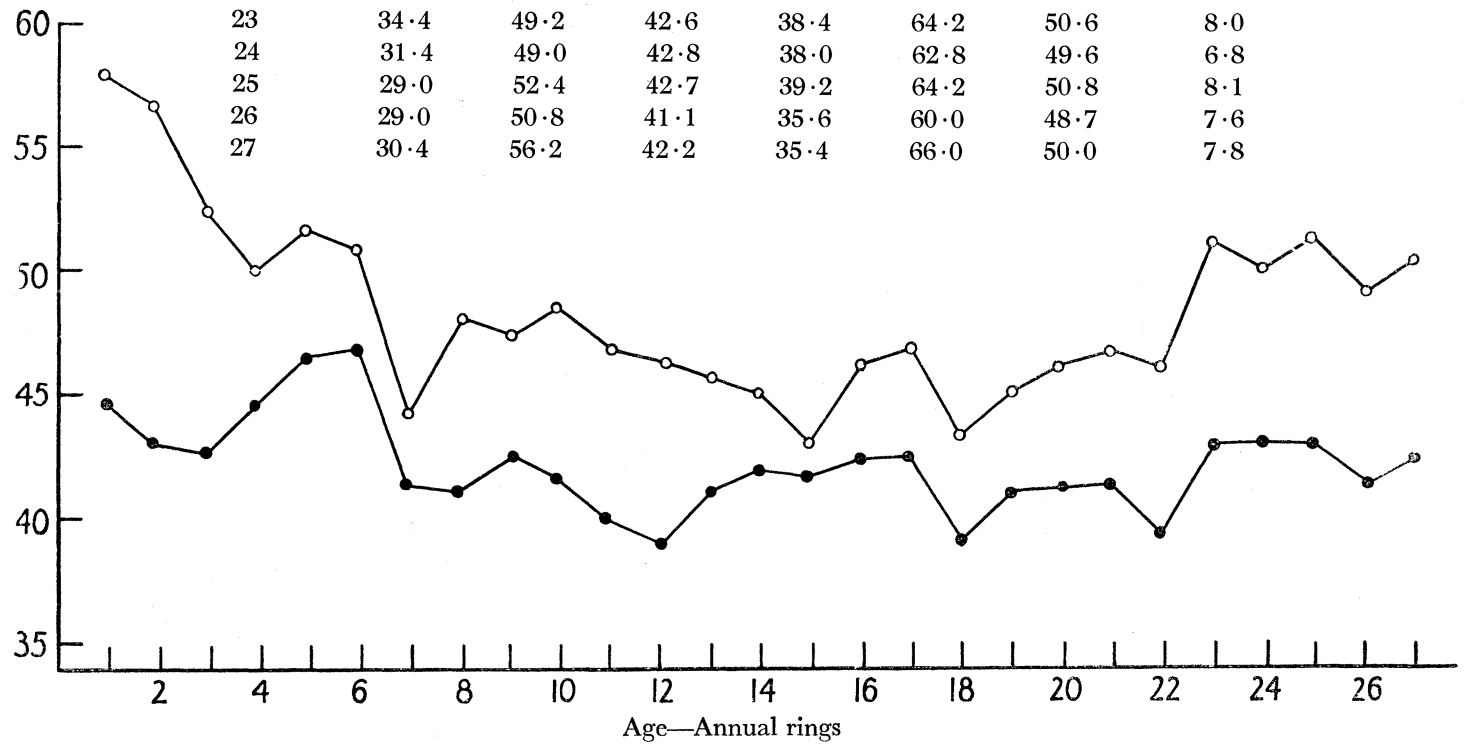

FIG. 12—Variation of Inclination with age for Cedar. ○, radial walls ; ●, tangential walls.

this, so that there is no doubt as to the reality of the difference between the two inclinations. This is emphasized in Table II which shows the readings for radial and tangential walls taken from the same cell for three rare specimens in which, by chance, one radial wall and one tangential wall were cut off leaving the remaining walls for observation. Subsequent operations caused these to be pressed out flat so that reliable measurements could be made.

TABLE II

Species	*Radial inclination*	*Tangential inclination*	*Difference*
Japanese Larch	45·2°	40·4°	4·8°
	47·0°	39·6°	7·4°
Cedar	46·4°	41·2°	5·2°

This difference between the two walls is emphasized here merely as an immediate indication of some difference either in the method of production of the walls, or during their differentiation. The sign of the difference is quite fortuitous, depending on the various changes in length and breadth occurring during growth and differentiation.

The second fact of importance from the point of view of the present theory is the rapid steepening of the spiral on radial walls during the first few years. This is exactly what we should expect from cells which are increasing in length at approximately constant breadth and forms the first, qualitative, indication that the present theory may represent the truth. This steepening goes on for the first fifteen years, after which the curve seems to rise again. It is not at present quite clear to what this rise is due. Perhaps, since only in this one experiment has such a long series of readings been taken, the result is quite accidental ; but we are concerned mainly with the wood of the first few years, so that no explanation of such a rise is at present put forward. It should be understood that this part of the curve lies completely outside the scope of the present considerations, the complete curve being given here mainly to show experimentally that a change in the fibrillar direction on radial walls, which has been predicted in the theory already outlined, does actually occur only during the first few years.

The series of readings for the tangential walls fits in equally well with the suggestion already put forward. While in the first two years or so the spiral seems to become steeper, in later years the direction of the fibrils fluctuates about a mean, some of the fluctuations being of the same order as the initial fall in the curve. A certain rough agreement is obvious between the variations of the fibrillar directions on radial and tangential walls, and it is especially evident at the crest at about the fifth year, and at the troughs at the eighteenth, twenty-second, and twenty-sixth years. Some part of this fluctuation may be due to the treatment after the tracheids were removed from the wood, but one would not expect this to have much effect since the samples of the wood of each annual ring were removed almost at the same time, and were treated simultaneously by identical methods. On the other hand, the correspondence is too close and occurs too frequently to be explicable by pure chance. The obvious conclusion would seem to be that *the direction in which the*

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protoplasm lays down the fibrils of the tangential walls is affected by that on the radial walls, i.e., is affected by the direction of the fibrils on the old walls to a greater or less degree. Other minor fluctuations in the inclination on tangential walls, which are not reflected in that on radial walls may be due to some corresponding variation in some environmental condition affecting the protoplasm, over which we have no control and which we cannot take into consideration. Attention must now be focussed on the first few years ; in the remaining specimens only the inner rings are studied.

The conclusions already outlined receive equally good support from the seedlings of Japanese Larch (Table III, figs. 13, 14). The wood was obtained from two seven-year-old seedlings (A and B), which had been planted out after two years in a nursery. Two sets of readings were taken from each seedling, one at ground level and one at a height of one foot. At the higher level, where only five annual rings could be counted, both specimens showed marked signs of frosting, introducing random longitudinal divisions into the radial files of cells. As might have been expected this completely disturbed the run of the readings on radial walls, causing a variation otherwise found only on tangential walls. The latter two sets of readings were therefore discarded.

TABLE III—THE INCLINATION IN DEGREES FOR TWO SEEDLINGS OF JAPANESE LARCH, TAKEN AT GROUND LEVEL

STANDARD ERROR APPROXIMATELY 1°

Seedling	Annual Ring	Inclination on Tangential Walls: Min	Max	Average	Inclination on Radial Walls: Min	Max	Average	Difference
A	1	32·6	55·2	45·9	40·0	71·0	56·0	10·1
	2	30·0	54·0	43·4	36·0	73·0	53·3	9·9
	3	33·0	54·0	41·7	35·8	65·6	52·0	10·3
	4	35·0	51·0	42·0	35·0	69·4	50·8	8·8
	5	30·0	55·4	43·2	37·6	63·0	49·1	5·9
	6	30·0	48·2	41·3	34·0	58·4	45·5	4·2
	7	30·0	50·6	42·2	34·8	54·6	44·0	1·8
B	1	25·0	49·0	35·6	32·0	71·0	51·3	15·7
	2	30·0	49·6	40·9	37·8	79·0	57·5	16·6
	3	23·6	47·2	36·8	34·0	73·0	48·8	12·0
	4	30·0	46·0	39·2	38·2	66·6	48·1	8·9
	5	31·0	48·0	40·2	35·6	58·0	49·1	8·9
	6	28·0	49·3	38·7	31·4	62·0	44·8	6·1
	7	30·0	47·8	38·9	30·0	58·8	43·0	4·1

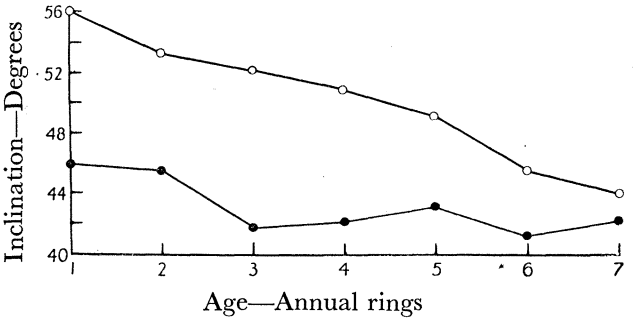

FIG. 13—Variation of Inclination with age for Japanese Larch A. ○, radial walls ; ●, tangential walls.

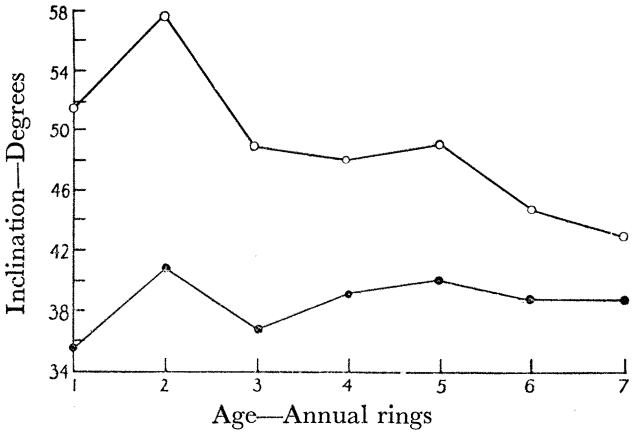

FIG. 14—Variation of Inclination with age for Japanese Larch B. ○, radial walls; ●, tangential walls.

From the fourth specimen, a 14-year-old trunk of *Abies nobilis* no readings were taken from the tangential walls. The approximate constancy of fibrillar inclination on tangential walls seems to be quite certain, and it now remains to test the theory by the more stringent quantitative test on radial walls. It will be noticed that the change in direction of the fibrils of the radial walls is of the same order for all specimens. This may be seen from fig. 15 where the readings are plotted on the same graph.

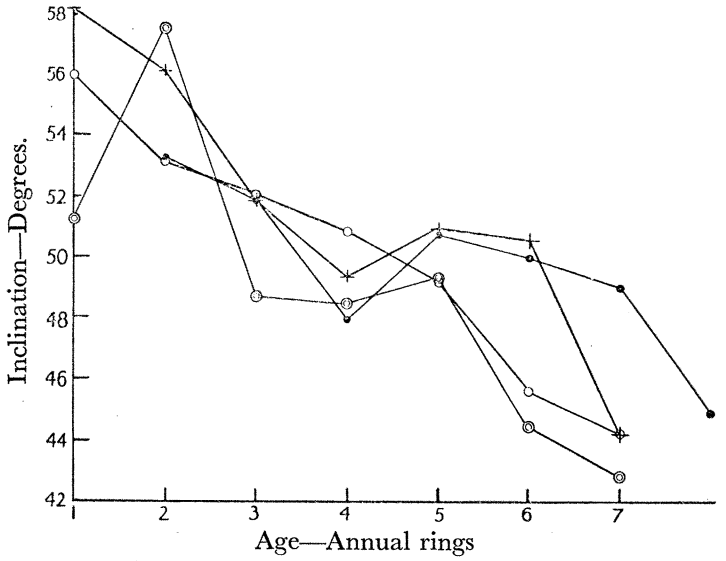

FIG. 15—Variation of Inclination on radial walls for all specimens. +, Cedar branch; ○, Japanese Larch A; ◎, Japanese Larch B; ●, *Abies nobilis*.

The readings of length, radial breadth, and fibrillar direction on radial walls are summarized in Table IV. In some annual rings the cells were too distorted after maceration to allow the extraction of accurate measurements. No readings were taken from these rings and they are consequently omitted from the table. In all cases the variation, mean, and standard error of the mean are given, as defined above. It will be seen that both **L/cot** θ and **L/B cot** θ are fairly constant for each

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TABLE IV

Species	Annual Ring	Inclination θ on radial walls (degrees) Variation	Inclination θ on radial walls (degrees) Average	cot θ (Average)	Length in μ Variation	Length in μ Average (L)	Breadth in μ Variation	Breadth in μ Average (B)	$\frac{L}{\cot\theta} = K_n$	$\frac{L}{B\cot\theta} = K_b$
Cedar	2	36·0—67·0	56·0 ± 1·0	0·687 ± 0·030	729—1560	1071 ± 27	10·0—27·0	18·1 ± 0·5	1558 ± 78	86·0 ± 5·1
	3	39·8—71·0	51·9 ± 0·9	0·796 ± 0·078	747—1700	1200 ± 33	11·2—30·0	19·2 ± 0·5	1507 ± 64	78·5 ± 3·9
	4	36·6—70·0	49·3 ± 1·1	0·891 ± 0·036	916—1880	1358 ± 36	13·2—26·2	17·8 ± 0·4	1524 ± 73	85·6 ± 4·5
	5	36·4—69·2	50·9 ± 1·0	0·840 ± 0·032	955—1760	1310 ± 24	11·2—22·5	17·1 ± 0·6	1557 ± 66	91·0 ± 5·0
	6	37·0—62·2	50·4 ± 1·0	0·850 ± 0·027	975—1720	1326 ± 35	11·3—26·2	19·1 ± 0·5	1558 ± 64	81·5 ± 4·0
Japanese Larch A	1	40·0—71·0	56·0 ± 1·0	0·697 ± 0·026	394—1320	728 ± 32	16·0—32·0	24·0 ± 0·5	1045 ± 59	43·7 ± 2·7
	2	36·0—73·0	53·3 ± 1·2	0·759 ± 0·038	339—1269	784 ± 33	16·0—36·0	24·0 ± 0·6	1034 ± 67	43·1 ± 2·6
	3	35·8—65·6	52·0 ± 1·1	0·780 ± 0·032	567—1210	850 ± 26	16·0—36·0	22·0 ± 0·6	1090 ± 55	49·6 ± 2·9
	4	35·0—69·4	50·8 ± 1·2	0·848 ± 0·035	434—1375	873 ± 33	16·0—36·0	24·0 ± 0·7	1018 ± 52	41·1 ± 2·4
	5	37·6—63·0	49·1 ± 0·9	0·899 ± 0·029	496—1374	931 ± 26	16·0—40·0	26·8 ± 0·8	1036 ± 44	39·6 ± 2·0
	6	34·0—58·4	45·5 ± 0·7	0·997 ± 0·027	459—1618	1034 ± 44	16·0—32·0	24·4 ± 0·6	1036 ± 53	42·5 ± 2·5
	7	34·8—54·6	44·0 ± 0·8	0·987 ± 0·027	620—1812	1094 ± 36	20·0—40·0	28·0 ± 0·9	1110 ± 47	39·8 ± 2·1
Japanese Larch B	1	32·0—71·0	51·3 ± 1·5	0·855 ± 0·044	471—1164	800 ± 22	12·0—24·0	17·6 ± 0·4	935 ± 57	53·0 ± 3·3
	2	37·8—79·0	57·5 ± 1·1	0·650 ± 0·032	462—1140	768 ± 24	12·0—28·0	19·6 ± 0·5	1182 ± 69	60·3 ± 3·8
	3	34·0—73·0	48·8 ± 1·5	0·903 ± 0·041	484—1212	800 ± 24	8·0—20·0	14·4 ± 0·4	886 ± 46	61·4 ± 3·6
	4	38·2—66·6	48·1 ± 0·9	0·928 ± 0·033	483—1204	868 ± 29	12·0—26·0	19·2 ± 0·5	935 ± 44	48·7 ± 2·6
	5	35·6—58·0	49·1 ± 0·7	0·880 ± 0·024	415—1276	889 ± 30	12·0—29·0	19·6 ± 0·6	1010 ± 44	51·6 ± 2·6
	6	31·4—62·0	44·8 ± 1·1	1·039 ± 0·039	532—1470	970 ± 32	—	—	933 ± 46	—
	7	30·0—58·8	43·0 ± 1·1	1·113 ± 0·041	611—1800	1028 ± 33	12·0—26·0	16·8 ± 0·6	923 ± 45	54·9 ± 3·3
Abies nobilis	2	42·8—68·8	53·3 ± 1·0	0·746 ± 0·024	754—2190	1467 ± 39	16·0—38·0	28·8 ± 0·3	1967 ± 81	68·4 ± 3·1
	3	42·4—65·0	51·9 ± 0·8	0·791 ± 0·023	862—2540	1799 ± 65	22·0—48·0	37·2 ± 0·8	2270 ± 104	61·4 ± 3·1
	4	34·8—64·8	47·9 ± 1·0	0·920 ± 0·032	1130—2540	1925 ± 49	18·0—44·0	31·6 ± 0·8	2090 ± 98	66·2 ± 3·5
	5	39·2—64·6	50·7 ± 0·8	0·831 ± 0·025	1462—2794	2205 ± 47	28·0—56·0	41·6 ± 1·0	2650 ± 66	63·0 ± 2·5
	6	35·8—62·4	49·8 ± 0·9	0·873 ± 0·027	1488—2712	2165 ± 40	28·0—56·0	39·6 ± 1·2	2483 ± 81	62·8 ± 2·9
	7	36·0—64·0	48·8 ± 1·0	0·956 ± 0·035	1485—3520	2408 ± 61	28·0—60·0	41·2 ± 0·9	2522 ± 110	61·4 ± 3·1
	8	30·0—52·8	44·7 ± 0·9	1·055 ± 0·034	1818—3510	2420 ± 53	24·0—60·0	40·0 ± 1·2	2292 ± 89	57·5 ± 2·8
	9	30·8—60·0	44·2 ± 1·0	1·056 ± 0·039	1560—2980	2424 ± 39	24·0—56·0	40·0 ± 0·8	2300 ± 92	57·6 ± 2·9
	11	23·0—47·6	34·7 ± 0·8	1·495 ± 0·046	1440—3699	2666 ± 68	20·0—46·0	33·2 ± 1·1	1782 ± 71	53·7 ± 2·9

specimen, one or the other varying only within the limits of the standard error. Considering the enormous variation in the individual readings from cells in any one year, the constancy of the last two columns in the table is of a degree quite unexpected, and forms a striking confirmation of the presence of some element of truth in the theory. In all specimens except *Abies* the average breadth of the cells is too constant to allow a definite decision to be made as to the relative constancy of the last two columns. In Table V, however, the averages of the "constants"

TABLE V

Specimen	K_n	Coefficient of Variation of K_n	K_b	Coefficient of Variation of K_b
Cedar	1541	31	84·5	38
Japanese Larch				
A	1053	37	42·8	40
B	972	37	55·0	42
Abies nobilis	2262	30	59·1	37

for each specimen are given, together with the standard deviations of the individual figures, expressed as a percentage of the corresponding mean "constant." One is tempted to say that in Japanese Larch the first column is the more constant. In *Abies* and cedar, however, the standard deviation of the first column is much smaller than that of the second, so that we should conclude fairly definitely for these specimens that the variation in radial dimension of the tracheids in spring wood is, on an average, connected with a similar variation in the corresponding cambium initials.

THE NATURE OF THE MOLECULAR SPIRAL ON TRACHEID WALLS

It has already been stated that in most elongated cells the molecular structure is such that some sort of a spiral runs round the cell—a spiral which is not visible microscopically but whose inclination can be measured in the polarizing microscope. Several assumptions have been made as to the exact character of this spiral, and the grounds upon which these were made must now be clearly defined. It was assumed that, in the tracheid and the cambial initial, the wall structure corresponded to a single spiral consisting of a series of fibrils running the whole length of the cell. In this section and the next, a justification of these assumptions will be made.

The spiral formation of elongated cells with thick secondary wall deposits is readily deduced under the polarizing microscope, and is widely recognized in the literature. REIMERS (1922) by a special swelling technique showed that the fibrils themselves run in a spiral round the cell; STEINBRINCK (1927) quotes examples in which different wall layers have spirals of different steepness and occasionally of different sign; FREY (1930) gives descriptions of the spiral structure of many types of cell as determined under the polarizing microscope; and many other workers have produced evidence for the universal nature of this spiral form in elongated

cells. Some evidence already exists to show that in certain types the cellulose fibrils in the various layers of the wall run in the same direction, so that the whole wall corresponds to a single set of fibrils. Though numerous examples of cells with two or more sets of fibrils have been cited in the literature they refer exclusively to fibres taken from the bark. There seems to be little evidence for a crossed fibrillar structure in the walls of tracheids of the softwoods under consideration here. Perhaps the strongest argument which the worker with the polarizing microscope is tempted to put forward for wood cells is the sharp extinction of the single wall as compared with that of the double wall (where the chains are certainly crossed). Unfortunately this argument loses much of its force in view of the recent work on *Valonia* (Astbury, Marwick, and Bernal, 1932) which is being continued by the author in collaboration with Mr. Astbury. In this, the first single cell membrane to be examined directly by an X-ray method, we have a wall whose cellulose chains run in two directions at an angle varying from about 80° to about 60°, and yet the extinction can be perfectly sharp. On the *Valonia* wall two sets of striations are visible under the ordinary microscope which are parallel to the two sets of cellulose chains. In some wood walls striations may also be detected which run in a single spiral round the cell. But in the absence of any such markings on the walls under consideration in the present paper it is possible that these walls, like that of *Valonia*, consist of a basketwork of chains. Other evidence must be sought before any reliance can be placed on the structure of the wood cell wall as deduced under the polarizing microscope alone. Refractive index measurements by Frey (1926) on cells of the cotton hair, which also have a spiral structure, show that the refractive index parallel to the spiral winding, whose direction was determined under the polarizing microscope, is identical with that of ramie fibres parallel to their lengths. Now X-ray analysis has shown that in ramie we have a limiting case of a spiral structure. The cellulose chains are oriented nearly parallel to the length of the fibre, and may be regarded as a single set of parallel chains. Any considerable crossing of chains in the cotton hair would certainly have led to a lower refractive index. Hence in the cotton hair it is probable that there is only one spiral round the wall. The X-ray work of Herzog and Jancke (1928) points to the same conclusion. These authors have found that the inclination of the fibrils in cotton is the same whether measured by an X-ray method or under the polarizing microscope. This indicates that the direction of the cellulose chains in the wall corresponds to the " major extinction position " so that there must be only a single set of chains in the wall. Similar determinations were carried out by them, on other cells, sometimes with the same result. While their work is of considerable value from the present point of view it is not definitely conclusive. The X-ray work was carried out on *bundles* of cotton hairs and the determination under the polarizing microscope was an average of readings from many cells. It seemed, therefore, essential to the present purpose to determine by an X-ray method the direction of the cellulose chains in a *single* wood cell and to relate this with the extinction positions of the wall. This is rendered somewhat simpler by the fact that some tracheids on maceration are found to show

certain cracks, parallel to the major extinction position which previous workers have assumed parallel to the cellulose chains, forming a single spiral round the cell. If we can show that the cellulose chains are, in fact, parallel to the cracks, any doubt as to the existence of a single spiral in these walls would be dispelled. The X-ray photography of such minute cells presents a difficult problem; their scattering power is far too small to give a diagram in the ordinary spectrometer. Under the direction of Mr. ASTBURY, and with his help and that of other workers in his laboratory to whom the author is greatly indebted, a camera has now been constructed to overcome this and other difficulties. In this camera it is possible to obtain good photographs, showing characteristic diffraction spots, from such minute vegetable cells. The camera, a description of which will be given elsewhere by Mr. ASTBURY, gives photographs a few millimetres in diameter, which can later be enlarged to a size suitable for reproduction.

For this part of the work tracheids of *Sequoia* were used on account of their greater size.* The wood was macerated in five per cent chromic acid, washed, and then pressed quite flat between two glass plates so that the cracks, which were otherwise not in focus at the same time under the microscope, appeared as a cross and focussed simultaneously. A single cell so treated was placed in the beam with the flattened faces perpendicular to the beam. The resulting photograph showed that the *whole* cell, when flattened, possessed only two sets of cellulose chains, parallel to the cracks in the walls. This has been verified for several cells, a typical result being shown in figs. 20, 21, Plate 17. Fig. 22, Plate 17, shows the photomicrograph of that part of the walls of the cell whose X-ray diagram is given in figs. 20, 21. It will readily be seen that the cracks are parallel to the sets of cellulose chains. Now it is quite clear that one set of cracks belongs to each wall, and it seems equally certain that only one set of chains belongs to each wall, each wall containing the set of cellulose chains parallel to the cracks in it. Now there seems to be no reason why this result cannot be generalized to cover all tracheids, so that we may conclude that in the tracheids we are considering in the present paper there is, in each wall, only a single chain direction. The whole cell wall is wound with a single spiral.

The question as to whether the fibrils composing the walls of the cambial initials can be treated as rigid spirals is of a completely different character. No direct experimental test of this point is as yet possible and, as far as the author is aware, the results put forward in the present paper are the first indications, even of an indirect experimental nature, that the assumption that these spirals may be rigid in the present sense may be valid. As will be emphasized later, however, the fibrils cannot always be treated as continuous threads. It will be clear from the brief account of the structure of the cellulose wall given above that the membrane of plant cells cannot be regarded in any strict sense as consisting of continuous, rigid spirals. Its organization is more in the nature of small, probably elongated, particles of wall substance laid down in a spiral fashion, so that each

* Since the communication of this paper, similar results have been obtained with the tracheids from *Abies nobilis*.

small unit has the same inclination to the long axis of the cell. Now if the wall is free from tension, but is nevertheless increasing in dimensions, then the process of extension may be defined as one of pure growth. The insertion in the wall of new micelles (and, since the wall is free from tension, such insertion is essential) will probably take place with no change in the direction of the micelles already in the wall, and we have the phenomenon of cell expansion with no change in the direction of the fibrils. On the other hand, the application of a longitudinal tension may produce an entirely different effect. The micelles are almost certainly not separate discrete entities, and are in any case closely interleaved and imbedded in a medium composed of pectin and probably other organic complexes. Now if the cell is elongating, any line drawn round it in the direction of the spiral formation (*i.e.*, the spiral winding of an ordinary helical spiral) must have increased in length. In the structure of the wall this line corresponds to a series of closely overlapping micelles which must therefore, at first glance, have slipped past each other. The longitudinal tension will, however, have a secondary effect ; it will tend to turn the micelles in a direction more closely parallel to that of the tension. Whether the micelles alter their direction, producing a change in the steepness of the spiral, or whether the whole deformation is taken up by sliding depends on the relative value of the forces involved. If the force required to produce slip is much smaller than that to change micellar direction, then we have a case closely analogous to a pure growth process. If the converse is true, then it is possible for the wall to behave as though consisting of rigid spirals. This latter case seemed to merit a more detailed discussion and the work described in the present paper was first begun in order to test this point. It will be shown in the next few pages that the experimental results quoted above are in full agreement with these conclusions. While a pure growth process cannot produce any considerable change in the fibrillar direction, growth by tension has the effect of steepening the spiral.

The Origin of the Spiral Structure of Tracheid Walls

Thus of the assumptions made in the argument prior to the experimental work one, that the tracheid wall has a single spiral, has been supported by direct experimental evidence and another, that the hypothetical spiral on the wall of the cambial initial can be treated as an ordinary rigid spiral, has been briefly discussed. It now remains only to refer to the third assumption, that the wall of the cambial initial has a spiral structure essentially similar to that of the tracheid. In the present section an argument is presented, based on the structure of the tracheid wall, which suggests that this assumption is a fair approximation to the truth.

It is therefore proposed to examine how the micellar spiral of the tracheid originates and to discuss in greater detail than previously its modification during growth and development. Before proceeding with the argument it will be profitable to consider briefly some relevant data put forward by Jaccard and Frey (1928).

These authors have attempted to correlate the micellar spirals of tracheids with the forces active during growth, but the chief interest from the present point of view centres upon the distribution of right- and left-hand spirals among the tracheids, and upon certain deductions made as to the wall of the initial. Their investigations were concerned with horizontal branches of certain species of softwood. On the lower side of such branches, where the wood is orange in colour, the annual rings are wide and the wood is under pressure. This wood is often spoken of as "rotholz" or pressure wood. Its elements have thicker walls than those in the narrower part of the ring on the upper side of the branch. Here the wood is white and the thinner walled "tension" tracheids are sometimes longer on the average than the pressure tracheids at the same "level." JACCARD and FREY found the spirals on tension tracheids to be considerably steeper than on pressure tracheids, but they hesitate to ascribe this to the differences in the forces acting on the corresponding cambial initials. They prefer to compare these tracheids to those in spring and summer wood, basing the difference on the varying speeds of growth of the wood. Their argument is that tension tracheids, like summer tracheids, are produced more slowly. Ring width then increases less rapidly and they suggest, therefore, that the cambial initial has more time to elongate between two "horizontal" divisions, so that the spiral is steeper. Their argument would appear to involve two misconceptions. The cambial initial is dividing frequently by *longitudinal tangential* walls, but *transverse* division as a result of their extension in length occurs, in any individual initial, only once in several years (PRIESTLEY, 1930)—far too seldom to have any effect on the steepening of the spiral. Again, after such a division the authors appear to assume a sudden return of the inclination of the spiral to its original value in the unextended initial. Such an assumption is supported by no experimental evidence and seems entirely contrary to everything we know as to the nature of the cellulose wall and its gradual displacement during growth. The mechanism put forward by JACCARD and FREY to explain their observed difference in inclination is thus based on questionable assumptions, and further investigation of the connection between ring width and the steepness of the spirals is certainly necessary.

All the tracheids examined in the present paper had right-hand spirals. JACCARD and FREY, on the other hand, found species with both right- and left-hand spiralled tracheids. In the softwood tree it is possible to find both right- and left-hand spiralled tracheids in various proportions. Now in the same paper JACCARD and FREY report an investigation of spiral grain in *Picea excelsa*, which merits somewhat closer attention. Spiral grain in a softwood is the result of a spiral inclination of the whole cylinder of narrow cambial initials, which has the effect that all the tracheids of the wood are tilted in a common direction in the tangential plane. JACCARD and FREY find that the tracheids have always the same (micellar) spiral sign as that of the grain. They admit, however, that there may be, here and there, a tracheid with opposite sign. Assuming that the structure of the cambial initial is the same as that of the corresponding tracheid, they then attempt to connect the

spiral of the micelles with that of the grain, and to correlate their discovery with a conception originally put forward by Braun (1854), and later developed by Hartig (1901), that the transverse walls of initials with right-hand spiral grain always fall to the left, and of initials with left-hand spiral grain to the right. The proposed correlation is clear from fig. 16 (*d*) and (*e*). The position of the cross wall is supposed

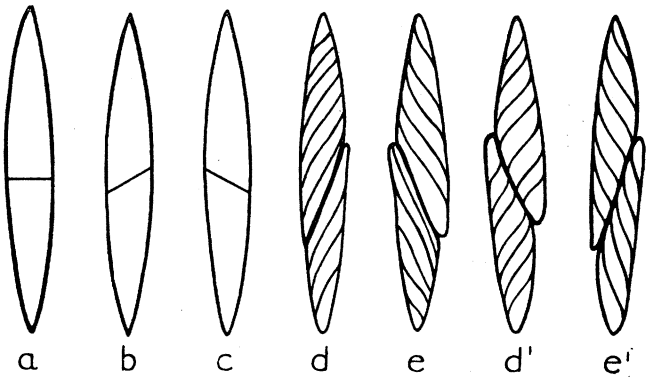

Fig. 16—(*a*), (*b*), (*c*), (*d*), and (*e*) after Frey.

to determine the sign of the micellar spiral. On account of the spiral winding itself this idea seems to be highly improbable. Fig. 16(*d'*) and (*e'*) is a reproduction of the cells shown in fig. 16(*d*) and (*e*), turned through 180° about the axis of the cell so that the walls previously at the back are now visible. The transverse walls are now seen not to be parallel to the micellar rows ; in fact, in a cylindrical cell, the cross wall and the micellar direction could only be parallel at one point, and in a cell of square cross-section only one tangential wall can have micelles oriented parallel to the cross wall. It seems fairly certain that no exact correlation can exist between the micellar spiral and the transverse wall.

This work of Jaccard and Frey does, however, impose two conditions on the mechanism of the development of the micellar spiral. Such a mechanism must be able to produce tracheids with different spiral signs, and must be such that the variation in ring width causes a corresponding alteration in the inclination of the micelles. To these we must add the conditions which have been shown to be necessary during the present investigations. The mechanism must explain how the law $L = K \cot \theta$ (or $L = KB \cot \theta$) comes about for tracheids and must enable the transverse wall at each transverse division to become structurally identical with a radial wall.

All tracheids are formed from fusiform initials and these in turn originate by elongation of the iso-diametric cells of the apical meristem. The spiral must therefore be either inherent in the wall of the apical meristem cell, or evolved during the complicated elongation process. Now, whilst the spiral structure of a tracheid can readily be deduced in the polarizing microscope, the walls of the meristem cells appear to be quite isotropic, *i.e.*, devoid of such doubly refractive effects as would imply a regular cellulose structure. This may be due either to the extreme

thinness of the wall, or to a lack of parallel arrangement of the particles of cellulose composing it. The former view is held by FREY, and seems to be the most probable. The fact that primary walls do not give double refractive effects does not prohibit a space-latticed structure, and the following considerations lead us to suspect that these walls have, in fact, just such a structure as the thicker, double refractive ones later deposited on them.*

In the laying down of new wall layers two factors are obviously concerned :

(1) The structure of the old wall upon which the new layer is deposited.
(2) The structure of the protoplasmic surface at the time of deposition.

The actual manner in which the protoplasmic surface offers an orienting mechanism is not yet clear. It may be chemical as favoured by DEVAUX (1928), in spite of FREY'S assertion to the contrary ; it may be mechanical, *e.g.*, streaming as favoured by DENHAM (1923) ; but the actual mechanism itself is not of immediate importance. The developing tracheid does not undergo much deformation as the secondary wall is being deposited, so that the protoplasmic surface will be in very much the same condition as when the last layers of the primary wall were being laid down. No considerable effects due to change in area or shape of the protoplasmic surface are to be expected. If, then, the orienting force is in the protoplasm we should anticipate that the structure of the primary and secondary walls would be similar. If the orienting force is in the old wall, then the molecules in the new wall will be oriented by those in the old wall so as to be parallel to them. In either case, therefore, it is highly probable that the inner layers of the wall of a fusiform initial are spirally wound. Now it seems impossible that a continuous process of elongation, independent of the existing wall, could so modify the protoplasmic surface as to initiate a spiral winding. The considerable elongation of the cell more probably only modifies conditions already existing, so that the wall of the meristem cell itself must be lined with a spiral winding of some kind. Since the conditions inside a normal apical meristem cell have been the same ever since the cells were formed, or at any rate have repeatedly gone through the same cycle, it is highly probable that all wall layers are spirally wound in a similar way. The wall of the meristem can thus be regarded as a uniform sheet in which the micelles are arranged approximately parallel. Since all the walls in the apical meristem, except the outside walls of the surface layer, originate from new walls formed at division, the fundamental orienting mechanism must be in the protoplasm.

We have thus reached the conception of an iso-diametric meristem cell with some sort of spiral winding. It now becomes pertinent to enquire into the nature of this winding. Now although a specimen of wood may have both right- and left-hand spiralled tracheids, yet there is no evidence that a single cambial initial can give both right- and left-hand spiralled tracheids. That this process is impossible may be

* [*Note added in proof, September* 17, 1934—At the same time, different "layers" in this primary wall may have somewhat different spiral inclinations. It is necessary and sufficient that the inner layers of the wall of the initial are wound with a spiral similar to that on the secondary wall.]

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judged on *a priori* grounds. If the original initial, shown in cross-section in fig. 17, is dividing and has spirally wound walls, we may imagine that the process which originated the winding is still available. Now the mechanism orienting the particles

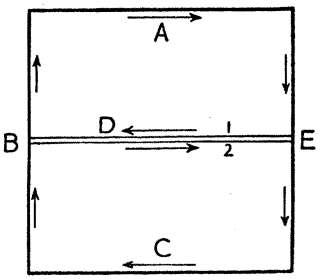

FIG. 17.

of wall substance must be such that each wall is constructed in a similar manner; if, for instance, the micelles in one wall of such a spirally wound cell are pointing from the bottom left-hand corner of the wall to the top right-hand corner then the same must be true for the other three walls. The mechanism is in a sense cyclic, and may be represented diagrammatically by arrows as in the figure. When the cell divides at D, the conditions influencing the direction of the micelles in the wall D_1 will be the same as they were at C; the conditions at D_2 will also be the same as they were at A. Hence the arrows corresponding to the two walls denoted by D must be in the direction shown in the figure. Hence if ABCE is spirally wound the cells ABD_1E and D_2BCE will be wound with spirals of the same sign as that of the original. If in the new wall D_2 of the figure the micellar direction was opposite to that required to complete the spiral on the walls BCE, then the micelles in D_2 and C would lie parallel, which is not what is observed. Observations on many thousands of tracheids have failed to reveal one in which the micelles on opposite walls did not run in opposite directions.

It would seem then, since wood sometimes consists solely of right or left wound tracheids, and sometimes of a mixture of both, that a similar distribution of sign occurs in the corresponding cambial initials, and that the structure of the wall of an initial in this respect is decided in the meristem itself. Only one type of cell exists in this apical meristem, and both right- and left-hand spirals must be inherent in it. Taking the cell as cubical for simplicity, a spiral can be wound on it which has this remarkable property. In fig. 24, Plate 18, a model of such a cell is shown. We wind a left-hand spiral on the walls shown vertical (the sign is obviously immaterial), join the " fibrils " on these across the top wall as shown and across the bottom in the opposite direction. Then on the vertical walls we have a left-hand spiral, while if we turn the model so that the edge CD is vertical, D uppermost for correct perspective, we have on the walls now vertical a right-hand spiral. It is of interest to note that it is quite a simple matter so to wind the spiral that each winding is in the form of a loop; in the model shown the winding consists of one endless loop of string. Thus, travelling along the winding from any given point in it, we always finally reach the point from which we started. The model is completely

cyclic in the sense already used above. If, now, either by lateral pressure or by longitudinal tension the cell extends in length, the elongated cell will have a spiral winding whose sign will depend on the forces acting. If the model as shown in fig. 24*a* is caused to extend parallel to XX′ the resulting initial will have a left-hand spiral, as in fig. 24*b*. If it elongates parallel to ZZ′ the spiral will be right-handed with respect to the long axis of the cell, as in fig. 24*c*. Elongation along Y Y′ may not be possible; in the extending walls the "spiral" runs in opposite directions in *adjacent* walls, so that elongation would involve its rapid distortion and the elongated walls would not have a spiral organization. It is worth noting that no softwood tracheids have yet been examined in which such a spiral organization is not present.

Thus in the apical meristem there may exist only one type of cell with various orientations. Yet all these can be traced back to a single cell, having been produced therefrom by division, after a purely random fashion. This is quite conceivable, for while regular division in parallel planes cannot change the sign of a spiral, it seems that division in a random fashion may, in effect, turn the cell round. Four of the edges of the proposed cubical model possess strikingly different properties from those of the remaining eight, and it is to this difference that the properties of the model as a whole can be traced. Especially is the possibility of the effective change of orientation on random division to be attributed to the properties of these four edges. If the walls adjoining any one of the remaining eight edges were made coplanar, then the direction of the fibrils on the two walls would be somewhat alike, because, on following the fibrils across the edge from one face to the next, they continue in the same spiral direction. On the other four edges this is not so for the fibrils, passing over from one wall to the next, turn back along themselves to a greater or less degree. Hence we have here adjoining walls where fibrils may be said to lie in opposite directions. Now although in the model the fibrils are given the same inclination to the edge of the cube, for reasons of symmetry, we cannot assume in general that this is even approximately representative of the actual structure. This is particularly obvious since the cell is actually a polyhedron with 12 or 14 sides and the orientation of the ideal cube is quite arbitrary. Hence the direction of the fibrils in a new wall at division, which meets two walls on which the fibrils run in opposite directions, approximates to the one or the other of the directions on these walls according to the relative inclination of their fibrils and the relative proportions of wall surface.

It must be noted that the transverse wall of the initial can only become identical with a radial wall if the four "unique" edges are in tangential pseudo-transverse planes.* In this case, rotation of the new transverse wall around a *radial* line (which actually occurs in the cambium) causes a closer approximation of the direction of the fibrils in it to that in the longitudinal radial wall, as this transverse wall assumes a position approximating more closely to that of a radial wall. Any tilt

* That is to say, these four edges lie in tangential planes and are somewhat inclined to the horizontal.

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about a tangential transverse line would cause an apparent lack of homogeneity in the tangential walls. We cannot say that this does not occur to some extent in the case of the initials, but the radial expansion during their differentiation into tracheids will reduce such tilt considerably.

The process whereby a cell can be turned in three dimensions is far too complicated to follow without the use of an actual model. An illustration can, however, be given in two dimensions, though it is not suggested that this constitutes any rigorous proof.

Suppose ABCD, fig. 18, be a longitudinal section of a cell dividing in a random fashion, and that AD be a direction along which it can extend, parallel to the arrow. If division takes place along EF, then the direction of the micelles in the wall EF_1 will be somewhat the same as the direction of those on ED and DF. Similarly the subsequent division along, say, GH, will produce a wall GH_1 whose micelles are in a similar direction to those on FC and CH. Also similarly for EI_1 and IK_1. In

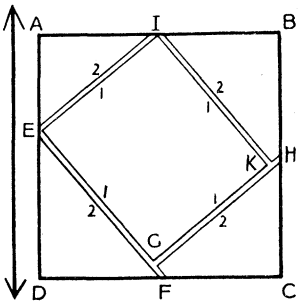

FIG. 18

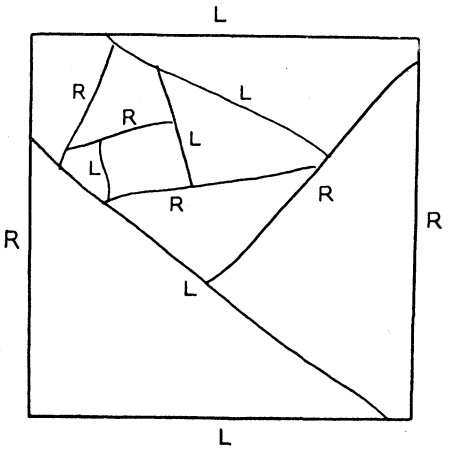

FIG. 18*a*—Two-dimensional representation of the divisions necessary to " turn " a cell. A wall with a right-hand spiral is denoted by R and with a left by L.

two opposite walls, say, IK_1 and EG_1, of the new cell EGIK the micelles will run in opposite directions. The micelles on IB run in an opposite direction to those on DF ; those on BH run in an opposite direction to those on ED ; hence in the walls EG_1 and IK_1, whose micellar directions are roughly determined by those on the constituent walls mentioned, the micelles will run in opposite directions. Suppose

extension along AD yields a right-hand and along AB a left-hand spiral. Then, especially if AD is more strongly right handed (*i.e.*, its inclination is great) than DC is left-handed, extension of the new cell along EG yields a right hand spiral; in GH, on the other hand, since the new wall is more nearly parallel to FC than CH, we can have a left-hand spiral. Another set of divisions may turn the new wall corresponding to GH so that it lies parallel to the original old wall AD. When conditions then cause cells to extend parallel to the arrow, cells such as ABCD will give a right-hand spiral and cells such as this new one a left-hand spiral. The necessary divisions are given in more detail in fig. 18*a*. By such divisions, tracheids developed from essentially similar apical meristem cells can have different spiral signs. At the same time it is to be noticed that more regular divisions (in more or less parallel planes) will produce a meristem whose cells can give tracheids of one sign only. Hence the distribution of spiral signs in the wood may be traced to the method of division in the meristem.

Perhaps the interest of the proposed model of the cell of the apical meristem is more clearly expressed when the winding is drawn upon a sphere, *i.e.*, when we use as a model the form which a meristematic cell would presumably take when isolated from its neighbours. In addition, such a spherical model may be of use later on in comparing the structure of other cells, whether spherical or of some other shape, which may later become available for investigation. Drawings of this spherical model are given in fig. 19. Two different aspects of the model are given, the com-

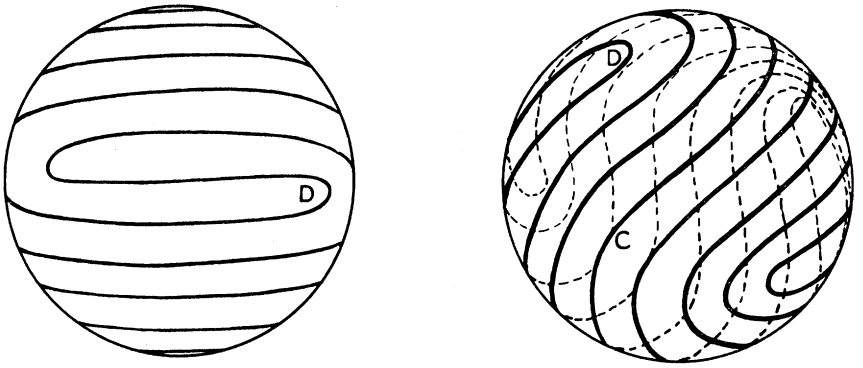

FIG. 19 (*a*) and (*b*)—Spherical models for the proposed structure of the wall of apical meristem cells. The letters C, D correspond to those on Plate 18.

plete " spiral " winding being included in (*a*) only. The winding obviously resembles the seam in a tennis ball to some extent, though the model as a whole possess three axes of two-fold symmetry. In making any attempt to compare this structure with that of any other cell whose form is that of a polyhedron, it must be remembered that on any face of the polyhedron the " fibrils " will probably run in continuous straight lines. Any " turning back " of the fibrils will probably occur at an edge, so that we may expect some small distortion of the winding. This may be illustrated by a comparison of the cubical and the ideal spherical model.

We may therefore visualize a series of meristematic cells at the shoot apex with a wall structure such that a spiral of each sign is inherent in it. These cells are oriented in a random fashion. Now those which can elongate parallel to the axis of the shoot to give a spirally wound cell proceed to differentiate into the cells of the procambium. This is probably taking place under the influence of the extension in the surrounding vacuolating cells, so that the wall spiral may be expected to become steeper. Considerations of the tangential walls of tracheids, discussed below, lead us, however, to assume that the inclination of the micelles in a new wall at division, deposited free from the influence of an old wall, will correspond more closely with that of the apical meristem cell from which the procambial cell in question arose. We may therefore deduce that upon older walls the spiral will be very steep (with an inclination of the order of 20° if the inclination of the meristem cell is 45°), while younger walls will have a somewhat flatter spiral. After this stage, the inner tissues cease to vacuolate and divide so that the shoot ceases to increase in length. Longitudinal extension of the initials can now take place only with difficulty, and tangential extension is impossible except so far as it is allowed by the increasing girth of the stem. The easiest direction for increase in volume would seem to be radial. It may be significant that after the procambial stage the cells divide exclusively by tangential walls and never by radial walls. The tangential walls of tracheids in a file, being produced time after time under almost identical conditions, will have almost the same inclination. On the other hand, radial walls should have constantly varying inclinations. The wall is extending in length, and it seems that the effect of this extension on the inclination of the micelles will not be considerably disturbed by the lateral expansion of the cell during differentiation. The case of the radial walls is analogous to that described above (p. 143) and to which the formula $L = K \cot \theta$ applies if we make one assumption, an assumption which is, in fact, forced upon us. If the radial expansion of a cambial initial after each longitudinal division causes any flattening of that part of the spiral on it, we should expect the inclination of the micelles in the radial walls of the tracheids cut off to be almost 90° within a very short time. Actually no such increase in inclination was observed, the part of the spiral on radial walls becoming steeper with time in all cases. We are compelled provisionally to assume that this radial extension during an active growing period has no effect on the inclination ; as shown in the last section, this extension during a growing period does not necessarily contradict the general principles laid down in this paper. Making this assumption we have, *in effect*, cells which are elongating at constant girth since change in girth causes no change in inclination, and we should expect the formula $L = K \cot \theta$ to apply more or less accurately. This applies only to radial walls. Tangential walls are constantly being renewed by longitudinal division, each new wall proceeding to differentiate into a tracheid wall before any appreciable elongation has taken place. On the other hand, radial walls are always in contact with tangential walls whose fibrils complete the spiral, and those walls which remain meristematic are always extending. We have, then, cells which are extending in length with no alteration in circumference, the change

in inclination being only observable on radial walls. The radial walls of tracheids in a file should then form a series in which the inclination of the fibrils to the vertical steadily decreases as we proceed from pith to bark.

It is thus possible to relate the modifications in wall structure occurring in the cells under consideration to the simultaneous changes in dimension of the cell. There is no obvious reason why this conclusion cannot be generalized to cover all extending cell walls of the plant. A possible explanation of the results of JACCARD and FREY is immediately obvious. The greater length of tension tracheids may account for the steeper spirals. It is very doubtful, however, whether this is the correct explanation. Although tension tracheids are in general longer than pressure tracheids, evidence exists to show that the cambial initials giving rise to pressure tracheids have divided more often and therefore must have elongated more than tension tracheids.

CONCLUSION

The striking agreement between the experimental results and the predictions from theory is sufficiently obvious to require little further emphasis. While this agreement does not necessarily prove that the present theory is correct in detail, it suggests that it is sufficiently true in outline to encourage further research along similar lines. The walls of the meristematic cells of the shoot apex have been shown to possess a structure such that spirals of both sign are inherent in any individual cell. It has been suggested that, by a process of random division, these cells can so change their orientation that those which elongate parallel to the axis of the shoot can produce procambial cells whose walls are wound with one spiral of either sign. The variation of the numbers of cells with each spiral sign is probably to be traced to the method of division among the iso-diametric cells of the shoot apex. In turn, these procambial cells elongate to form the cambium. Up to this stage all the walls of each cell can be regarded as undergoing the same transformation and, on the lines of the argument developed in this paper, may be expected to correspond to a rather steep spiral. In the cambium, however, conditions are somewhat different. While the radial walls of the cambial initials continue to elongate enormously, each individual tangential wall, being renewed frequently by the repeated tangential division, only undergoes a slight specific elongation. The fibrils of the tangential walls make, therefore, a much greater angle of inclination with the cell axis than do those on the corresponding radial walls. The differentiation of such a cell into a tracheid causes a considerable flattening of that part of the spiral on the radial walls, so that the walls of the tracheids should have similar fibrillar inclinations. This is found to be so. Moreover, the variation of this inclination on tracheid walls from one annual ring to the next can be explained adequately by a corresponding change in the fibrillar direction in the walls of the cambium cells as they elongate.

Some parts of the theory are certainly not, as yet, quite as clear and concise as could be wished, but it must be remembered that the aim of the present paper is

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not to formulate any settled account of the problem but to indicate the first preliminaries towards its solution. The analogy between the cambial initial and a cell growing at constant girth is by no means strict. The element of uncertainty which enters here, due to the different fibrillar inclinations which we have assumed as corresponding to the radial and tangential walls of the initials, is rather unsatisfactory and the analogy would seem to require further consideration.

A conception which seems to be of fundamental importance, however, has arisen in the theoretical considerations. We have deduced that while the longitudinal extension of a cambial initial causes a change in the inclination of the cellulose spiral, the radial expansion after each tangential division must have no corresponding effect. It is significant that these two changes in cell dimension seem to occur by totally different methods. The radial expansion certainly takes place against comparatively large pressures, and is solely due to a growth process. The longitudinal extension, on the other hand, is to be attributed, in part at least, to a passive deformation caused by these pressures. While the actual difference in the manner in which a cell can expand under these two conditions is not yet quite clear, it may be pointed out that during the deposition of new primary wall substance the micelles are probably not linked together as firmly as we consider them to be in a secondary wall. The conception of a rigid spiral winding is then somewhat erroneous, particularly when considering only the insertion of new particles of wall substance. The experimental results presented above give indirect indication that any change in pitch due to wall deformation is probably to be connected with the *excess* of the deformation over that which would have occurred by a pure growth process in the absence of any external forces. This would suggest that while the radial expansion of an initial after each tangential division is due solely to growth, the longitudinal extension is due mainly to external forces in the first few years of radial growth. The radial expansion of a differentiating tracheid, on the other hand, taking place under the influence of a rapid intake of water, cannot be regarded in any sense as a pure growth process. Hence the flattening of the spiral during this expansion in no way contradicts these conclusions.

One or two points which arise from the experimental results require a little further consideration. In the first place the approximate constancy of fibrillar inclination on tangential walls seems to suggest that there is in the protoplasm some mechanism which can not only orientate the micelles laid down in a new wall, *but can continue to do so over long periods in an exactly similar manner.* It must be pointed out that this is only an inference and is not directly contained in the results. The actual known fact is that the *average* inclination on tangential walls remains constant from year to year ; the individual readings may be varying wildly in any file of tracheids within the measured limits of variation, although this seems quite improbable. Since now the radial walls of an initial seem to have quite a different inclination from the tangential walls, the directing influence of an old wall must overrule any orienting tendency in the protoplasm. We may conclude, therefore, for the cases studied, that in the absence of any old wall the protoplasm can lay down

cellulose micelles in a definite direction over a considerable period, but that *when an old wall is present the direction of the micelles in a new layer conforms more closely to that in the old wall than to that imposed on it by the protoplasm*. There seems to be no reason why this cannot be generalized to cover the majority of vegetable tissues.

The fact that here and there we get fluctuations in the inclination on tangential walls, corresponding to those on radial walls, indicates that the micelles in the radial wall can influence in some way the direction in which the micelles are laid down in the tangential walls. The number of " coincidences " in the curves for cedar (fig. 12) is too great to have arisen by pure chance. These fluctuations are not due to any variation in the protoplasmic mechanism, for the new radial layer is laid down under the influence of an old wall. An alternative explanation can be put forward if the new wall layer is laid down as a continuous layer surrounding each daughter protoplast. The fibrils will be more or less continuous on passing from a radial to a tangential wall. It may be that at the point where a fibril is turning the corner from a radial to a tangential wall, it can impose upon that part of it which forms the tangential wall an approximation to its own direction in the radial wall. The conception is, of course, extremely vague as all such speculations must be until a direct method of attack on the cambium is developed, but such a mechanism would not seem to be *a priori* absurd. It is thus possible that *the existing walls of a cell undergoing division can to some extent affect the structure of a new dividing wall*. Any considerable variation in the protoplasmic mechanism might be expected to lessen the effect of such a process, so that we can also observe variations in the direction of the micelles in the tangential walls independent of any fluctuations in the radial walls.

It will be observed that, in spite of the conclusions of JACCARD and FREY that ring width has an effect on the spiral pitch of tracheids, the present account does not include any correction for this factor. In the first place, the variations in ring width were not considered to be sufficiently great to cause any serious disturbances, assuming the validity of their results for both radial and tangential walls. In the second place, on making certain assumptions, considerations can be put forward to show that variations due to such a cause may be expected on tangential walls only. Consideration of this effect of ring width are, however, postponed until further experimental evidence is available.

ACKNOWLEDGMENTS

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Summary

(1) The tracheids of the softwood or conifer are produced by repeated tangential division of the cambial initials, which are in turn developed from the procambial tissue and finally from the meristematic cells of the shoot apex. Both cambial initials and tracheids are extremely long, thin cells with approximately rectangular cross-section, two longitudinal walls lying in the tangential, and two in the radial, plane.

(2) While the cambial initials continue to elongate for years, the tracheids cut off from them are dead, and do not change in dimension.

(3) The cellulose portion of the tracheid wall consists of micelles which lie at a fairly constant angle to the long axis of the cell, forming a single spiral round it. This spiral can be either right- or left-handed.

(4) The inclination of the micelles to the long axis is rather less on tangential than on radial walls.

(5) The average inclination of the micelles on the tangential walls of the tracheids in the spring wood of any annual ring is the same as that of any other, to a fair approximation.

(6) The average inclination θ of the micelles on the corresponding radial walls varies from one annual ring to the next according to one of the formulæ,

$$L = K \cot \theta$$
$$L = KB \cot \theta,$$

where L = average length of tracheids in the spring wood of the annual ring,
B = average radial breadth,
and K = constant.

(7) It is deduced that the walls of the cambial initials are constructed similarly to those of the tracheids, except that the inclination of the micelles on tangential walls is greater than on radial walls.

(8) The variation of length with micellar inclination is adequately explained by a corresponding change in the inclination on the walls of the initials as they elongate, according to the formula

$$L = K \cot \theta$$

i.e., the cambial initials are analogous to spirals increasing in length at constant girth.

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(9) A structure is suggested for the walls of the meristematic cells of the shoot apex, in which spirals of both sign are inherent. These cells exist at the apex in all orientations, and those which can elongate parallel to the axis of the shoot to give a spirally wound cell differentiate into tracheids. The variation of spiral signs among the tracheids thus depends on the orientation of the apical meristem cells.

(10) It is suggested that this random orientation of cells, which have all been developed from a single cell, is to be connected with the process of random division.

(11) From the details of the variation of the micellar inclination on tangential and radial walls, certain deductions are made as to the method of orientation of cellulose particles in a wall, as follows.

(12) The micelles in a new layer deposited on an old wall are so oriented by the *old wall* that they lie parallel to the micelles in it.

(13) The protoplasm itself can orientate the cellulose particles of a *new wall* at division, and can continue to do so for considerable periods of time in an exactly similar manner.

(14) At the same time, the structure of a new wall at division may to some extent be influenced by the existing walls.

(15) The principles underlying the present investigations may well be applied to other growing cells. It would seem that, in general, when a cell is changing in dimension owing to the application of external forces the change in wall structure is to be associated with the *excess* of the change in cell dimension over that which would have occurred under a pure growth process. On the other hand, no change in wall structure is anticipated in cells which are expanding by a pure growth process.

References

Ambronn and Frey (1926). "Das Polarisationsmikroskop," Leipzig.

Anderson, D. B. (1928). 'Jahrb. wiss. Bot.,' vol **69**, p. 50.

Astbury, W. T., Marwick, T. C., and Bernal, J. D. (1932). 'Proc. Roy. Soc.,' B, vol. **109**, p. 443.

Bailey, I. W. (1920, *a*). 'J. Gen. Physiol.,' vol. **2**, p. 519.

—— (1920, *b*). 'Amer. J. Bot.,' vol. **7**, p. 363.

—— (1915). 'Bot. Gaz.,' vol. **60**, p. 66.

Balls, W. L. (1919). 'Proc. Roy. Soc.,' B, vol. **90**, p. 542.

Beijer, J. J. (1927). 'Rec. Trav. bot. néerland,' vol. **24**, p. 631.

Braun, A. (1854). 'SitzBer. preuss. Akad. Wiss.,' p. 432.

Denham, H. J. (1923). 'J. Text. Inst.,' vol. **14**, No. 4, p. T 85.

Devaux, A. (1928). 'Bull. Soc. bot. Fr.,' vol. **75**, p. 90.

Fisher, R. A. (1932). "Statistical Methods for Research Workers," Oliver and Boyd.

FREY, A. (1926). 'Jahrb. wiss. Bot.,' vol. **65**, p. 195.
FREY-WYSSLING, A. (1930). 'Z. Wiss. Mikr.,' vol. **47**, p. 42.
HARTIG, R. (1901). "Holzuntersuchungen, Altes und Neues," Berlin.
HAWORTH, W. N. (1929). "The Constitution of Sugars," London.
HENGSTENBERG, J., and MARK, H. (1928). 'Z. Krystallogr.,' vol. **69**, p. 271.
HERZOG, R. O., and JANCKE, W. (1928). 'Z. phys. Chem.,' A, vol. **139**, p. 235.
JACCARD, P., and FREY, A. (1928). 'Jahrb. wiss. Bot.,' vol. **68**, p. 844.
KLINKEN, J. (1914). 'Bibl. Bot.,' Stuttgart, vol. **19**, p. 1.
MEYER, K. H., and MARK, H. (1928). I. 'Ber. deuts. chem. Ges.,' vol. **61** B, p. 593.
—— (1929). II. 'Z. phys. Chem.,' B, vol. **2**, p. 115.
—— (1930). "Der Aufbau der hochpolymeren organischen Naturstoffe."
NÄGELI, C. (1928) 'Ostwald's Klass. exakt. Wiss.,' vol. **227**.
PRESTON, R. D. (1931). 'Proc. Leeds Phil. Soc.,' vol. **2**, p. 185.
PRIESTLEY, J. H. (1930). 'New Phytol.,' vol. **29**, nos. 1, 2, and 5.
—— (1932). 'Rep. Brit. Ass.,' Section K, at York.
REIMERS, H. (1922). 'Mitt. deuts. Forsch. Inst. Textlilst.'
ROBINSON, W. (1919). 'Phil. Trans.,' B, vol. **210**, p. 49.
SEIFRIZ, W. (1931). 'J. Phys. Chem.,' vol. **35**.
SPONSLER, O. L. (1925). 'J. Gen. Physiol.,' vol. **9**, p. 221.
—— (1925-6). 'J. Gen. Physiol.,' vol. **9**, p. 667.
SPONSLER, O. L., and DORE, W. H. (1926). 'Colloid. Symposium. Monogr.,' vol. **4**, p. 174.
STEINBRINCK (1927). 'Naturwiss.,' vol. **15**, p. 978.
VAN WISSELINGH (1924). "Die Zellmembranen" Linsbauer K. d. Pflanzenanatomie, vol. **3**, Berlin.
WIENER, O. (1912). 'Abh. sächs. Ges. (Akad.) Wiss.,' vol. **32**.

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Description of Plates

Plate 17

Fig. 20—X-ray microphotograph of a single tracheid of *Sequoia*. The outer diffraction spots represent the 3·9 A spacing.
Each row of spots is perpendicular to the cellulose fibrils producing it.
Tracheid parallel to the longer edge of the paper.

Fig. 21—Enlargement of fig. 20. The angle θ between the rows of spots is 47°.

Fig. 22—Photomicrograph of that part of the specimen whose X-ray photograph is given in the above figs. The cracks in the wall (which are parallel to the major extinction positions) are clearly perpendicular to the rows of spots on the X-ray photographs, and the angle θ between them is 46°.

Fig. 23—Photomicrograph of a longitudinal section of a shoot growing point (bud) of *Pinus sylvestris*. *a*, cells of the apical meristem, *b*, *b*, vacuolating cells, *c*, *c*, strands of procambium cells which later develop the vascular tissues.

Plate 18

Fig. 24—The proposed models for the wall structure of meristematic cells in the conifer.

(*a*) Meristematic cell of the shoot apex, made cubical for simplicity. Both spiral signs are present in this cell.

(*b*) Cell of cambium (and procambium), produced from (*a*) by elongation parallel to XX′, and having a left-hand spiral.

(*c*) Cell of cambium (and procambium), produced from (*a*) by elongation parallel to ZZ′, and having a right-hand spiral.

For further description see text.

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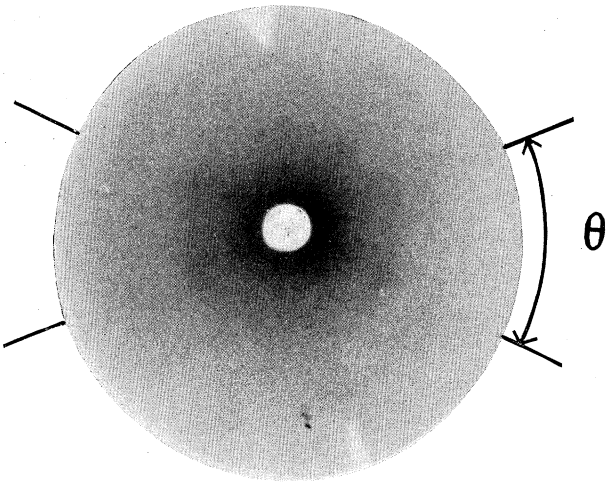

FIG. 21

FIG. 22

FIG. 20

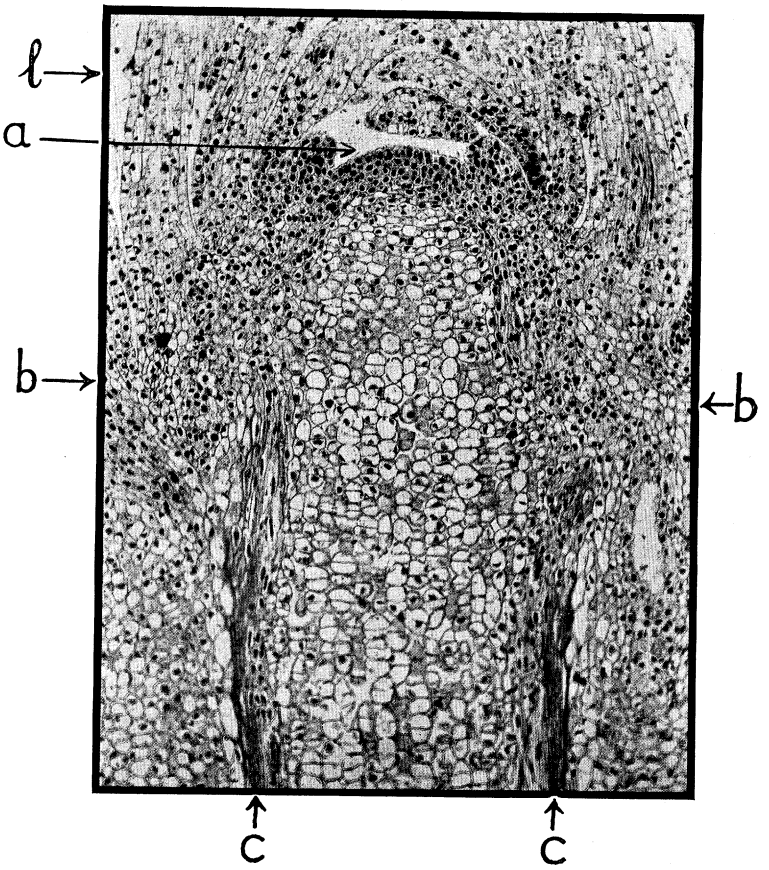

FIG. 23

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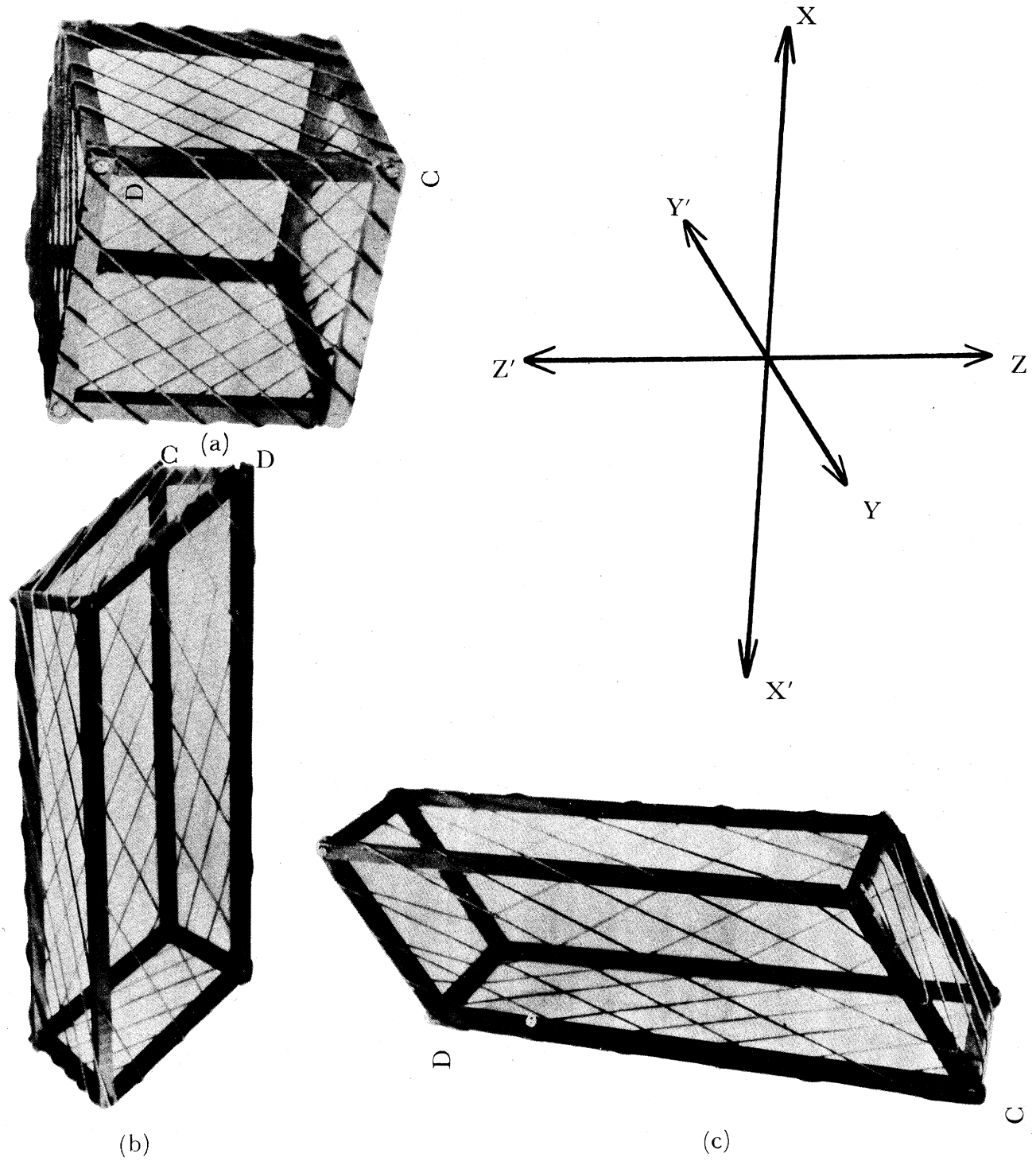

Fig. 24.

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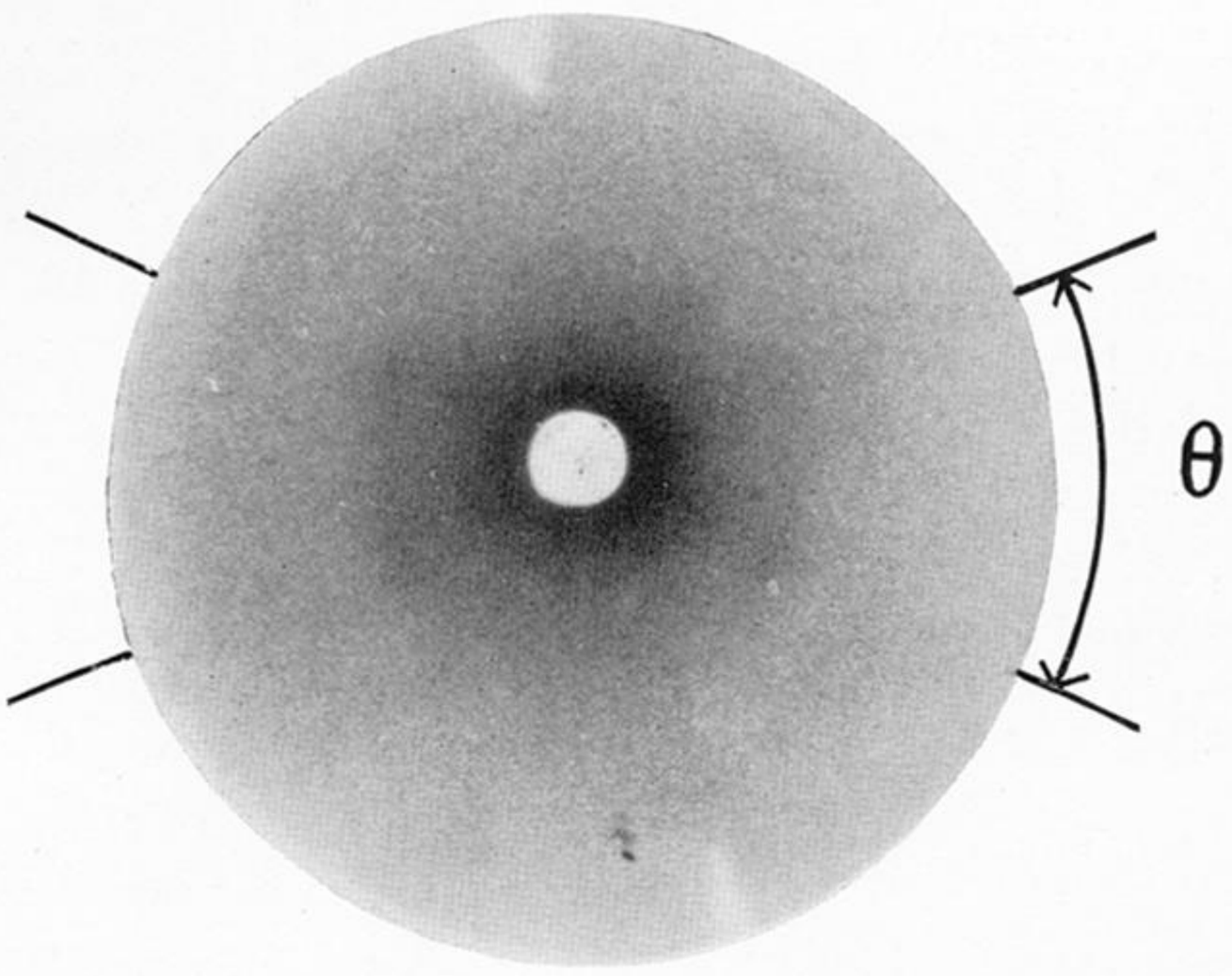

FIG. 21

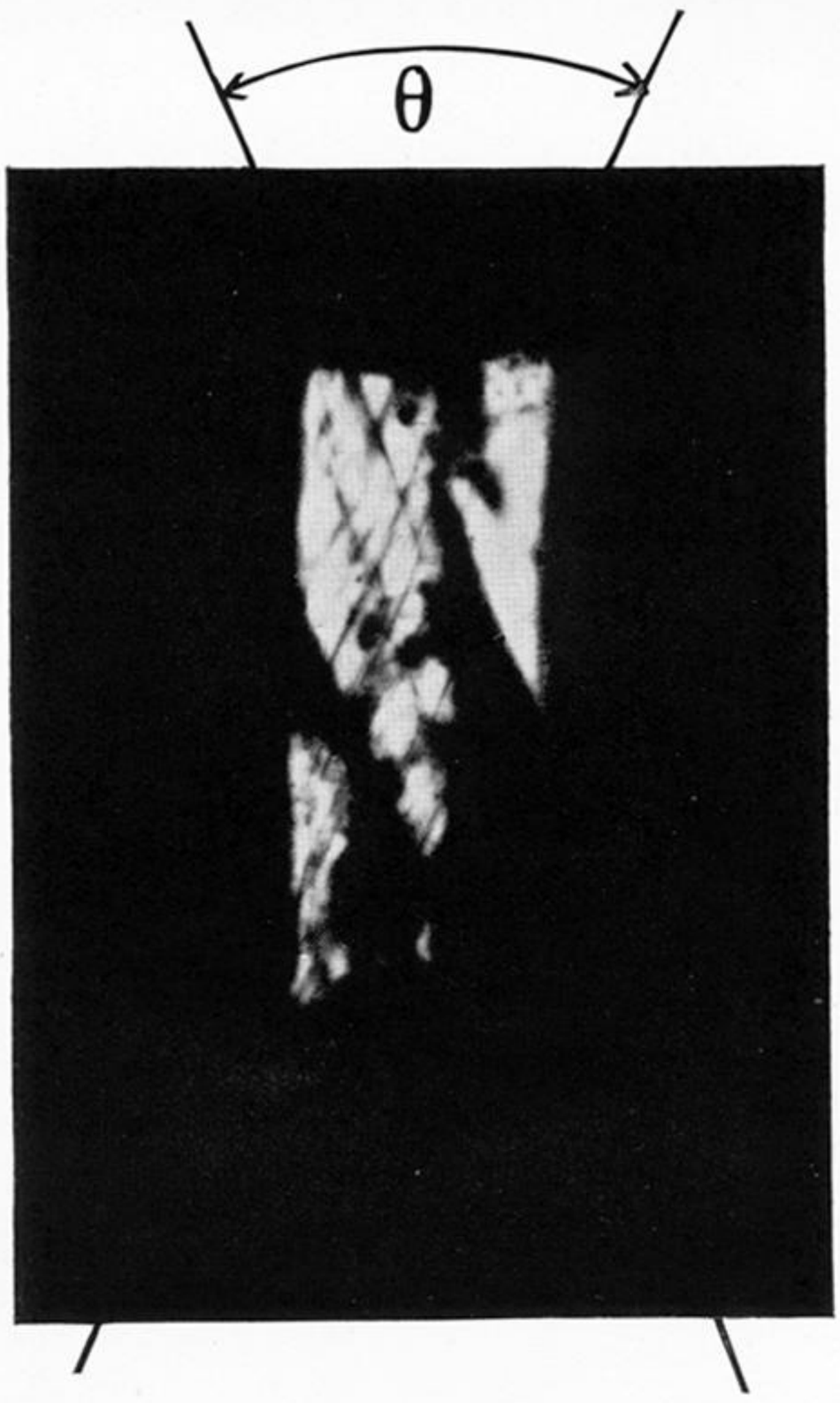

FIG. 22

FIG. 20

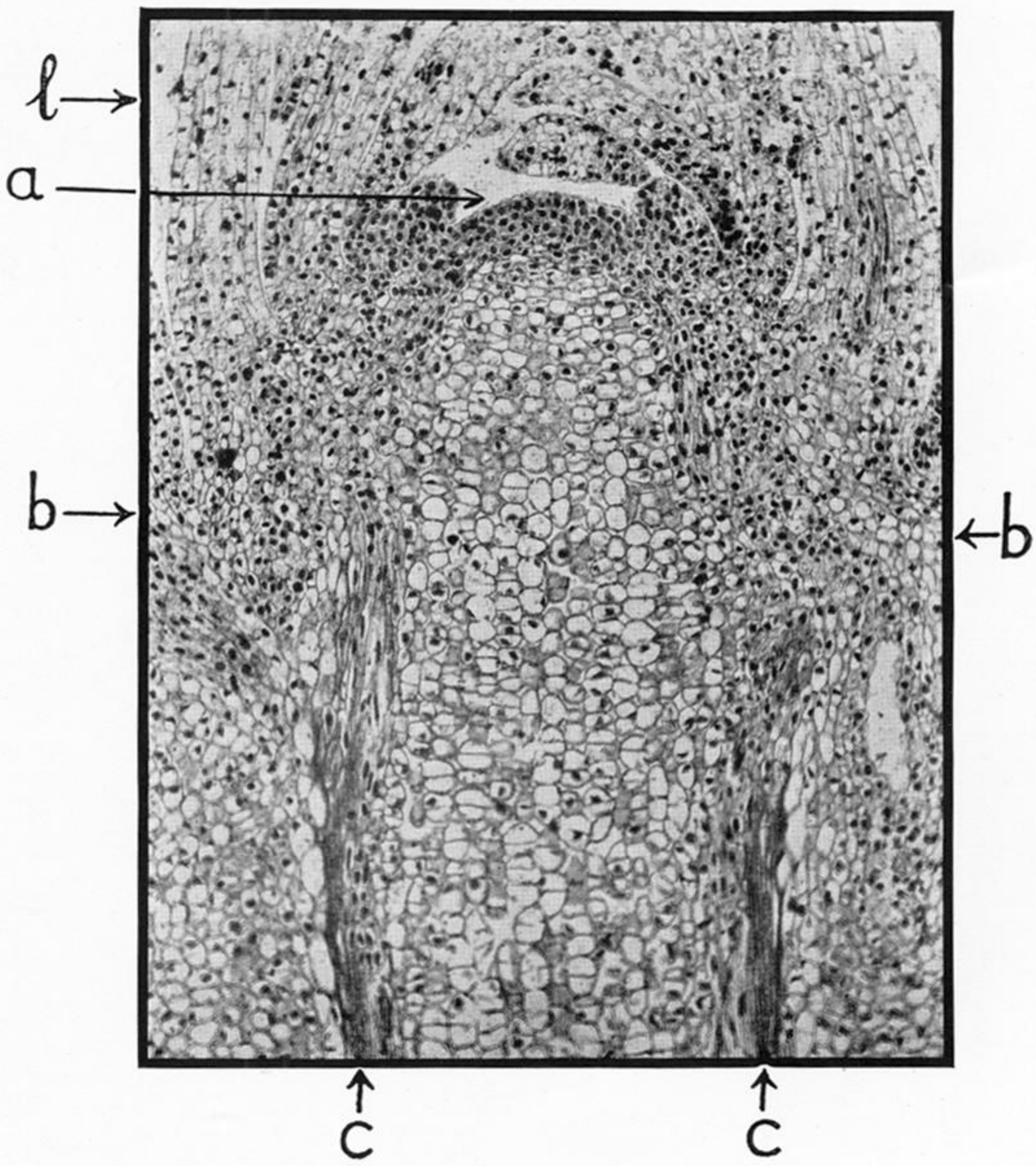

FIG. 23

PLATE 17

FIG. 20—X-ray microphotograph of a single tracheid of *Sequoia*. The outer diffraction spots represent the 3·9 A spacing.
Each row of spots is perpendicular to the cellulose fibrils producing it.
Tracheid parallel to the longer edge of the paper.

FIG. 21—Enlargement of fig. 20. The angle θ between the rows of spots is 47°.

FIG. 22—Photomicrograph of that part of the specimen whose X-ray photograph is given in the above figs. The cracks in the wall (which are parallel to the major extinction positions) are clearly perpendicular to the rows of spots on the X-ray photographs, and the angle θ between them is 46°.

FIG. 23—Photomicrograph of a longitudinal section of a shoot growing point (bud) of *Pinus sylvestris*. *a*, cells of the apical meristem, *b*, *b*, vacuolating cells, *c*, *c*, strands of procambium cells which later develop the vascular tissues.

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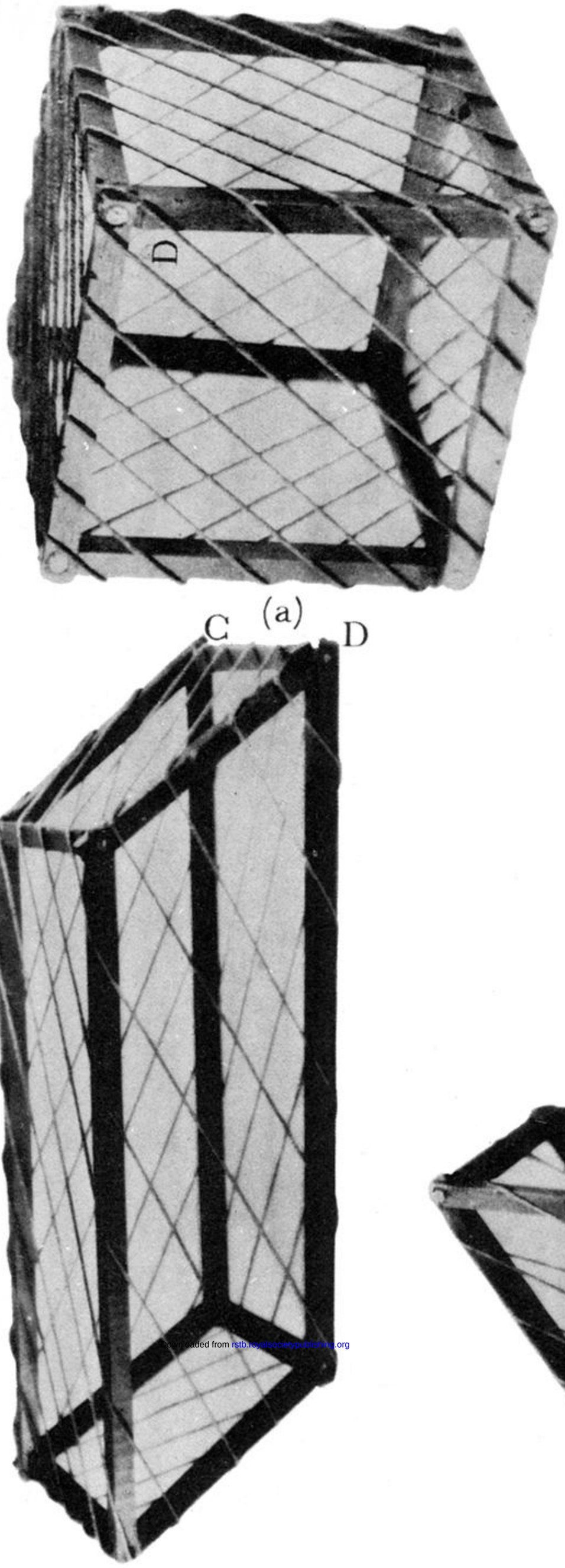

FIG. 24.

PLATE 18

FIG. 24—The proposed models for the wall structure of meristematic cells in the conifer.

(*a*) Meristematic cell of the shoot apex, made cubical for simplicity. Both spiral signs are present in this cell.

(*b*) Cell of cambium (and procambium), produced from (*a*) by elongation parallel to XX′, and having a left-hand spiral.

(*c*) Cell of cambium (and procambium), produced from (*a*) by elongation parallel to ZZ′, and having a right-hand spiral.

For further description see text.