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A HISTOLOGICAL STUDY OF THE SELF-DIVIDING LAMINAE OF CERTAIN KELPS.*

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Among the brown algae the family Laminariaceae or the kelps, besides comprising the largest species of algae, display in many other ways peculiarities of extreme interest. One of these is a novel and unusual method of branching, exhibited by several of the genera, a character which has caused them to be placed in a sub-family, the Lessoneatae. In this group branches are formed, not as outgrowths from the growing point, which in all the kelps is intercalated between the stipe and blade, but by the formation of a perforation through the growing region, which upon elongation divides the lamina and to a greater or less extent, the stipe also. Taking Nereocystis as typical of the subfamily, a glance at Fig. 1 will make clear this peculiar branching habit. The figure shows a very young plant in which the primary split has divided the original blade and secondary splits are seen fairly started. In Nereocystis lines of modified tissue are always seen running out in advance of the splits. These appear even before the basal perforation is developed, as seen in Fig. 1, b. The repetition of this process of division goes on until hundreds of laminae are found attached to the basal bladder by a system of branching more or less dichotomous in character.

A few writers on the Lessoneatae have given some attention to the histological processes involved in developing the fissures. MacMillan (1899) in his observations on Nereocystis gives a

* Contribution from the Botanical Laboratory of the Ohio State University, 59.

paragraph to the dividing of the lamina, in which he says: "I have been able to determine the origin and nature of the cleft. A single row of cortical cells immediately below the epidermis deliquesces or collapses and the epidermis furrows along the depression. The delequescence is propagated to adjacent cells, right and left, and continues down the middle lamella. The furrowing may take place along one surface of the leaf or along both surfaces until the epidermal cells come to lie against the middle lamella. The latter then breaks down and the two epidermises are contiguous. The split takes place along the base of the furrow and leaves the two halves of the lamina with apparently normal unwounded edges. * * * * The epidermis suffers no disintegration during the process. * * * * The furrow of the epidermis seems to deepen destroying the inner cells of the lamina as it progresses." In other accounts the central idea has likewise been an association of a process of cell disintegration or gelatinization with the inception and advance of the split. Rosenthal (1890) in his discussion of Macrocystis holds that the inception of the split occurs as a parting of the superficial layers, which is eventually followed by a swelling or gelatinization (quellungen) of the pith-web. In this modified pith-web a cavity is formed, which, enlarging, finally meets the gaps already developed in the upper layers and the lamina is divided. No discussion of the origin of either the inner cavity or superficial clefts was given. Reinke (1903) writing on Macrocystis gives Will's (1897) account of the splitting process, which account is also confirmed by Skottsberg (1907). The fissure occurs by the formation of an elongated cavity filled with a jelly-like substance, arising through gelatinization of the inner tissues. The furrow or depression which precedes the cleft is formed by a sinking or pushing in of the epidermis due to increased division of the cells overlying the gelatinized portion.

Because of the brief and fragmentary nature of the accounts heretofore given, it was believed that a fuller investigation of the matter would be desirable. Further it was thought important to make a comparative study of the splitting processes in the different genera available, in three of which, Postelsia, Lessoniopsis and Dictyoneuron, these processes have heretofore never been described. Material for the five genera investigated was in the collection of Prof. Robert F. Griggs of the Ohio State University, to whom I am greatly indebted, not only for material, but for much valuable advice and criticism throughout the course of the study. In view of the diversity found between the different genera, it would be very interesting to study Lessonia and Pelagophycus also, but material of these genera could not be obtained.

A part of the plants studied were killed in chrome-acetic acid and part in formalin. The usual methods of microtomy were followed; paraffine forming the embedding medium and the sections cut 10 mic. thick. The single stain aniline safranin or the same in combination with gentian violet were used. The first stain gives the middle lamella of gelatinous interlacing food conducting hyphae a characteristic tint which is of much value in distinguishing it from the adjacent cortex. All drawings were made with the camera lucida.

To understand the splitting of the kelp lamina and its relation to the tissues through which it passes, a digression must be made to set forth the manner of growth in the kelps, with special consideration of the derivation of the tissues. Three systems of tissues make up the kelp thallus: the epidermis, the underlying cortex and the central pith-web. Sections of stipe or lamina show the hypha-like elements of the pith-web to be highly stretched and modified cortex cells and the cortex cells are clearly seen to be derived from the epidermal cells, which form therefore the meristem in these plants. By periclinal walls the epidermal cells build the cortex; by anticlinal ones the epidermal area is enlarged. Hypodermal and outer cortical cells are often seen dividing, but the total meristematic activity of these internal cells is not nearly so great as that of the epidermal cells. The cells pushed inward from the epidermis reach their maximum size in the middle cortex. On the outside of this expanding cortical zone, the epidermis correspondingly enlarges its area by a constant increase in the number of its relatively smaller cells; the division walls of course being anticlinal. On the inner side of the expanding cortical zone the passive pith-web is seen to consist of much elongated cortical cells (trumpet hyphae) between which are large intercellular spaces filled with a gelatinous matrix. By this method the large and complex kelp thallus originates and the various structures peculiar to the several genera, including the method of branching under discussion, have their origin in variations of this simple process.

NEREOCYSTIS.

Nereocystis with its prominent splitting line extending far in advance of the cleft, forms an especially favorable type for study as portions of the lamina through which the line passed could be successively investigated down to the actual fissure and the various stages of the process clearly observed. Fig. 2 shows a section through the splitting line at a point corresponding to Fig. 1, a. The changes from normal lamina are at once seen to be a diminution in the thickness of cortex and pith-web, resulting externally in the formation of a broad furrow on each side. A comparison of the affected region with that of normal lamina at either side, discloses the fact that in the middle region the ratio of periclinal divisions to anticlinal ones has increased as is evidenced by the increased number of pith-web elements, together with the incipient cell rows from which they were derived. The unusual number of cortex cells formed rapidly stretches and attenuates the pith-web and inner cortex until the original medulla, locally, has been almost replaced by the newer and but slightly modified cortical elements. Fig. 3 illustrates the process in a more striking manner. This as well as the remaining figures are of the same magnification. At this stage periclinal divisions have been so rapid that small ridges have been formed in the middle of each broad furrow. The enlarging inner cells give the cortical structure a fan-like aspect. The inner cortical cells in the middle region pass into the middle layer before they reach their maximum size due to the rapid development of the cells over them. This accounts for the local massed condition of the medulla in the splitting region, causing the dark splitting line when the blade is viewed by transmitted light. The final result of all this activity is the intercalation of a region, made up entirely of new tissue, which has no strength to resist wave action and is easily torn apart. Fig. 4 shows the lamina at the critical point with the tear partly through it. The inner cortex cells of the preceding figure have passed into the middle layer condition and the lamina is markedly reduced in thickness. When severely whipped the laminae are often ripped at their distal ends; the tear if of any depth always following the weakened zone of the splitting line (Fig. 1.)

The wounds formed are shown in Fig. 5 which in the serial sections was taken from the same slide as Fig. 4. By normal activity the epidermis and cortex are built out and around the exposed part of the medulla (Fig. 6), finally coming in contact (Figs. 7-8). Generally the two epidermal layers do not exactly meet and the edges of the new laminae show a scar in section. Subsequent to the healing, the cells of the inner cortex, overlying the edge of the middle layer, do not develop to normal size but become sclerenchymatized (Fig. 8). Growth above presses them inward, noticeably bending the hyphae of the middle layer. This condition disappears later when the thick walled cortical elements pass over to the medulla.

This method of splitting was observed in several specimens but in no instance was any deliquescence or cell disintegration observed as reported by MacMillan in his observations on this plant.

POSTELSIA

Postelsia and the other Lessoneatae differ from Nereocystis in the absence of a long splitting line formed in front of the actual cleft. A close inspection shows, however, the presence of a very short line indicating that the modification of the inner tissues is not begun until the fissure is very near. But for the most part the tissue changes involved are confined to the region immediately around the advancing fissure.

The splitting process in Postelsia cannot be correlated with that of Nereocystis. Instead of a mass of new tissue being formed by periclinal activity, there obtains a relative lessening or inhibition of all cell division, while the modification of the various cells in the dividing region into cortex and pith-web continues unabated. Thus the lamina locally becomes thinner and thinner until the critical point is reached. Fig. 9 shows a normal portion of a lamina at one side of the splitting region. The epidermal and hypodermal cells are markedly elongated perpendicular to the surface; the larger middle cortex cells have their usual isodiametrical form and the inner cortex and pith-web are stretched horizontal to the surface. Contrasting with this is Fig. 10 from the middle of the furrow on the same section as Fig. 9. Here the large cortical cells have become prematurely elements of the middle layer and even the outer cortical elements show evidence of horizontal stretching due to the expansion of the superficial layers, while there are few divisions in the epidermis.

This lack of meristematic activity fails to build out the lamina to the normal thickness as shown in Fig. 9, and reduction in thickness continues progressively as the cortical cells are stretched into the weak and yielding pith-web elements. In Fig. 11 the lamina is shown at the critical point where ripping apart may occur. The cortex has practically disappeared. The development of the remaining hypodermal cells has parted the inactive epidermis on each side and the lamina is now in condition to be torn apart by the slightest twist. Throughout the entire process of reduction to the critical point no cell gelatinization occurred. After fission the wounds are healed exactly as in Nereocystis, by activity of the adjacent epidermal cells building tissue out and around the exposed edge of the medulla.

LESSONIOPSIS.

The lamina of Lessoniopsis is characterized by a thickened mid-rib made up chiefly of sclerenchymatized cortex. Upon division the reduction of the lamina to the critical point may be divided into two rather definite stages. In the first place as seen in the development of the perforation, broad furrows are formed in the basal portion of the mid-rib, resulting in the intercalation here of a small area of normal undifferentiated lamina. Then through this, rather than through the mid-rib proper, the cleft is propagated. These two stages are more sharply differentiated in the case of older and advancing splits, for here the portions of the divided normal lamina or the reduced mid-rib retain their thin blade-like character and broaden out until the daughter laminae are symmetrical and the mid-ribs occupy their normal median position.

The origin of the primary shallow furrows is different from anything seen in either of the preceding genera. In Lessoniopsis the relative increase in anticlinal activity in the epidermis seems to be the factor operative in reducing the thickness of the cortical layers. The undue stress brought to bear on the cortex by the rapidly expanding superficial layer results in the premature transition of the inner and middle cortex to the pith-web condition. Ordinarily expansion at the surface in inanimate objects results in buckling. In this case the transmission of the stress to the inner cortical layers stretches their elements into the thinner or highly elongated pith-web condition, thus markedly lowering the upper layers so as to produce concavity instead of convexity at the surface. No figure is given to illustrate this condition as the area concerned was far too extensive to be drawn on a scale sufficiently large to show the histological changes.

The second stage or splitting proper is by a process as different from that observed in Postelsia as that is different from Nereocystis. The central part of the mid-rib, after its reduction to the thickness of ordinary lamina, is locally still further reduced by the action of an internal cortical meristem, associated with a quiescent epidermis. On both sides of the medulla in the region concerned the cortical cells are seen dividing with anticlinal walls (Fig. 12). This has resulted not only in severely attenuating the pith-web but on one side the epidermis itself has parted and the critical point has been reached, for the thin-walled cells of this newly formed tissue cannot withstand the ripping tendencies in the wave swayed and twisted lamina. At approximately the same stage or on the same slide from which the drawing was made, the lamina portions were already separated.

In healing, the wounds first are covered for a time with a callus formation but later the epidermis and cortex heal them over exactly as in Nereocystis.

MACROCYSTIS.

The splitting process in this genus was studied by the German writers already quoted, but they evidently confined their attention to the development of the original perforation and did not study the elongation of the cleft, which is carried out by a different process than that forming the perforation. This fact at once sets Macrocystis apart from the preceding genera in which the processes originating the perforation are also operative in elongating the cleft. Sufficient material was available showing the incipient and older splits, to make four or five series of sections illustrating each of these stages. Part of the material studied came from the ruvian coast, part from Vancouver's Island. Small and large venile laminae, having splits in about the same stage of development, were used to determine whether the splitting process varied in any way with the size of the Lamina.

The writer is able to confirm in part Will's account of the origin of the basal perforation. Broad shallow furrows appear on either side of the thallus. These depressions may arise as in Nereocystis by a relative lessening of anticlinal divisions, but the area concerned is so extensive and the slightly constricted portion passes so gradually into the normal lamina, that no marked difference of tissue structure could be observed. After these shallow depressions become well defined, another and wholly different process is inaugurated. The inner cortical cells lying under the base of each furrow become gelatinized. A single cell disintegrates followed by its immediate neighbors until all the heavy walled cortex under the central part of the furrow has disappeared. Associated with this process of gelatinization is a marked local deepening of the furrows at their central parts (Fig. 13-14.) Will states that this originates by a pushing in of the epidermis due to increased division of the cells over the gelatinized portion. The evidence for this does not appear conclusive as can be seen by comparing the size of the hypodermal cells beneath the furrow with those on either side. The cells lying in the base of the furrow (Fig. 14) give evidence of relative inactivity, showing a diminution in anticlinal and especially periclinal divisions. To the lessening of periclinal activity chiefly, can be ascribed the origin of the more sharply defined secondary furrow, for the epidermal cells in this region fail to build out the thallus. This is somewhat similar to the condition in Postelsia. At about the stage figured normal growth begins to close in on the gelatinized cavity separating schizogenetically the remaining cortical cells underlying the furrow. The usual gelatin filled pith-web together with the newly gelatinized regions of the cortex forms a sort of internal cavity or wound which is healed by a process identical with that seen in Nereocystis (Fig. 15). Here normal development is bringing the respective sides around the edges of the middle layers. The remnant of one of the gelatinized portions is still present, stretched across below the furrow.

After the original basal perforation is formed in the manner just described its advance through the lamina is by a wholly different process. Unexpected as this might seem the evidence for it is quite conclusive. Fully formed splits 5 mm. in length and upward were studied, occurring in laminae of different size and thickness and from different waters, British Columbia and Peru, but in no case was there any deviation in the process.

Local and excessive meristematic activity of the cortex is the fundamental factor in the advancing cleft. First, however, there is a local increase in the normal growth process which results in piling up slightly modified cortical cells in the medulla. Next a few hypodermal and outer cortical cells begin to divide rapidly forming a wedge-like mass, which tears the epidermis apart and the process which succeeds in dividing the lamina is begun (Fig. 16.) The cells exposed by the parting of the epidermis become passive and subject to the tearing tendencies of the rapidly expanding tissue beneath them. They are separated and in this manner the cleft is carried clear through the lamina. So great is the meristematic activity that before the cleft reaches the pith-web this layer locally has been entirely replaced by dividing cortical elements, through which the cleft is propagated. The final separation of the last thin walled cortical cells is of course mechanical. By the continual extension of this cortical activity distally, the whole lamina is finally divided, while proximally, the separation is carried some distance down the stipe by the same sort of activity except the meristematic wound tissue is formed in larger masses and the cleft advances in a more irregular manner.

In healing, the superficial cells of the exposed wound tissue are transformed into epidermal elements. There is however a tendency to close the wound as previously described, by the crowding or pressing around of the tissue adjacent to it.

Material containing clefts of proper age to show the transition stages, by which the initial gelatinization process gives way to the secondary process of cortical activity, was not available so this nteresting phase of the problem cannot be taken up in the present diiscussion.

DICTYONEURON.

In Dictyoneuron only the method of advance of the older cleft was studied, as the collection contained no material showing the incipient or perforation stages. The process involved in the advance of the cleft was essentially the same as that in Macrocystis but the cortical meristem is more definitely localized than in that genus and only occurs at first on one side of the medulla. Fig. 17 shows a section of a young lamina in which a split 5 mm. in length was present. The half of the section not shown was normal like the region at the edges of the drawing. Cell division and growth in the cortex has resulted in the formation of a mass of tissue which presses slightly into the pith-web. When this mass has become somewhat more extensive than that figured, a few cells near its center begin dividing very rapidly and build up a new secondary mass within the first (Fig. 18), which pushes out the older cells on all sides of it, notably below into the pith-web. On account of this rapid internal division, the original epidermis is pulled apart from a to b and the beginning of the cleft has been started by the wedging action of the ball like mass of new tissue. This cleft shown at Fig. 18, c, next enters the central mass and passes rapidly to its center. After the development of the cleft, the superficial and hypodermal cells lining the gap divide chiefly with periclinal walls until the newer tissue comes to have a fan-like structure, similar to that seen in Nereocystis (Fig. 19). By the same process described in that plant though on a much larger scale, the furrow is strongly widened and deepened. This results as in Nereocystis not only in attenuating the lamina locally but in filling up the middle region with young cortical tissue (Fig. 20) which has no strength and is easily torn apart by wave action.

After ripping apart the cortical cells exposed from a to b (Fig. 20) take on the aspect of epidermal elements with which is associated a tendency of the contiguous tissues to close around the injured portion. The two lamina edges resulting from the entire process of division are lined with new tissue, the superficial layer of which becomes epidermal in character and remains so. These edges show for some time a groove running in them which is wholly accounted for by the peculiar splitting process obtaining in this plant.

GENERAL CONSIDERATIONS.

That in all cases the split actually progresses through the blade is clearly shown. The exposure of the inner tissues lying just behind the apex of the advancing cleft is evidence enough to prove that the fissure is cleaving the blade and is not a pseudo-cleft brought about by intercalary growth of the portion divided by the perforation. This latter, however, is of much importance in the apparent elongation of the split.

As to the rapidity of advance of the cleft, nothing whatever was determined. It is doubtful that this can be worked out even in the field with any degree of accuracy. The facts of intercalary growth and mechanical ripping would tend to vitiate any measurements that might be made.

In the study of the various genera special attention was given to the advance of the distal end of the split, or the division of the blade. In each genus however observations on the proximal end of the cleft were made, which not only showed that the stipe was dividing, but the splitting process was in each instance identical with that described for the blade. Owing to the thickness and compactness of the stipe the changes in it are much slower than in the blade. The elongation of the stipes is chiefly by intercalary growth.

An arrangement of the five genera on a basis of specialization in the splitting would be as follows: Postelsia, Nereocystis, Lessoniopsis, Macrocystis and Dictyoneuron. The relative arrangement of the first three might be questioned but that the process in Macrocystis and particularly Dictyoneuron is a very definite and specialized one admits of no doubt.

Upon taking up the present investigation, the writer expected to find that the division of the laminae in the different genera, was brought about by the same process with of course some minor variations. It was then very surprising to find the widest differences prevailing among the various genera, differences in some instances so great as to make the histological processes involved appear diametrically opposed. The end result, the branching of the plant, in all cases is the same so we have in these forms a most striking example of those numerous instances in nature in which a common end is attained through totally different means.

SUMMARY.

1. The splitting of the lamina of Nereocystis is due to a relative increase of periclinal divisions resulting in the intercalation of weak new tissue which is mechanically torn apart. The wounds heal by normal growth, building the tissue out and around the exposed edges of the medulla.

2. In Postelsia cell division in the meristematic epidermis ceases almost entirely at the point where splitting is to occur and the lamina becomes so thin by the continued differentiation of the tissue already present that it is torn apart by the impact of the waves. The wounds heal as in Nereocystis.

3. In Lessoniopsis an area within the mid-rib is reduced to the thickness of normal lamina by relative increase of anticlinal divisions. Within this area further reduction and weakening occurs by anticlinal divisions in the cortex. The wounds after mechanical ripping are at first covered with callus; later healing as in Nereocystis.

4. Macrocystis shows the perforation to originate by local gelatinization of the inner and middle cortex and cessation of periclinal activity in the epidermis over the gelatinized portion, resulting in a deep sinus on either side of the lamina. The adjacent tissues are finally forced in on the gelatinized places until the epidermis breaks apart forming the perforation. Healing as in Nereocystis. When once formed the fissure advances by excessive cortical meristematic activity which first tears apart the epidermis and finally the whole lamina, which meanwhile has locally become filled with cortex cells. Healing is by transformation of the exposed cortex into epidermis.

5. In Dictyoneuron only the advance of the cleft was studied. An internal wedge-like mass of tissue, a cortical meristem, tears the overlying layers apart. Periclinal division of the cells lining the gap forms a fan-like structure which reduces the lamina in thickness and strength until mechanical tearing follows. The superficial cells of the new tissue formed on the respective edges are changed to epidermal elements and remain so.

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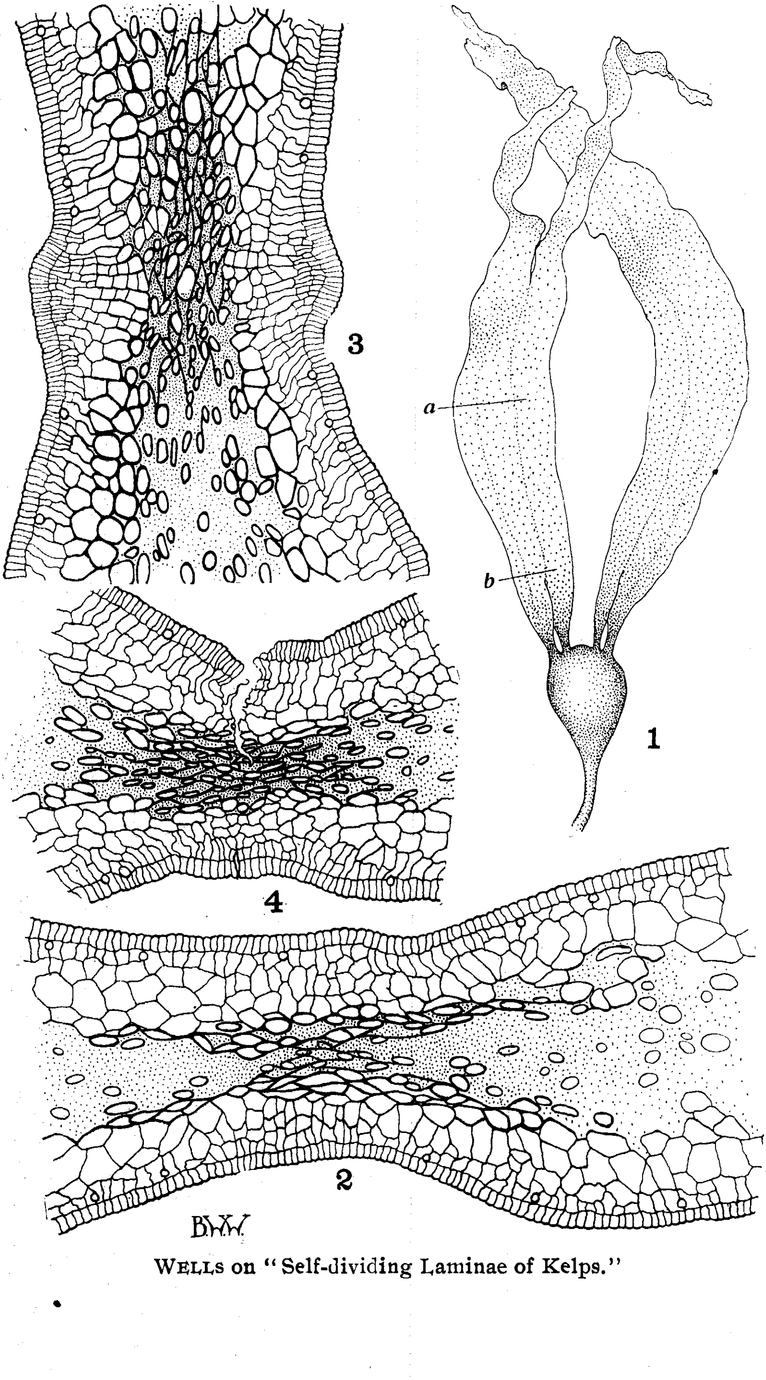
EXPLANATION OF PLATES.

FIG. 1. A Young Nereocystis plant showing splits and splitting lines.
FIG. 2. Cross section of Nereocystis lamina at a point corresponding to to Fig. 1, a.
FIG. 3. Cross section of Nereocystis lamina at a point near end of split corresponding to Fig. 1, b.
FIG. 4. Cross section of Nereocystis lamina showing mechanical tear.
FIG. 5. Cross section of a newly divided lamina of Nereocystis showing wounds.
FIG. 6. Healing lamina of Nereocystis.
FIG. 7. Healing lamina of Nereocystis, later stage.
FIG. 8. Completely healed lamina of Nereocystis.
FIG. 9. Cross section of normal Postelsia lamina.
FIG. 10. Middle stage in splitting Postelsia lamina.
FIG. 11. Final stage in splitting lamina of Postelsia.
FIG. 12. Last stage of splitting in Lessoniopsis.
FIG. 12a. Entire section showing position of Fig. 12.
FIG. 13. Origin of perforation in Macrocystis, middle stage.
FIG. 14. Structure of portion enclosed in dotted line, Fig. 13.
FIG. 15. Final stage in the development of the original perforation in Macrocystis.
FIG. 16. First stage in the advance of the mature cleft in the Macrocystis lamina.
FIG. 17. Primary cortical meristem in splitting region of Dictyoneuron.
FIG. 18. Secondary cortical meristem within the first in Dictyoneuron.
FIG. 19. The cleft and fan shaped structure developed in the splitting of the Dictyoneuron lamina.
FIG. 20. Final stage in the dividing process in Dictyoneuron. Cortical meristem present.



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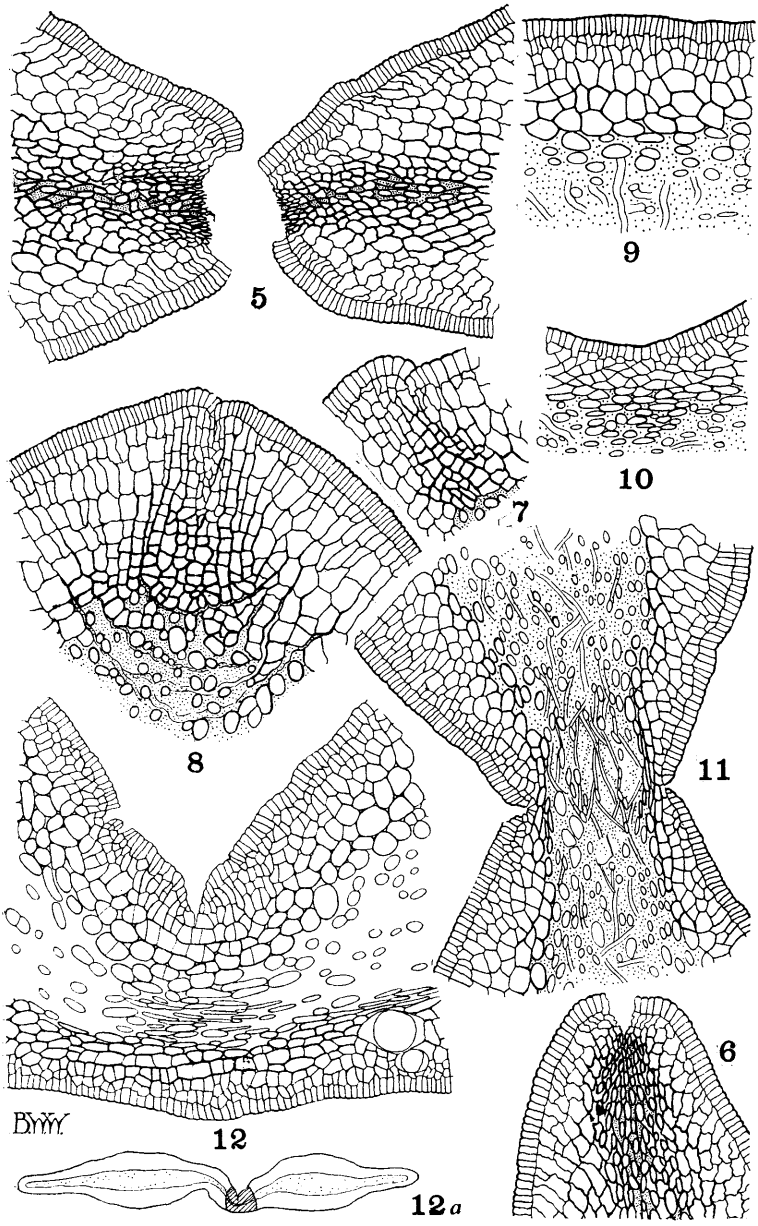

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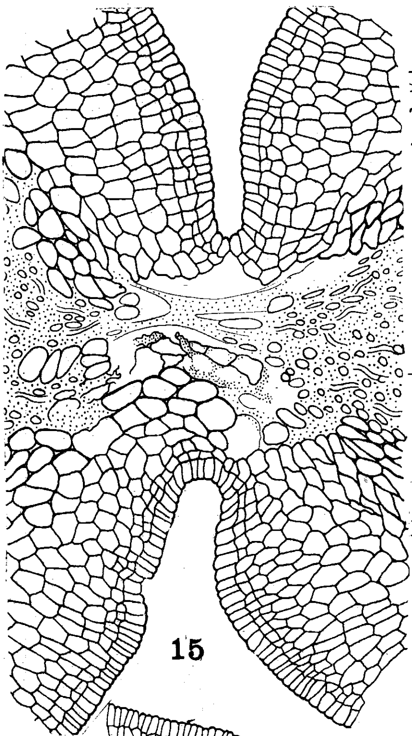

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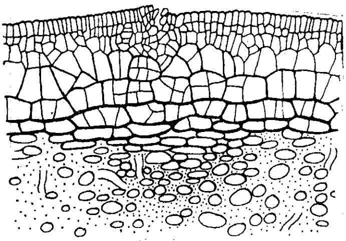

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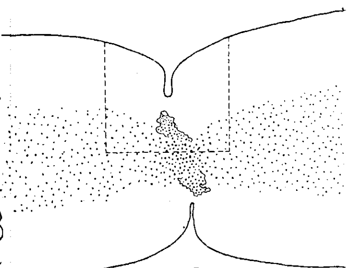

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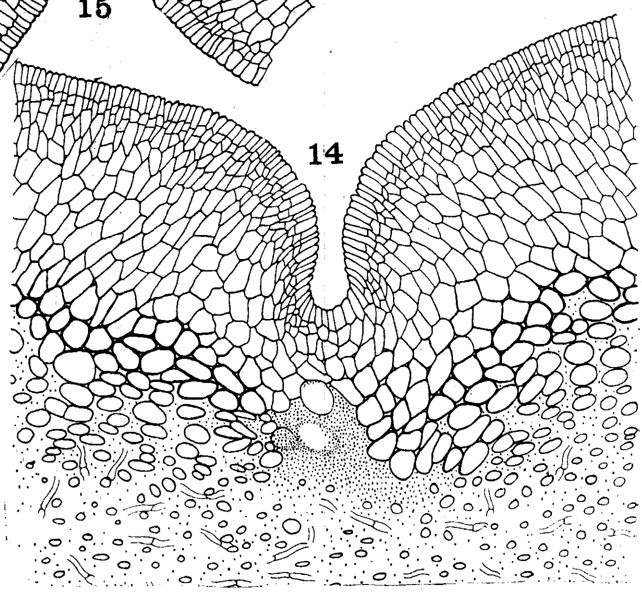

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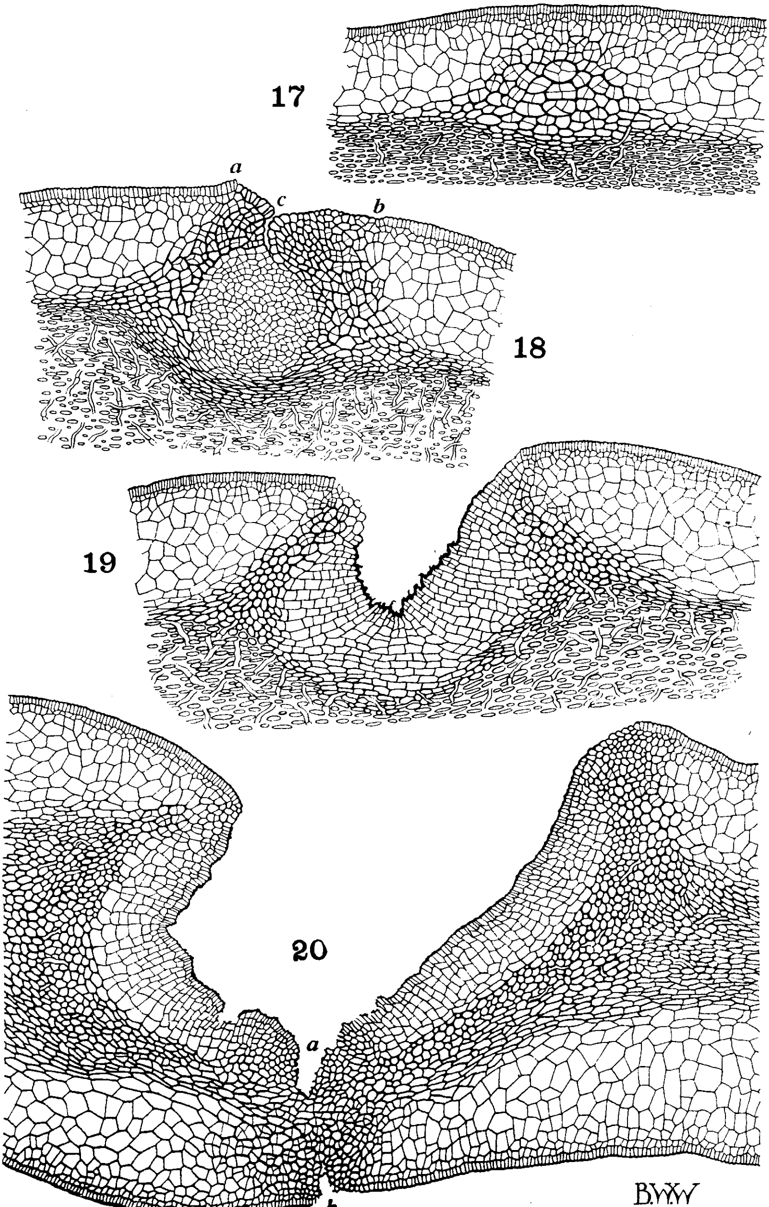

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