THE SIGNIFICANCE OF THE CAMBIUM IN THE STUDY OF CERTAIN PHYSIOLOGICAL PROBLEMS.

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PLATE 1.

(Received for publication, January 20, 1920.)

INTRODUCTION.

During the last few years, I have had occasion to devote considerable attention to the study of the cambium of the higher plants, in endeavoring to determine to what extent variations in the structure of timber affect certain of its physical and mechanical properties. In conducting these investigations, I have been impressed by the fact that the cambium is an unusually favorable medium for the study of certain theoretical problems, particularly the working sphere of the nucleus, the much discussed nucleocytoplasmic-relation, and the dynamics of karyokinesis and cytokinesis. It seems advisable, accordingly, to call attention to certain phenomena[1] in the cytology of this meristematic tissue which may be of general interest to physiologists as well as to cytologists.

Description of the Material.

As is well known, the stems and roots of gymnosperms and dicotyledons increase in diameter through the activity of a jacketing layer of undifferentiated tissue which forms xylem internally and phloem externally. In the case of most gymnosperms and arborescent and fruticose dicotyledons, the initials of this lateral meristem or cambium continue to divide throughout the life of the plant, and their more or less highly differentiated derivative cells constitute the bulk

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[1] These phenomena will be described and discussed in detail in a series of papers to be published in the *American Journal of Botany.*

of the tissue of an adult individual. The cambial initials are of two distinct shapes and sizes: (1) numerous, large, much elongated (parallel to the long axis of the stem or root) elements; and (2) scattered aggregations of small, more or less isodiametric cells which divide to form the horizontal sheets of radially disposed parenchyma, the so called medullary rays. The principal divisions in both types of initials are periclinal or parallel to tangents to the circumference of the stem or root. In other words, the large cells divide in a tangential longitudinal plane, which is a division plane of *maximal* area, whereas the ray initials form partitions that commonly are surfaces of *minimal* area.

The tangential diameter of the cambial initials increases to a certain extent during the earlier stages of the enlargement of stems and roots, but falls far short of being sufficient to compensate for the rapid increase in the periphery of the cambium. Nägeli inferred from this that the elongated initials must divide periodically in a radial longitudinal plane. He even elaborated formulas for computing the frequency of such divisions during a given increase in the radius of a stem. However, as is not infrequently the case with *a priori* mathematical deductions concerning complex biological phenomena, Nägeli's generalization is supported by few, if any, of the actual facts. Although the hypothetical radial longitudinal divisions are described and figured in many botanical text-books, I have been unable to find them in any of the gymnosperms and less highly specialized dicotyledons that I have studied. The cells slowly elongate, sliding by one another, until they have reached a certain size. They then divide by means of a more or less oblique transverse partition into two short halves, which in turn elongate and divide.[2] Thus, the increase in the periphery of the cambium is due primarily not to radial longitudinal divisions of the large initials accompanied by lateral enlargement of the products of such divisions, but to the formation of transverse partitions, followed by elongation or longitudinal "sliding growth."[3]

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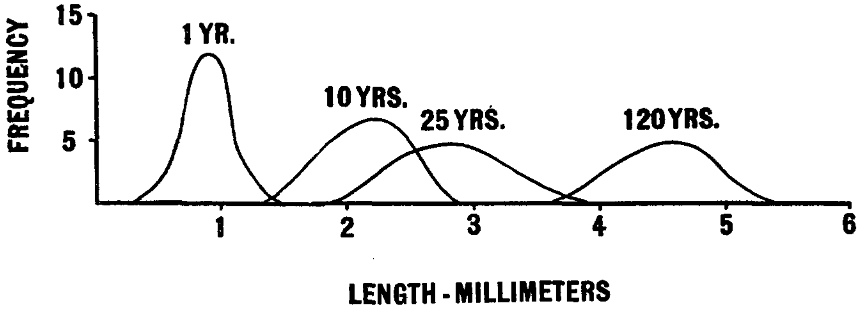
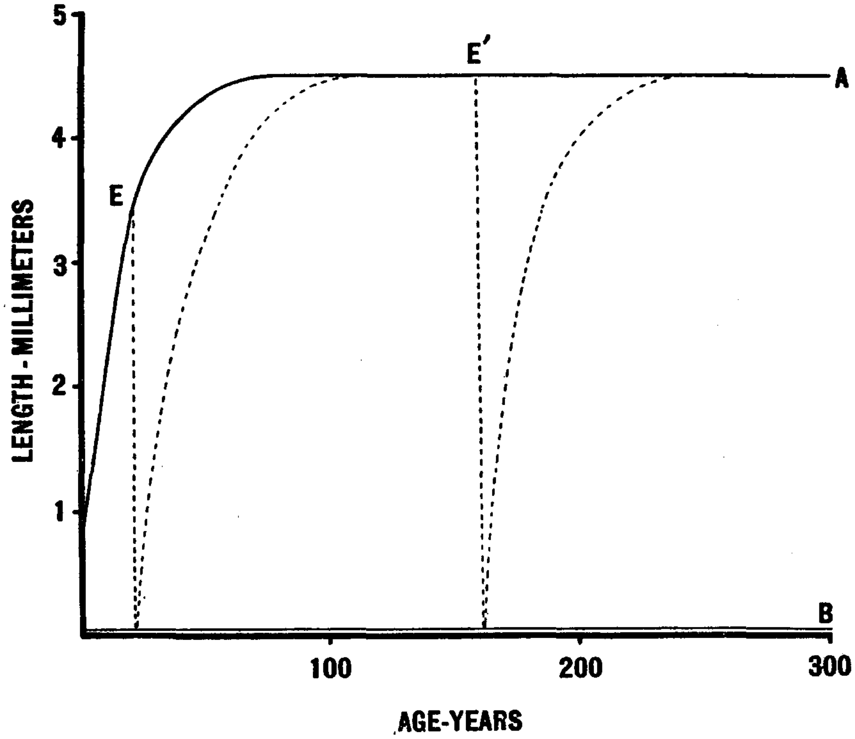
[2] It should be kept clearly in mind, in this connection, that during the process of elongation, between successive transverse divisions, the cells continue to divide in the tangential longitudinal plane.

[3] This phenomenon of sliding growth is of considerable practical significance in the study of the properties and utilization of timber.

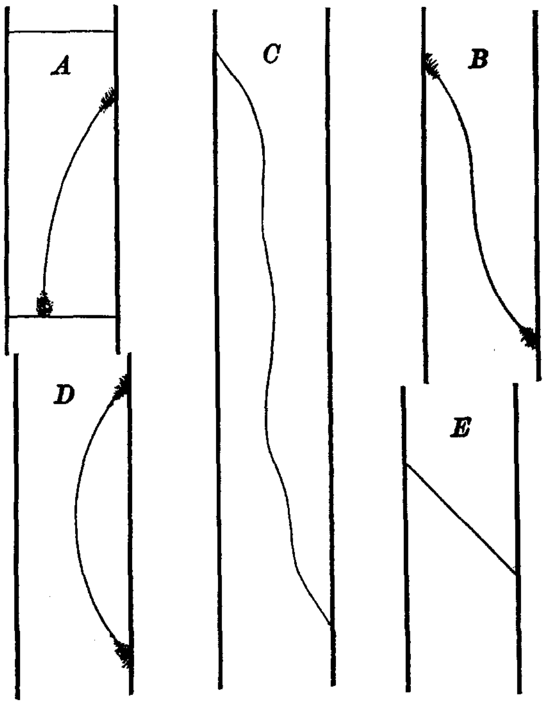


Text-Fig. 1. Frequency curves showing variation in the size of cambial initials in stems of different ages.



Text-Fig. 2. A, curve showing variations in the average size of large cambial initials with increasing age of a stem. B, size level of cells of the general order of magnitude of the cells of the embryo and growing points. E and E′, curves showing effects of experimentally induced changes in cell size.

Owing to the fact that the initials do not divide and elongate in unison, there is usually a very considerable variability in the length, and *pari passu* in the volume, of adjacent elements. As shown in Text-fig. 1, the average, maximum and minimum lengths of the cells are considerably less in very young, slender, than in old, robust stems and roots; but the values do not continue to increase throughout the entire life of an individual. On the contrary, the curve of average length at successive ages is of the general type illustrated in Text-fig. 2 (A). There is a rapid increase in length for a period of years until a certain size is attained, which then remains constant during succeeding growth.[4] A similar, though much less striking, increase



TEXT-FIG. 3. Acute intersections of division membranes. A, from initial dividing to form callus. B, C, and E, from initials dividing pseudotransversely. D, from initial dividing unequally to form new ray initials.

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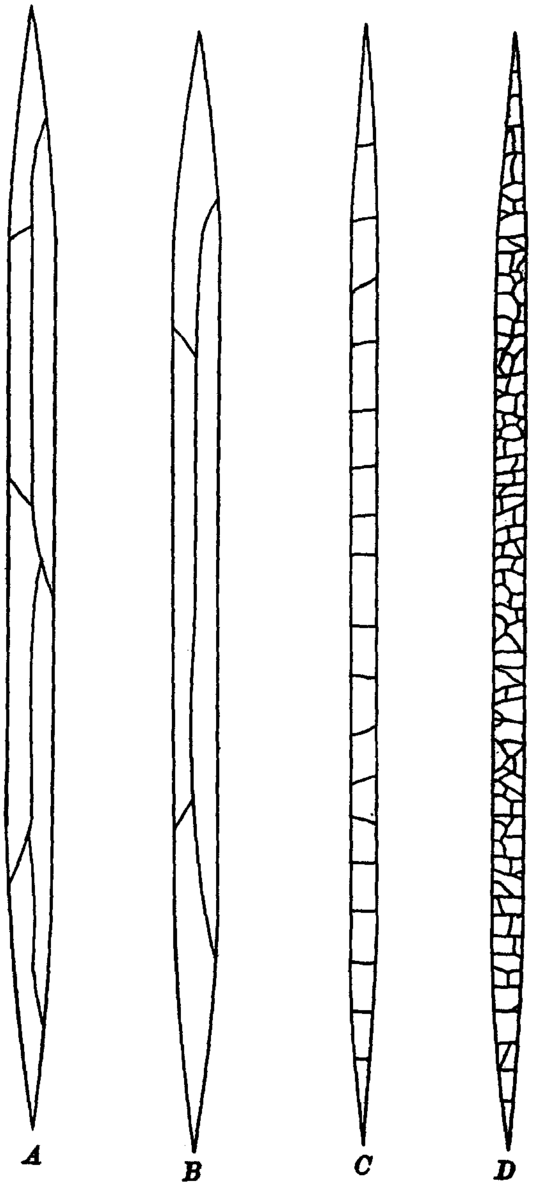
[4] The curve for any particular level or radius of a tree usually fluctuates about this norm, owing to the effects of various environmental factors.



TEXT-FIG. 4. A and B, acute intersections of division membranes in the daughter cells of the cambium of certain dicotyledons. C and D, angles of intersection in initials which are dividing to form callus.

commonly occurs in the tangential diameter of the cells; but in the gymnosperms the length of the initials is so great, in proportion to their breadth, that the volume curve closely parallels that for the longitudinal dimension. Under normal conditions the volume of the more or less isodiametric ray initials is very much less than that of even the smallest elongated initials (Figs. 1 and 2) and is of the same general order of magnitude as that of the undifferentiated cells of the embryo and terminal meristems or growing points.

To maintain a proper ratio between the two types of initials, in a layer whose periphery is continually increasing in area, new aggregations of ray initials are periodically carved out of the large initials by appropriate divisions of these elements (Text-fig. 3, D). Furthermore, under abnormal environmental conditions, *e.g.* injuries, all the elongated initials in a given area may be induced to divide into small, more or less isodiametric cells (Text-fig. 4, C and D). During the subsequent growth of this region of the meristem, certain of these small cells elongate and finally regenerate initials of normal dimensions, as shown by dotted lines in Text-fig. 2, E and E′.

The Problem of the Working Sphere of the Nucleus and the Maximum Size of Undifferentiated Plant Cells.

Sachs and Strasburger almost simultaneously called attention to the fact that undifferentiated, actively dividing and growing cells of plants, such as occur in embryonic and meristematic tissue, are relatively minute, and concluded that this was undoubtedly due to the fact that the working sphere of the nucleus is very restricted. Strasburger found that, in the case of "embryonic" cells of the growing points of various plants, the ratio between the average diameters of the nuclei and of the cells is as 0.003–0.016 mm.: 0.005–0.024 mm., or as 2:3; and Sachs pointed out that, although plants vary enormously in their linear dimensions (0.001 to 100,000 mm.), there is not a proportional variation (0.001 to 0.05 mm.) in the size of their constituent cells. Both investigators emphasized the fact that, even in highly differentiated tissues, unusually large or much elongated protoplasts[5] tend to be multinucleate.

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[5] Such as have been critically studied by Schmitz, Treub, Kallen, Johow, Haberlandt, Pirotta, Buscalioni, and many others.

In view of these and similar investigations, it might have been expected *a priori* that the large, elongated, undifferentiated cells of the lateral meristem or cambium, which in certain plants attain a length of more than 10,000 micra and a volume of 10,000,000 cubic micra, should contain more than one nucleus each. Such is not the case, however, in any of the somewhat extensive series of gymnosperms and angiosperms that I have studied. Nor do the initials contain abnormally elongated, giant nuclei, such as have been described and figured by Molisch for highly specialized tissue cells of certain monocotyledons. Each initial contains a single nucleus, which is centrally located and retains this position during the processes of growth and cytokinesis.

It is evident, accordingly, that there is a very much greater variability in the size of meristematic cells in plants than was suspected by Sachs or Strasburger, and that in elements of this type the nucleus may extend its "energizing" influence to a distance of several thousand instead of a few micra.[6]

The Relation between Cell Size, Nuclear Size, and Chromosomal Mass.

Strasburger's measurements led him to believe that there is a close correlation between cell size and nuclear size in the meristematic tissue of plants, a conclusion that was strongly supported by the experimental investigations of Gerassimow. The importance of this relation between cell size and nuclear size was further emphasized by Hertwig, and by Boveri, who endeavored to prove that "The size of the larval cells is a function of the quantity of chromatin which they contain, and the volume of the cell is in direct proportion to the number of chromosomes." The subsequent painstaking and detailed investigations of a number of zoologists have indicated that, although in general large cells tend to have larger nuclei than small cells, the nucleocytoplasmic-relation is not invariably a constant and self-regulating ratio. In many animals, it fluctuates within rather wide limits, not only as between different tissues, but even in embryonic

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[6] Sachs considered that the conditions in large animal eggs strengthened rather than weakened his case, since these highly specialized, yolk-containing cells are "inactive" until "energized" by numerous nuclei.

cells during different stages in ontogeny and under different environmental conditions. Conklin has emphasized the fact that in many cases the size of the nucleus is determined by the volume of protoplasm in which it lies, rather than by the number of chromosomes.

On the botanical side, Gates, Gregory, Winkler, and Tupper and Bartlett have shown that a number of races of plants, which have the tetraploid, instead of the diploid, number of chromosomes, are composed of cells larger than those of normal varieties. Winkler's paper is a particularly suggestive one, not only owing to the interesting experimental methods used in his work, but to his general discussion of the relation between cell size and chromosomal number in plants. He reaches the following conclusions, as a result of his own observations and those of a number of other investigators. In embryonic somatic tissue, terminal and lateral meristems, the cells are of nearly uniform size, are roughly isodiametric, and under normal conditions contain the diploid number of chromosomes. Multinucleate protoplasts, nuclear fusions, and changes from the diploid to the tetraploid and polyploid condition are of common occurrence in non-meristematic somatic tissue. In the latter tissue many cells depart widely from the inherited specific cell size of the plant. Such cells tend to be hyperchromatic; much elongated elements containing more than one nucleus each, and other types of large cells an abnormal number (tetraploid or polyploid) of chromosomes. In other words, Winkler considers that there is a very significant correlation between cell size and chromosomal mass, both in the embryonic and non-meristematic somatic tissues of plants.

As I have suggested at the beginning of this paper, the cambium appears to possess certain distinct advantages as a medium for studying various phases of the nucleocytoplasmic-relation. In this tissue adjacent cells vary greatly in shape and size and it is possible not only to compare elongated initials of very different lengths and volumes, but also to contrast them with adjoining ray initials which are of the same general order of magnitude as the cells of the embryo and terminal meristems. By proper experimental methods the long initials may be induced to divide into smaller and smaller units, until more or less isodiametric cells, which resemble the ray initials, are formed. These may subsequently elongate and regenerate elements of normal size. In regions having cold winters, there is a more or less prolonged resting period, during which the cambial cells are inactive (not undergoing division). This period offers a favorable opportunity for measuring and computing the relative sizes of the initials and their nuclei. Furthermore, during the active growing season, adjacent cells (ray initials and elongated initials of varying sizes) may be found in equivalent stages of karyokinesis and cytokinesis, which facilitates comparisons within the limits of a single section.

TABLE I.

Pinus strobus L.

Type.	Nucleus.				Cell.				Ratio between volume of nucleus and volume of cell.
	Dimensions.			Approximate volume.	Dimensions.			Approximate volume.	
	L	R	T		L	R	T		
	Cambium from 1 yr. old stem.								
	micra	*micra*	*micra*	*cu. micra*	*micra*	*micra*	*micra*	*cu. micra*	
Ray initials......	10.8	8.7	6.5	350	22.9	17.8	13.8	5,000	1:14
Large "	63	3.2	5.8	1,000	870	4.3	16.1	60,000	1:60
	Cambium from 60 yr. old stem.								
Ray initials......	12.4	12.5	9.9	850	24.8	26.6	17.0	10,000	1:12
Large "	82	5.9	8.9	3,500	4,000	6.2	42.4	1,000,000	1:286

Basis: dimensions of cells and nuclei are averages of 50 measurements.

In 1917, 1918, and 1919, the writer collected numerous specimens of the cambium of *Pinus strobus* L.—from different parts of the stem and root, from trees of different ages and sizes, and from varying environments—at frequent intervals during the resting and growing seasons. As shown in Table I and in Figs. 1 to 6, the larger initials of the common white pine tend to have larger nuclei, but the ratio between cell size and nuclear size fluctuates greatly in the case of the elongated initials. So far as I have been able to determine, however, all the nuclei, regardless of their size, contain approximately the diploid number (twenty-four) of chromosomes. The larger size of the elongated initials is not associated with a tetraploid or polyploid condition. Nor is it a concomitant of a marked increase in the size of the individual chromosomes, for I have repeatedly found small ray initials in which the chromosomes were fully as long and thick as those of adjoining large, elongated initials. The staining reactions of the various types of initials indicate that the chromatic material is more concentrated in the smaller than in the larger nuclei except during certain stages of karyokinesis; and that the increase in the size of the nuclei is due primarily to an increase in the volume of achromatic substances. Although there is no constant and striking difference in the number and size of the chromosomes in the large and small initials, *the volume of the nucleoli is conspicuously greater* in the larger cells (Figs. 3, 4, 5, and 6). This increase is associated with a *corresponding increase in kinoplasma* during karyokinesis and cytokinesis.

My observations upon *Pinus strobus*, therefore, do not support Strasburger's and Winkler's conclusions in regard to the constancy of "specific" cell sizes and nuclear sizes in the meristems of plants and are in opposition to Winkler's assumption that giant cells are hyperchromatic. In lateral meristems there are relatively great variations in cell size without corresponding changes in the number of chromosomes. In other words, in dealing with the nucleocytoplasmic-relation it is essential to distinguish between (1) those cases in which there is a correlation between cell size, nuclear size, and chromosomal mass, and (2) those in which chromosomal number (chromosomal mass) *is constant and nuclear size and cellular size are variable.*

Karyokinesis and Cytokinesis.

In the gymnosperms, as illustrated by *Pinus strobus*, the polar axis of the division figure usually does not stand at right angles to the long axis of the protoplast, but is placed diagonally across the cell (Fig. 7). This position of the karyokinetic figure is not an artifact, *i.e.* due to displacement of an ordinary spindle, since the whole figure is asymmetrically developed in conformity with its diagonal position. The formation of a cell plate, starting from one of these obliquely placed spindles, is a truly remarkable cytological phenomenon. The spindle becomes greatly extended laterally by the addition of peripheral "fibers," and gradually assumes a more or less curved form (Figs. 8 and 9). As more peripheral fibers are successively added, the remains of the original central fibers disappear (Fig. 9) from about the cell plate, leaving two separate aggregations of fibers that are connected by the first formed portion of the cell plate (Fig. 10). These aggregations of kinoplasmic fibers, which I have called *kinoplasmasomes*, have a very characteristic form and structure, both in gymnosperms and angiosperms. When the initials are dividing in the usual tangential longitudinal plane, the kinoplasmasomes extend across the cell—at right angles to the longitudinal axis—from one radial wall to the other (Fig. 12) and are located in the center of the protoplast, midway between its tangential surfaces (Fig. 10). In sectional view, they have a somewhat wedge-shaped outline, bluntly convex in front and tapering to a point at the rear along the cell plate (Fig. 10). They move in opposite directions towards the ends of the cell (Figs. 11 and 12). As they move forward the cell plate is extended until it eventually reaches the two ends of the cell, thus dividing the protoplast into two similar halves, each of which contains one of the daughter nuclei (*n*). The latter remain close together, near the center of the cell, during the process of cell plate formation, and I have been unable to demonstrate any visible connection between the daughter nuclei (*n*) and the kinoplasmasomes (*k*) or their constituent fibers. Not infrequently the distance traversed by the kinoplasmasomes, in passing from the vicinity of the daughter nuclei to the ends of the protoplast, may be from 500 to 5,000 micra. The two kinoplasmasomes usually, although not invariably, move forward at *equal rates*, so that at any given stage they appear to be equidistant from the starting point and daughter nuclei. Preliminary investigations indicate that the total time consumed in the process of cell plate formation is considerable, in all probability a matter of many hours rather than minutes.

In the normal, tangential longitudinal divisions of the large initials, the kinoplasmasomes, once they have curved into a position midway between the tangential walls of the cell, move in a straight line towards the ends of the protoplast; thus the division is a plane surface. This is frequently not true, however, in the case of the semitransverse divisions of the initials. The kinoplasmasomes meander more or less in certain cases and curved or undulating surfaces are formed in consequence (Text-fig. 3).

Dynamics of Cytokinesis.

The phenomena of karyokinesis and cytokinesis in the cambium are in direct contradiction to most generalizations concerning cell division. According to Sachs' Law, successive division planes should intersect at right angles; but in the cambium the successive longitudinal partitions are parallel. Hertwig's development of Sachs' Law hypothecates that the axis of the mitotic figures typically lies in the longest axis of the protoplasmic mass, and division therefore tends to cut this axis at a right angle; but in the cambium the axis of the mitotic figure is usually placed either at right angles to the long axis of the cell (angiosperms) or in a diagonal position (conifers, angiosperms). Errera's (Plateau's) Law of Minimal Area is based upon the assumption that the recently formed membranes of cells are semiliquid films which "tend to assume a form which would be assumed, under similar conditions, by a liquid film destitute of weight;" but in the cambium the partitions are commonly division planes of maximal area. Furthermore, according to this law, the division membranes of most plant cells[7] should intersect the sides of the cells at right angles. De Wildeman, Thompson, and others contend that even the occurrence of oblique divisions in elongated plant cells does not necessarily invalidate the minimal area hypotheses, provided these partitions are sigmoid and intersect the side walls at right angles.

It is significant, accordingly, that in the cambial initials—as also in their daughter cells—of gymnosperms and dicotyledons, many partitions are formed which intersect the older, rigid walls at angles of varying degrees of acuteness (Text-figs. 3 and 4). These acute angles are not due to sliding growth and displacement of protoplasts. The peculiar type of cell plate formation in the cambium facilitates the study of successive stages in the formation of the division membranes and it is evident that the kinoplasmasomes, cell plates, and new cell walls frequently intersect the sides of the cells at very acute angles (Text-fig. 3).

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[7] The older walls of most cells of the higher plants are relatively thick and rigid.

These facts, as those cited by Chambers, raise the question whether in dealing with cytokinesis we actually are concerned with protoplasm in liquid or semiliquid phases.

In any case, this type of cytokinesis, in which the process of cell plate formation is so greatly extended—both as regards space and time—and so clearly dissociated from the usual phenomena of karyokinesis, promises upon further and more critical analysis, to be of some significance in any general discussion concerning the dynamics of cell division.

SUMMARY.

1. The adjacent, undifferentiated, uninucleated cells of the lateral meristem or cambium are of two distinct shapes and sizes: (1) small, more or less isodiametric initials which are of the same general order of magnitude as the cells of the terminal meristem and embryo; and (2) large, elongated initials which in certain cases may attain a length of more than 10,000 micra and a volume of 10,000,000 cubic micra. The large initials may be induced to divide to form small initials, and the latter to regenerate elongated cells of normal dimensions. Thus, the cambium affords an unusually favorable medium for the study of a number of fundamental physiological and cytological problems.

2. A study of the cambium reveals the fact that there is a very much greater variability in the size of meristematic cells in plants than was suspected by Sachs or Strasburger, and that the working sphere of the nucleus is by no means so restricted as assumed by these investigators.

3. Although the larger cambial initials of *Pinus strobus* tend to have larger nuclei, the nucleocytoplasmic-relation varies within wide limits and the diploid number of chromosomes is constant. The conditions in the cambium do not support Winkler's view that there is a close correlation between chromosomal number (chromosomal mass) and cell size in the somatic tissue of plants, and that giant cells are hyperchromatic.

4. The process of cell plate formation in the cambium is a remarkable phenomenon, and one which is significant in discussing the relative merits of various theories concerning the dynamics of karyokinesis and cytokinesis.

5. The newly formed partition membranes in the cambial initials frequently intersect the side walls at angles of varying degrees of acuteness, which is in contradiction to Errera's (Plateau's) Law of Minimal Area.

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EXPLANATION OF PLATE 1.

FIG. 1. Scale drawing, showing the relative sizes of ray initials and long initials in the cambium of a 60 year old stem of *Pinus strobus* L. × 100.

FIG. 2. Scale drawing, showing the relative sizes of ray initials and large initials in the cambium of a year old stem of *Pinus strobus*. × 100.

FIG. 3. Nucleus from large initial shown in Fig. 1. × 1,000.

FIG. 4. Nucleus from large initial shown in Fig. 2. × 1,000.

FIG. 5. Nucleus from ray initial shown in Fig. 1. × 1,000.

FIG. 6. Nucleus from ray initial shown in Fig. 2. × 1,000.

FIG. 7. Karyokinetic figure in longitudinally dividing cambial initial of *Pinus strobus*. × 3,000.

FIGS. 8, 9, and 10. Early stages in the formation of kinoplasmasomes and cell plate; (*n*) daughter nuclei, (*k*) kinoplasmasomes. × 3,000.

FIG. 11. Radial longitudinal extension of cambial initial, showing later stage in the formation of the cell plate. × 100.

FIG. 12. The same. Tangential longitudinal extension. × 100.

PLATE 1.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

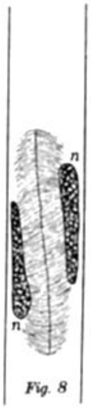

Fig. 8

Fig. 9

Fig. 10

Fig. 11

Fig. 12

(Bailey: Significance of cambium.)