

Anatomy and Histology of *Macrocystis pyrifera* and *Laminaria saccharina*.

BY

M. G. SYKES,

Girton College, Cambridge, and Bathurst Student of Newnham College, Cambridge.

With Plates XIX, XX, and XXI.

AS much of the previous work on the anatomy of the Laminariaceae has led to contradictory conclusions, it appeared desirable that some further investigations should be made. Since only spirit or herbarium material has so far been examined, very little is known about the histology of these plants, and Mr. Hill therefore suggested that an examination of material preserved by more careful methods might yield useful results. I then undertook the study of some material of *Macrocystis pyrifera*, Ag. and *Laminaria saccharina*, Lamour., which had been previously collected and preserved by him, and I have since pickled and examined other material of *Laminaria saccharina*.

I wish to express my sincere thanks to Mr. Hill for so liberally placing his material at my disposal, and also for much help and advice throughout the work. This research has been carried out at the Cambridge University Botany School.

I. HISTORICAL SUMMARY.

(a) *Anatomy.*

The trumpet hyphae in the pith tissues of the Laminariaceae were originally discovered by Reinke¹, but Wille² in 1885 was the first to describe them accurately, and to suggest that they may possess a conducting function.

The larger sieve-tubes in *Macrocystis* had been noticed by J. J. Parker of Otago, but it remained for Will³ to give a careful account of them with

¹ Reinke, Beiträge zur Kenntnis d. Tange. Prings. Jahr. f. w. Bot., 1876, Bd. 1, p. 317.

² Wille, Siebhyphen bei den Algen, Ber. d. deutsch. bot. Ges., 1885; and Bidrag til Algernes Physiologiske Anatomi, Kongl. Svenska Vetens. Akad. Handl., B. xxi, No. 12, Stockholm, 1885.

³ Will, Zur Anatomie von *Macrocystis luxurians*. Bot. Zeit., 1884.

figures both in *Macrocyctis* and in *Nereocyctis*. His description was followed by a paper from Rosenthal¹, who disagreed with him on many points concerning the anatomy and histology of *Macrocyctis*. Three years earlier had appeared Oliver's² results, on the subject of the obliteration of the sieve-tubes in *Macrocyctis* and *Nereocyctis*.

In 1897 Macmillan³ described the external features of *Nereocyctis*, paying, however, only very small attention to the anatomy. Finally Wille⁴, in 1897, again investigated the trumpet hyphae in *Alaria* and other Laminariaceae, and made an attempt to group the tissues in this family into various physiological systems.

Will⁵ and Rosenthal⁶ both studied the development of the young tissues in *Macrocyctis*, and Setchell⁷, during the course of his description of *Sacchoriza dermatodea*, gives a short and useful account of the youngest stages in that plant. A short summary of most of the above papers is given by Oltmanns⁸. The conclusions arrived at by these various authors are sometimes at variance, but the following is an attempt to sketch some of their principal points.

i. *Development in young plants.* The general ground-plan of structure in all Laminariaceae is the same. The tissues of the adult may be broadly differentiated into 'cortex' and 'central body'; though no sharp line can be drawn between these two regions, since it is found that in the course of development the inner layers of the cortex give rise to the outer layers of the central body. According to Setchell⁷ the development of the central body in *Sacchoriza* takes place in the following manner. In the very young plant the innermost cells of the thallus soon cease to divide, and thus form the first constituent of the medulla, becoming much stretched owing to the great growth in length of the surrounding tissues. Later the adjoining layers of cells add to the medulla, and are, in a similar manner, stretched and elongated. The longitudinal walls of all the cells forming the central body become greatly swollen by the conversion of the middle lamella into slime, giving rise to the appearance of a number of isolated rows of filaments. These rows are connected by short cross connexions, which later become stretched by the swelling of the walls; they are considered by Setchell to be formed from the pit canals between the original central cells. In addition to these a large number of hyphae

¹ Rosenthal, Zur Kenntnis von *Macrocyctis* und *Thalassiphyllum*. Flora, 1890, p. 105.

² Oliver, On the obliteration of the sieve-tubes in the Laminariaceae. Annals of Botany, i, 1887, p. 95.

³ Macmillan, Observations on *Nereocyctis*. Mem. Torrey Bot. Club, 1897, p. 273.

⁴ Wille, Festschrift til H. Maj. Kong. Oskar II, 1897, Regieringsjubilant.

⁵ Will, l. c., 1884.

⁶ Rosenthal, l. c., 1890.

⁷ Setchell, W. A., Concerning the life-history of *Sacchoriza dermatodea*. Proc. Amer. Acad., 1891, 26, p. 177 (Crypt. Lab. of Harvard Univ., 17).

⁸ Oltmanns, Morphologie und Biologie der Algen, vol. i, pp. 445-458.

grow in from the inner cortical layers and anastomose between the pith-tubes.

The descriptions given by Rosenthal¹ and Will² of young plants of *Macrocystis* bear a remarkable resemblance to Setchell's account of *Sacchoriza*, for in *Macrocystis* the original central cells also become stretched in the same way to form elongated filaments, and the number of these filaments is later added to from the inner 'cortical' cells. According to Rosenthal the formation of cross-connexions may take place in one of two ways. The first method is that noticed by Setchell in *Sacchoriza*, and consists in the pulling out of a pit canal, owing to the swelling of the longitudinal walls of the pith elements, and in the formation of cross walls in the canal thus produced. The second method, which is, he says, more common, begins by the formation of a lateral protuberance which grows out from the longitudinal wall of one cell, forming a true hypha-like outgrowth, and, after a shorter or longer period of wandering, makes a secondary connexion with some other cell, the two cells connected being either quite near together or at some distance from one another.

A large number of hyphae also grow in from the inner cortical cells, and, wandering in the substance of the swollen middle lamellae of the cells of the medulla, are responsible for much of the later growth of that region. Setchell states that in *Sacchoriza* broken cross connexions can grow out and form hyphae, and he also thinks that some of the hyphae formed from the inner cortex may assume a longitudinal course in the pith, and hence resemble, except for their smaller diameter, the original pith filaments. From the various accounts it is uncertain whether the ingrowing hyphae ever form secondary connexions in the medulla. Rosenthal³ and Oltmanns⁴ assert that they never do so, but always have free endings, and on the whole this appears to be the opinion of most authors except Will⁵ and Wille⁶, who believe that new connexions may sometimes arise.

ii. *Growth in thickness.* The method of increase in diameter in the stem of *Macrocystis* has been a difficult question to solve. It is known that in the Laminariaceae growth in thickness is entirely due to the outer layers of the cortex, which divide rapidly by tangential and radial divisions. The same kind of meristematic growth certainly does take place in *Macrocystis*, and it is principally to this method of cell division that Rosenthal ascribes the increase in thickness in that plant. He points out that, while in the young stem the width of the cortex is approximately equal to that of the pith, in the old stem it may be ten times as great. On the other hand there can be no doubt that the diameter of the pith in the old plant is much greater than in the young one, but as to the method by which it has been

¹ Rosenthal, l. c., 1890.

³ Rosenthal, l. c., 1890.

⁶ Will, l. c., 1884.

² Will, l. c., 1884.

⁴ Oltmanns, l. c.

⁶ Wille, l. c., 1885 and 1897.

increased there is some difference of opinion. Rosenthal considers that its growth is to be wholly ascribed to the slow addition of new elements on its periphery by transformation from the inner cortex, the only meristematic layer being the outer layer of the cortex. He lays great stress on the distinct radial rows to be traced, in the young plant, from this outer layer right into the centre of the pith. He supposes the outer cortex to be continually added to on its outer edge, and transformed slowly into inner cortex on its inner edge. The inner cortex differs from the outer in being composed of more elongated cells, and it is these which, in their turn, give rise to those peculiar elements found only in *Macrocytis* and *Nereocytis*, which are usually regarded as true sieve-tubes, and which are found, in all except the very young stages, arranged in radial rows at the periphery of the pith.

In a stage slightly older than that in which sieve-tube formation begins, when most of the original inner cortex has been already used up in their production, the remainder of that tissue presents a rather striking appearance, and it is to this narrow band that we will return below when discussing Will's theory of secondary growth in *Macrocytis*.

The later formed inner cortex is made up of cells much less elongated than are those of the earlier layers, and hence there is a more or less sharp distinction between the two tissues. No more sieve-tubes are formed after a certain stage (probably, according to Rosenthal, when all the original inner cortex has been used up), and the whole of the further growth in thickness of the medulla is due to the expansion of the already formed tissues, and to the ingrowth of cortical hyphae.

The alternative view, due to Will, is that the production of new sieve-tubes may go on indefinitely, and Oliver¹ appears to have accepted his theory. Will attributes the formation of these elements, in the young plant, to a special 'thickening ring' which corresponds both in position and appearance with the narrow band of tissue referred to above, and which he believes functions as a separate meristem.

Will states that in the older plant this 'thickening ring' has no definite boundary, but a gradual transition between inner cortex and sieve-tubes is then found. It is not at all clear from his account whether he means to imply by this statement that there is only an apparent transition, or whether he wishes to draw the inference that actual transition from inner cortical cell to sieve-tube is at the moment taking place. One would suppose, however, from the general terms of his description, that, unlike Rosenthal, he considers growth in thickness, by means of a secondary formation of new sieve-tubes, to be a long continued process, and that he therefore believes in the production of new young sieve-tubes in the old plant.

¹ Oliver, l. c., 1887.

Neither does Oltmanns' ¹ account of the production of the sieve-tubes in *Macrocystis* help to clear up the difficulty.

No definite secondary growth in thickness has ever been described in any other Laminariaceae except *Nereocystis* and *Thalassiophyllum*. The former appears to agree closely with *Macrocystis* ², both in its mode of development and in its adult anatomy, but the latter, described by Rosenthal ³, has an inner cortical meristem which takes the place of the original outer cortical meristem, the latter soon ceasing to function.

iii. *Morphological nature of the various elements composing the medulla in the Laminariaceae.* The terminology of the various elements composing the medulla has been a matter of some difficulty.

(a) It is fairly agreed that the large outer tubes in *Macrocystis* and *Nereocystis* are probably true sieve-tubes (Oltmanns, Oliver, Will, Wille, Rosenthal), but it is uncertain whether the original pith cells should be included in this category. These have been termed 'trumpet hyphae' by Oliver, but Oltmanns ⁴ has recently pointed out that, whether they are to be looked upon as sieve-tubes or not, they are certainly not hyphae. The latter suggests that probably both they and the large sieve-tubes will be found to possess sieve-plates, whose manner of formation will prove to be similar to that of the true sieve-plates in the Phanerogams.

Wille ⁵ also considers the 'sieve-cells' or central pith cells, both of *Laminaria* and of *Macrocystis*, to be homologous with the larger sieve-tubes of *Macrocystis*; whilst Oliver, on the other hand, maintains that they are of an entirely different nature, and are not to be regarded as true sieve-tubes. He lays stress on the fact that he found no cross connexions in *Macrocystis* between the pith-cells, or 'trumpet hyphae', and the true sieve-tubes.

Oliver ⁶ and Wille ⁷ both bring forward the suggestion that the presence of these large sieve-tubes in *Macrocystis* and *Nereocystis* is correlated with the habit of these plants.

Oltmanns, however, suggests that their function may be in part mechanical.

(β) *Hyphae.* Various elements in the medulla have been termed hyphae by different authors, but it seems generally agreed that this loose use of the term is not to be commended, and that only the ingrowing filaments, arising from the cortex, can be really looked upon as true hyphae. It is as yet uncertain whether these form secondary connexions.

iv. *Mucilage canals.* The mucilage canals in *Macrocystis* and other Laminariaceae have been carefully described and figured by Will ⁸ and others, and do not immediately concern this account.

¹ Oltmanns, l. c., p. 452.

² Macmillan, l. c., 1897. Oliver, l. c., 1887.

³ Rosenthal, l. c., 1890.

⁴ Oltmanns, l. c., pp. 453-454.

⁵ Wille, l. c., 1897.

⁶ Oliver, l. c., 1887, p. 112.

⁷ Wille, l. c., 1897.

⁸ Will, l. c., 1884.

(b) *Histology.*

Owing to difficulties of technique very little histological work has been done on properly preserved material of the brown algae.

i. *General cell-wall.* The mucilaginous nature of the general walls has been noted by several authors and has been contrasted with the harder substance which forms the pit-closing membranes and the sieve-plates. The middle lamella is said to be largely composed of the calcium salt of tungstic acid, which is analogous to the calcium pectate of higher plants. Rosenthal¹ gives some account of the distribution of the pits in the cortex of *Macrocystis*, where they are confined chiefly to the end walls, but are also present occasionally on the lateral walls; protoplasmic threads have not been figured.

ii. *Development of the sieve-plate in Macrocystis.* In connexion with Rosenthal's hypothesis of the formation of sieve-tubes from the cortex of that plant, he brings forward a remarkable theory to account for the origin of a sieve-plate from the cross wall of a cortical cell. The latter has usually a single ring of pits in its end wall, but towards the pith Rosenthal says that cross walls are found in which the inner edges of these pits become indistinct, and finally the intervening wall in the centre of the ring is partly dissolved. Only a thin membrane is then left across the cell, having still a thick edge which represents the unaltered part of the wall outside the ring of pits. The thin membrane becomes the sieve-plate, and is finally found to be perforated by numerous holes, which are larger in the middle and smaller towards the periphery; the origin of these holes appears to be somewhat obscure. Rosenthal found but few lateral plates on the walls of the sieve-tubes, and notes that the lateral pits of the cortical cells would naturally become much separated after the latter had given rise to sieve-tubes by a stretching process.

Oliver² describes the formation and obliteration of the pores of the sieve-plate in *Macrocystis* and *Nereocystis*. He describes and figures protoplasmic threads in the cross walls of the young sieve-tubes, and also gives some details of the formation of callus and slime-strings in the sieve-plates; his paper, however, is chiefly concerned with the older stages in which callus has already begun to accumulate. He considers the callus masses which are formed on either side of the sieve-plates, and the long strings of callus which often entirely fill the lumen in the old trumpet hyphae, to be formations from the cell-wall, and he figures cases where the callus-stain is seen to fade into the wall, showing the change to be a gradual one.

¹ Rosenthal, l. c., 1890.

² Oliver, l. c., 1887, Figs. 89, &c., Pl. VIII.

Callus and slime-string formation are also described by Will¹, Wille², and Rosenthal³ in *Macrocystis*, and by Wille² in *Alaria*, and the suggestion is several times advanced that the development of the sieve-plate will be found, on further investigation, to be similar to that in Angiosperms. Since all these authors worked on dried or alcoholic material, the finer histological details of the protoplasmic threads and of callus-formation could not be studied.

iii. *Protoplasmic continuity.* Hick⁴ in 1885 attempted by various and somewhat drastic methods to demonstrate protoplasmic continuity in *Ascophyllum* and *Fucus*. The chief interest of his experiments lies in the demonstration which they afford of the great resistance of these algal cell-walls to powerful chemical reagents.

Rosenthal's attempts to demonstrate continuity of protoplasm appear to have been more successful. He does not figure his results, but describes slime-strings in the sieve-tubes and connexions through the pit-closing membranes of the cortical cells.

Oliver also describes protoplasmic continuity in the young trumpet hyphae and sieve-tubes of *Macrocystis*.

(c) *The objects of the present research.*

From the foregoing review it will be seen that several points require investigation.

I. *Anatomical.*

i. The morphological nature of the 'trumpet hyphae' and so-called 'true sieve-tubes' in *Macrocystis* and *Laminaria*.

ii. The nature of the growth in thickness of the medulla in *Macrocystis*.

iii. The formation of secondary connexions by the hyphae growing into the medulla in both plants.

II. *Histological.*

i. The question of the presence or absence of protoplasmic connecting-threads across the cell-wall.

ii. The development of the sieve-plate in *Macrocystis* and *Nereocystis*, and of the cross walls of the trumpet hyphae in these and other species, and its comparison with the development of the Angiospermous sieve-plate⁵.

iii. The nature of the callus found obliterating the older sieve-plates and formed throughout the length of the trumpet hyphae and old sieve-tubes. The question whether it is a cell-wall production, due to alteration of already formed cellulose, as described by Oliver, or whether it is deposited by the

¹ Will, l. c., 1884.

² Wille, l. c., 1885.

³ Rosenthal, l. c., 1890.

⁴ Hick, Protoplasmic Continuity in the Fucaceae. *Journal of Botany*, xxiii, 1885.

⁵ Hill, A. W., Histology of the sieve-tubes in *Pinus*. *Ann. of Bot.*, xv, 1901. Historical Summary, pp. 576-585.

protoplasm in the same way as cellulose, as has been suggested by Russow¹ and Strasburger², and found by Hill³ in *Pinus*.

II. MATERIAL AND METHODS USED IN THIS INVESTIGATION.

Material. The material of *Macrocystis* which was at my disposal consisted of the young apex of a growing plant, and of two collections of older portions of the stem. One of the two collections was made in the Falkland Islands, and consisted of pieces of stem of about one-third of an inch in diameter, while the other came from Talcahuano Harbour, Chili, and was obtained from rather younger plants whose stems varied from one-eighth to a quarter of an inch in diameter. The young apex was preserved in spirit, and the rest of the material was fixed in a solution of Iodine in Potassium Iodide, dissolved in sea-water, and preserved in a Thymol solution. All the material was obtained by Mr. Hill in December (1902), that is, early in the South American summer.

I had also the opportunity of examining all stages in the development of young plants of *Laminaria saccharina*, preserved in spirit. These young plants were used in investigating anatomical development, while older material, preserved in September by Mr. Hill, and in February by myself, had been treated in a manner which rendered possible more minute histological work. Some of the September material was fixed in a solution of Iodine in Potassium Iodide, and the rest in Picric acid, both being preserved in Thymol; but neither of these methods was found to be very successful. Various methods of fixation at different temperatures were tried as experiments on the February material.⁴

Methods. The methods used in swelling and staining were based on those already published by Gardiner⁵ and employed by Hill⁶ in *Pinus*. A solution of Iodine in 10 per cent. Potassium Iodide was found to be the most useful swelling agent, and gave good results when the material was left in the solution from two to three weeks.

For staining, a modification of the Safranin method⁷ was used in conjunction with Aniline blue⁸ and London blue. The chief difficulty met

¹ Russow, Sitzb. der Dorpater Nat. Ges., 1882; Ann. des Sc. Nat., 6^e sér., t. xiv, 1882.

² Strasburger, Bot. Pract., 1884.

³ Hill, l. c., p. 598. For a summary of the various views on the origin of callus, see pp. 597-600.

⁴ Some of the fixing agents used at the suggestion of Mr. Hill were Picric acid, Iodine in Potassium Iodide, Picro-Acetic acid, and Picro-Uranium Nitrate, all being dissolved in sea-water, and Thymol being used in all cases as a preservative.

⁵ Picro-Uranium Nitrate was found to give the best results, the composition of the solution used being as follows:

50 cc. sea-water.

.5 gr. Picric acid.

1 gr. Uranium Nitrate.

⁶ Gardiner, Proc. Camb. Phil. Soc., 1898; Proc. Roy. Soc., vol. lxii, 1897.

⁷ Hill, I. Connecting-threads in *Pinus* (Pt. 1 of Gardiner and Hill on the Histology of the Cell-wall), Phil. Trans. Roy. Soc., Bot., 1901, pp. 83-125. II. Histology of Sieve-tubes in *Pinus*, Annals of Botany, vol. xv, 1901, p. 576.

⁸ Gardiner, l. c., 1898, p. 508.

⁸ Water blue made up with aniline.

with was the impossibility of removing the Safranin from the walls by any of the ordinary methods, but a solution of dilute glycerine followed by London blue was found to produce the desired result. The London blue not only removes the Safranin from the walls, but also stains the callus, as does Water blue. Moreover it has the additional property of staining callus in places where Water blue has no effect, presumably because the callus is not sufficiently hydrated.

The acid-violet method¹ both by itself and in conjunction with Safranin was found to be useful, and Water blue made up with Aniline was occasionally employed alone.

London blue alone was used in the investigation of callus, and Methylene blue, Thionin, and Fuchsin were found to be convenient cell-wall stains.

III. MACROCYSTIS PYRIFERA.

A. GENERAL ANATOMY.

As no young plants of *Macrocystis* were available, my investigations of the development were confined to the study of the apical portion of an adult specimen.

Stage i. In the youngest part of the growing thallus the tissues are already differentiated into cortex and medulla, the breadth of the cortex being somewhat greater than that of the medulla.

The outermost layer of the cortex is composed of regularly arranged isodiametric cells, which are in a state of active division, and give rise to rows of elements, the radial arrangement of which can be traced in transverse section throughout the cortex, and often appears to be continued into the medulla. About eighteen layers of cells make up the entire cortex, the counting being made along any such radial row. The outer eight or ten layers are certainly of secondary origin. In longitudinal section they are seen to be composed of thin-walled parenchymatous elements, sparsely provided with contents, and on the extreme periphery of the section mucilage canals with small patches of secretory cells (cf. Will²) are present in abundance.

In longitudinal section this outer cortex is sharply marked off from the inner, which is made up of much more elongated cells, whose longitudinal walls are considerably swollen, while many of their transverse walls are very thin and give the impression of having been quite recently formed. It seems certain that this inner cortex represents the primary cortical tissue of the thallus, the cells of which have given rise to these characteristic elements by means of longitudinal stretching followed by rapidly succeeding cross

¹ Gardiner, l. c., 1898, p. 508.

² Will, l. c., 1884.

divisions. The cells of this inner cortex have very dense contents and large nuclei. Pits are present in their thin transverse walls, but they are not very easy to see, being more numerous, smaller and shallower than in any of the other walls. They do not appear, either in the inner or outer cortex, to be arranged in a ring as are the pits in the transverse walls of the cortical cells in older material. A few pits are also present in the longitudinal walls.

The innermost layers of primary cortex give rise to hyphae which run into the medulla. The free ends of these hyphae were never certainly seen, and it seems probable that secondary connexions are formed among them, but to find definite proof of this it would be necessary to study their development in young plants.

The medulla at this stage is nearly circular in cross section, and is composed of elements which are still more elongated than those making up the inner cortex, and have also more swollen walls. Between these elements, and in the mucilaginous substance formed from the swollen middle lamella of their walls, run numerous, much branched hyphae. In a transverse section at this stage there is no definite boundary line between the medulla and inner cortex, and the elements of the medulla themselves do not appear to be sharply differentiated as to size or other characters; but in longitudinal section it is seen that those forming the one or two outer rows pursue a considerably straighter course than the others, and are also somewhat wider, having more dense contents and larger nuclei. The elements composing these outer rows are the first of the true sieve-tubes, but at this stage are quite immature.

The central region of the pith or medulla is occupied by numerous sieve-tube-like elements, the 'trumpet hyphae' of Oliver. These are presumably the original central cells of the thallus which have become stretched by the growth of the outer tissues and separated by the hyphae growing in from the cortex and anastomosing between them, but never forming connexions with them. These central elements, which it will be useful to call the 'primary pith filaments', though they do not pursue a very straight course, are yet only seldom branched, except indeed at the origin of a leaf segment. At such a point it is usual to find numerous examples both of pith filaments and sieve-tubes dividing into two, one branch continuing its course in the main axis, while the other turns off into the leaf. Callus is apparently formed very early in the central part of the medulla, and even at this stage it is not unusual to find long strings of callus in course of formation. It seems probable, however, that in a slightly younger stage these elements must have functioned for conduction, but that, after the development of the sieve-tubes from the inner cortex, they have acquired a mechanical function, since their lumina have become obliterated by callus.

Stage ii. Commencement of rapid growth in thickness. In a stage slightly older than the one described above, the medulla has become oval in outline, and sieve-tubes are being rapidly produced by the stretching of the inner cortical cells, while the outer cortex is considerably enlarged by the divisions of the external meristem.

The inner cortex is still sharply marked off from the outer, while between the inner cortex and the medulla there is no clear line of distinction.

Every stage of transition between sieve-tube and cortical cell is present. The sieve-tubes on the periphery of the medulla consist of short elements hardly swollen at the cross walls, while the innermost ones are composed of elongated cells much dilated at the cross walls. Branching of the young sieve-tubes is fairly common, and cross connexions, developed from the pit canals by the stretching of the pits due to the swelling of the longitudinal cell-walls, are very easily traced at this level in their various stages of development. There is a considerable amount of callus in the primary pith filaments, but even in the innermost sieve-tubes callus formation has hardly commenced.

Stage iii. Sections taken about one and a half inches below the apex show a much more advanced stage of development. (Fig. 44, Pl. XXI.)

The cortex is now about four times the breadth of the medulla, and is no longer sharply differentiated into regions, but there appears to be a gradual transition, from the outer parenchymatous to the more elongated inner elements; the latter give rise to numerous hyphae. The whole of the characteristic inner or primary cortex described above¹ has been transformed into sieve-tubes, and all the cortex now present is of secondary origin, derived from the repeated divisions of the outer cortical meristem.

Young sieve-tubes are still present on the outer edge of the medulla, but it seems improbable that new ones are being formed at this stage. The zone of sieve-tubes now contains from seven to nine elements in each radial row, and the development of the sieve-plates is rapidly proceeding, callus being already present in the innermost rows. The 'primary pith filaments' all contain callus.

Stage iv. In a stem of about twice the thickness of that last described but little change appears to have taken place.

The almost isodiametric cells of the outer cortex have now deeply pitted walls, and the pits are distributed fairly evenly over the longitudinal, and arranged in a ring in the transverse, walls. In the more elongated and thicker-walled cells of the inner secondary cortex the pits are also arranged in a ring in the transverse walls, but in the longitudinal walls they are very few and isolated. The appearance of these latter cells, as well as the

¹ pp. 297-298.

arrangement of the pits in their walls, suggest that their function may be the conduction of water.

A much more definite boundary can be recognized between the cortex and medulla at this stage, at least in a transverse section, but this appearance is partly due to the fact that the ingrowing hyphae now originate only from the innermost cortical layer. From this layer they arise in large numbers and run more or less radially towards the centre; they often branch, and are thus to a large extent responsible for the increase in size of the medulla.

From six to nine sieve-tubes are present in each radial row, and the innermost element of each row is generally already quite filled with callus. The primary pith filaments are more widely separated than in the younger stages, and in many cases their lumina are choked by callus.

Stage v. The oldest stem which I examined was about half an inch in diameter (cf. Fig. 45, Pl. XXI).

From this stage nothing is gained to add to the description of the cortex already given in the last stage, beyond the fact that it is now eight or nine times broader than the medulla.

Not more than nine rows of sieve-tubes, the two or three innermost rows of which are now completely obliterated by callus, were found in any radial row; it would seem probable, therefore, that no new ones have been formed since the original primary inner cortex was transformed to the sieve-tube layer. On the other hand, young sieve-tubes composed of quite short cells without callus are still present, and in longitudinal section are often difficult to distinguish from the innermost cortical cells, though in cross section they are sharply differentiated from the latter by the much greater thickness of their walls. The innermost cortical cells have always a ring of pits on their transverse walls, while the young sieve-tubes have definite sieve-plates, and it is not easy to imagine how an adult wall with its ring of pits (such as that figured in Figs. 1 and 2, Pl. XIX) could give rise to a sieve-plate. Certainly no evidence of such a transition is forthcoming. In order to prove definitely that young sieve-tubes do not continue to be formed slowly from the inner secondary cortex throughout the life of the plant, it would be necessary to investigate some considerably older material. On the whole, however, from the evidence forthcoming, it seems probable that the increase in size of the medulla in the oldest specimens is due partly to the great increase in diameter of the elements composing it, but still more to the large number and repeated branching of the hyphae anastomosing among them.

In this old material hyphae which contain callus are occasionally met with. Some of these have taken a longitudinal course, and have very much the appearance of extremely narrow sieve-tubes. They are composed of elongated cells, swollen at the cross walls, and have dense contents;

there can be little doubt that Setchell¹ is referring to elements of this nature when he describes the transformation of some of the anastomosing hyphae of *Sacchoriza* into conducting-tubes.

B. HISTOLOGY.

i. *Nature of cell-walls.*

The mucilaginous nature of the cell-walls of both cortex and medulla in *Macrocystis* is well shown by their great affinity for Methylene blue. In this and other respects, however, both the pit-closing membranes of the cortical pits, and the sieve-plates, show a marked difference in reaction from the rest of the walls. The pit-closing membranes do not stain at all with Methylene blue, but the sieve-plates, with the exception of the small areas actually traversed by threads and slime-strings, take up the stain after remaining in it some hours; cf. Fig. 1, Pl. XIX.

With Congo red the outer layers of the cell-wall stain pink, but the middle lamella, pit-closing membranes, and sieve-plates remain unstained.

The cell-walls of both cortex and medulla give the ordinary cellulose reaction with Iodine and Sulphuric acid, but the middle lamella is very resistant, and remains yellow for a long time before it turns blue, whilst the actual membranes of the pits and the sieve-plates remain permanently yellow.

In their response to swelling agents, it is found that the pit-closing membranes and sieve-plates differ from the cell-wall. Picro-sulphuric and other acids cause the latter to swell considerably, but produce little or no result on either of the former. A solution of Iodine in Potassium Iodide, on the other hand, has not so marked an effect on the cell-wall, while prolonged treatment with this reagent finally causes the pit-closing membranes and sieve-plates to swell slightly. No swelling agent yet used has been able to overcome their powers of resistance to any great extent.

From the above it would appear that the chemical nature of the cell-wall is of the following constitution. The middle lamella is chiefly composed of pectic compounds with some cellulose, but in the outer layers of the wall the proportion of cellulose to pectose is increased. The pit-closing membranes and sieve-plates are evidently composed of some substance still more resistant than pectose.

ii. *Protoplasmic continuity in cortex and hyphae.*

a. *Cortex.* The demonstration of protoplasmic connecting-threads in the cortical cells of *Macrocystis* is, owing to the resistant nature of the pit-closing membrane, a matter of some difficulty, but satisfactory results were obtained by means of an adaptation of the Safranin method.

¹ Setchell, l. c., 1891.

In surface view (Fig. 2, Pl. XIX) a few threads, not more than six or seven, are seen in each cortical pit, and in longitudinal section (Figs. 3 and 4, Pl. XIX) these threads may be seen as very short lines which cross the pit-closing membrane and unite the protoplasm of adjoining cells. These connecting-threads were seen in old plants in the pit-closing membranes in the transverse and longitudinal walls of both inner and outer cortical cells.

b. Hyphae. Numerous single fine threads were demonstrated in the cross walls of the anastomosing hyphae in the young material (Fig. 24, Pl. XIX), but in the older material they are not visible, and it seems probable that the great swelling of the walls has stretched and broken them. It is impossible to be certain which of the cross walls are due to secondary fusions among the hyphae, and one cannot, therefore, say whether threads are developed at places where secondary fusions have occurred. To clear up this point an examination of young plants will be necessary.

iii. *Development of the sieve-plates and obliteration of the sieve-tubes.*

a. Primary pith filaments. Owing to the absence of sufficiently young material the development of the cross walls of the primary pith filaments could not be followed, since callus was already present in nearly every case investigated. In one instance (Fig. 26, Pl. XIX) knobs were seen on either side of the cross wall, but it was impossible to be certain that they were connected by threads, as this stage was observed in spirit material.

Callus is very soon formed, and Fig. 28, Pl. XIX, represents a very commonly occurring early stage, in which callus-rods are found traversing the sieve-plates and a callus-pad has also been already laid down on either side of the plate. There seems no doubt that the mass of callus shown here and in the later figures is laid down by the protoplasm, and is not formed by an alteration of the wall already present, for the original wall, undiminished in thickness, can still be seen, and it does not seem possible that such a large amount of callus could be produced merely by the swelling of an immeasurably small outer layer of this wall.

Fig. 29, Pl. XIX, represents a case in which the callus is accumulating in the centre of the plate, while Figs. 30, 31, Pl. XIX, are drawn from older stages in which a larger amount of callus has been formed. The callus usually accumulates much faster on the side farther from the apex. In Fig. 31, Pl. XIX, the protoplasm has begun to lay down callus throughout the length of the tube. It is formed first as a thin line on the edge of the protoplasm, but soon becomes irregular in its distribution, being laid down in small wedges or blocks, adjoining especially dense portions of protoplasm. These wedges (cf. Fig. 23, Pl. XIX) meet across the tube in places and nearly choke up the channel, but for some time a thin layer of protoplasm is left lining

the interior of the tube. Later, this also is often obliterated and the cavity becomes entirely blocked.

b. Secondary sieve-tubes. (i.) *Surface views.* A study of the earliest stages in the development of the terminal sieve-plates, both in the young material and in the sieve-tubes found on the periphery of the medulla in the older stems, yields very similar results. In the youngest sieve-plates there is a uniform distribution of the threads. Fig. 5, Pl. XIX, represents a slightly older and very much more frequent case in which the centre of the plate is still occupied by single threads, but towards the edge little groups of four or five threads are present; between the centre and the periphery groups are seen composed of two or three threads. Other cases are found in which the centre of the plate is in this last stage, while groups of four to seven threads are present on the periphery. In Fig. 6, Pl. XIX, is shown an older plate, just before callus formation, in which the threads are distributed throughout in groups, five being the usual number, though sometimes as few as four or as many as seven threads may be present in one group. These stages were seen in preparations stained with Safranin, Safranin and Aniline blue, Aniline blue only, and Benzyl blue, so that there seems little chance that any of them can be an artificial product.

Numerous estimations were made of the numbers, both of the separate threads in the youngest sieve-plates and of the groups of threads in the later stages, and there is no doubt that the two quantities broadly correspond. It seems impossible, therefore, that the groups are formed by the aggregation of the original single threads; rather, it seems likely that each group has arisen from the division of a single thread. This conclusion is also supported by the fact that groups of two or three threads are often found in an intermediate position between the earliest arrangement of separate threads and the final resulting groups of five or more.

In a surface view of a sieve-plate callus is first seen as a small ring surrounding each of the threads which make up such a group, and a young sieve-plate in this stage shows numerous small circles each made up of four to six blue points, as shown in Figs. 8 and 9*b*, Pl. XIX. By lateral fusion this group of separate spots soon gives rise to a single ring (Figs. 8 and 9*c*, Pl. XIX).

All this time the threads remain distinct, though some change takes place in their composition, for, simultaneously with the advent of the callus they stain more deeply than before with protoplasmic dyes.¹ Soon after the fusion of the separate callus-spots into a single ring, alterations take place very rapidly at the centre of each group of threads. The final result is that a single slime-string is formed, surrounded by its ring of callus, in place of the original group of independent threads (Fig. 7 and Figs. 8 and 9*c*, Pl. XIX). Before arriving at this stage two or three smaller slime-strings are often

¹ Cf. Hill, l. c., 1901, II, p. 589.

seen (Figs. 8 and 9 *d*, Pl. XIX) situated in a blue area which represents the original position of a group of threads, and these, together with the intervening callus and wall, when any unaltered wall is still present, appear to fuse and disorganize, giving rise to the single slime-string.

The sieve-plate is now fully developed and is a distinct sieve with open pores, through which, in optical section, slime-strings are often seen to run, though in many cases, owing to the thinness of the sections, they have fallen out of the pores during the processes involved in staining the preparation. Three to five sieve-tubes with sieve-plates in this condition may be found in any radial row in the oldest material of *Macrocyrtis* examined.

The obliteration of the innermost active sieve-tubes appears to be a very rapid process. Callus spreads over the surface of the sieve-plate in between the threads and then begins to accumulate at the centre of the plate. The mucilaginous and protoplasmic contents of the tube are at this stage collected into a densely staining mass over the central pad of callus (Fig. 17, Pl. XIX) and continue to deposit more callus, gradually increasing the size of the pad. A thick layer is thus spread over the whole plate and a callus-mass results (Fig. 19, Pl. XIX) on both sides of the plate, but is usually larger on one side than on the other. Meanwhile callus is often formed down the whole length of the tube (Figs. 20, 23, Pl. XIX), in a manner similar to that described above¹ for the primary pith filaments, and accumulates in small masses which are laid down by the dense protoplasm lying against the wall of the tube, till finally in many places the lumen is almost obliterated (Fig. 21, Pl. XIX).

ii. *Longitudinal sections of the terminal sieve-plates.* In longitudinal sections the course of events is not always easily followed. In the youngest sieve-plates examined, the threads are very delicate and often much broken, but they can be seen to be arranged singly (Fig. 11, Pl. XIX). More difficulty was experienced in following the formation of the groups of threads, as the sieve-plate at this stage often becomes very swollen. In several cases single threads were seen in the central portion of the plate, while groups occurred on the periphery. Fig. 13, Pl. XIX, shows the occasional appearance of groups of threads in a plate in which the separate arrangement is still prevalent. In numerous sieve-plates which as yet had no callus, the threads could be clearly seen to be arranged in groups throughout. In several cases a median node, such as that described in *Pinus*², was seen on each thread, and in one such instance callus had been formed and the threads were still visible traversing the callus-rod.

With the appearance of the callus the threads stain much more deeply with Safranin. These alterations are first noticed at the periphery of the

¹ p. 302.

² Hill, The histology of the sieve-tubes in *Pinus*. Ann. of Botany, xv, 1901, p. 589, and Figs. 8, 10, &c., Pl. XXXII; and p. 590 and Fig. 20, Pl. XXXIII.

plate and then proceed towards the centre, the periphery being therefore generally more advanced in development than the centre; they also always begin at the ends of each of the threads of a group and work towards the middle lamella. Sometimes it was found, as in Figs. 14 and 15, Pl. XIX, that callus formation had begun on one side only of the plate in any given group, and had gone as far as the middle lamella from that side, while on the other side unaltered threads were present, as yet not even changed with regard to their staining properties.¹

In many cases each thread can be clearly seen to have its own callus-rod, but the separate callus-rods soon fuse, and a single rod is formed, through which the deeply staining threads are seen to pass. These threads then disorganize and, together with the intervening callus, give rise to a single slime-string. Numerous preparations, such as those shown in Figs. 15, 16, Pl. XIX, were obtained in which callus and slime-string production had proceeded a certain distance from each side of the sieve-plate, and unaltered threads, or threads each enclosed in a separate callus-rod, could be seen stretching between the cones thus formed. In some sieve-plates (Fig. 16, Pl. XIX) this stage of development had been reached on the periphery, while in the centre groups of unaltered threads are still present. The middle lamella then becomes dissolved, at the point at which two cones finally meet, and a continuous slime-string enveloped in a tube of callus is thus obtained; the perforation of the sieve-plate is now complete.

The stages in the obliteration of the innermost sieve-tubes are more clearly seen in longitudinal than in transverse sections. In Fig. 16, Pl. XIX, callus formation can be seen to have spread, from the heads of the rods surrounding the slime-strings, over the intervening areas. In Fig. 17, Pl. XIX, a small pad of callus has been formed over the central portion of the sieve-plate, and in Fig. 19, Pl. XIX, such a stage as this has given rise to a large mass, extending right across the sieve-plate, and filling up a large portion of the sieve-tube. From this it seems clear that the obliteration, unlike the development of the sieve-plate, proceeds from the centre towards the periphery of the plate. In Fig. 18, Pl. XIX, a thin layer of callus is represented in process of being laid down against the wall of the tube; this layer soon increases in amount, generally starting from the sieve-plates, but sometimes arising separately in different parts of the tube². Finally we obtain a stage such as that represented in Figs. 19, 23, Pl. XIX, in which the lumen is almost entirely obliterated.

iii. *Lateral sieve-plates.* On the lateral walls of the sieve-tubes, the places at which branching has occurred, or from which lateral connexions have originated, develop normal sieve-plates (Fig. 10, Pl. XIX). Sieve-plates are also developed on the cross walls of the rows of cells forming the cross connexions between the sieve-tubes. In many of these cases the

¹ Cf. Hill, l. c., 1901, II, Pl. XXXIII, Figs. 18, 23.

² Oliver, l. c., 1887.

perforations are so extremely small that it seems impossible that groups of threads can have preceded them, and perhaps the single threads originally present never divide in such sieve-plates to form groups. In some young lateral plates, however, groups of threads have been distinctly seen.

Besides these lateral sieve-plates, small isolated pads of callus are often found on the lateral walls of the sieve-tubes. A highly magnified example of one of these is shown in Fig. 22 *c*, Pl. XIX, and no threads or slime-strings can be seen in it. These lateral pads often appear to be sunk right inside the wall, as shown in Fig. 22 *a*, Pl. XIX, but sometimes each pad appears to surround a hole, the edges of this hole being continuous with the inner wall of the sieve-tube (Fig. 22 *d*, Pl. XIX). In the lateral walls of young sieve-tubes a few cases have been found in which a small callus-ring was present and a collection of deeply staining protoplasm was noticed lying adjacent to the ring. I think it probable that these pads were originally developed round pits, the remains of the pit being still visible in such cases as Fig. 22 *d*, Pl. XIX. As stated above¹, the pits in the longitudinal walls of the primary inner cortical cells were very rare and widely separated, and after the stretching of these cells to form sieve-tubes they naturally became still further apart, and, while some of them gave rise to cross connexions, others might perhaps be ruptured by the rapid growth of the surrounding walls, and callus-pads might then be formed round the old pit entrance. The first evidence of such a pad appears as a ring surrounding a pit; this ring then spreads over the pit-closing membrane, and finally a small mass of callus is developed which nearly or completely fills the pit.

iv. *Nature of the callus in Macrocystis.*

The callus in the primary pith filaments, secondary sieve-tubes, and hyphae of *Macrocystis* gives all the reactions characteristic of the ordinary callus found in the sieve-tubes of Phanerogams. In addition to those already enumerated by Oliver², London blue, like Water blue, gives it a fine blue colour, Congo red stains it a bright pink, and Thionin gives it a purplish tinge. The fact that Thionin also stains mucilage a similar, though deeper, purple is of interest in connexion with the conception of callus as a hydrated or mucilaginous form of cellulose. The frothy appearance of the callus in *Macrocystis*, an appearance which is increased in swollen material, is very striking, and an attempt to figure this is seen in a lateral pad shown in Fig. 22 *c*, Pl. XIX.

IV. LAMINARIA SACCHARINA.

This species of *Laminaria*, though simpler than *Macrocystis* in many respects, is essentially similar as regards its general anatomy, while the histology of the two plants differs only in degree of development and complexity. I therefore propose to give but a short account of the anatomy of

¹ pp. 299-300.

² Oliver, l. c., 1887.

Laminaria saccharina, and then to pass on to its histology. The latter, more particularly the section dealing with the sieve-tubes, is of especial interest in connexion with the light which it throws on the evolution of the complex secondary sieve-tubes in *Macrocystis*, since these have hitherto been regarded as unparalleled among the Brown Algae.

(a) *GENERAL ANATOMY.*

Stage i. Young plant. The intercalary growing region in a very young plant of *Laminaria saccharina* already reveals a differentiation into primary cortex and medulla, though secondary growth by means of a cortical meristem has hardly yet begun. A cross section of such a stage is shown in Fig. 46, Pl. XXI.

The cells of the outermost layer of the cortex are just beginning to divide tangentially and radially to form the secondary cortex, but the main part of the cortex is primary, and can be seen in longitudinal section to be composed of rows of cells showing a gradual transition, from almost isodiametric cells on the outside, to much more elongated ones near the medulla. In these last, which are rapidly being stretched longitudinally, the swelling of the walls is more or less obvious, causing the pits in the longitudinal walls to become much stretched; hence giving rise to cross connexions between the cells. Hyphae are found arising from the inner layers of the primary cortex in various ways. They may originate as small projections, either near the cross wall of a cell, or sometimes at other places on its longitudinal wall, but always on the side nearest the medulla. They grow inwards towards the medulla and, since even at this stage free ends are but rarely found, it seems certain that secondary connexions are speedily formed. They often grow straight across, and thus connect together two inner cortical cells on opposite sides of the medulla, and during their course they may or may not give off branches. Others again run but a short way into the medulla, and soon form secondary connexions with other hyphae.

The medulla, a more magnified transverse section of which is shown in Fig. 47, Pl. XXI, is composed of much elongated cells, dilated at the transverse septa and with longitudinal walls much swollen, owing to mucilaginous degeneration. The invading hyphae anastomose in the jelly thus formed, but they never form connexions with the elongated elements. There can be no doubt that these last are derived from the original central cells of the thallus, and I propose to call them 'primary pith filaments', as in *Macrocystis*. They probably function as the primary conducting-cells in the young plant and at this stage contain no callus.

Stage ii. Beginning of growth in thickness. The next stage investigated was found in material taken from the base of the lamina, in a young plant about eight inches in length.

A transverse section of this plant is seen in Fig. 48, Pl. XXI. A zone of secondary cortex of considerable size has been added by the meristematic division of the outermost cortical layer, and the cells produced by this meristem are seen to be arranged in radial rows. In longitudinal section the outer cells composing these radial rows are found to be isodiametric, while the inner ones are slightly elongated in a longitudinal direction. There is thus a gradual transition, in the length of the cells, between the secondary and primary cortex, the outer elements of the latter being now more elongated than in Stage i, while the innermost cells have by this time attained a considerable length.

In the cross walls of the secondary cortex the pits are arranged in a ring and are much deeper, and also sharper in outline, than are the more numerous and smaller pits in the primary cortex. The cells of the secondary cortex have also many more pits in their longitudinal walls than have those of the primary cortex.

Many interesting changes have occurred in the primary cortex since the last stage. The hyphae running into the medulla originate from several of the inner layers of the cortex and separate the cells of which these layers are composed, causing them in transverse section to appear as if arranged in radial rows. The outermost cells of these rows are square-ended and somewhat thick-walled, while the innermost cells have much more swollen walls and are dilated at the transverse septa.

The last of these elements pass gradually into the original medulla and, becoming very similar in appearance to the primary pith filaments, add considerably to its size. The increase in size of the medulla, due to the addition of these secondary elements, is readily seen on comparing Figs. 46 and 48, Pl. XXI. I propose to term these elements 'inner secondary sieve-tubes', but it must be borne in mind that they are produced by a late development from the already formed cells of the primary cortex, as are the so-called secondary sieve-tubes in *Macrocystis*, and in neither of these cases are they 'secondary' in the sense in which the term is used when speaking of secondary phloem in Phanerogams, not being formed, like that tissue, from the divisions of a secondary meristem.

The original medulla presents much the same appearance as that described in the last stage.

Stage iii. Adult stem. Fig. 49, Pl. XXI, represents a cross section of the adult stem magnified to the same degree as are Figs. 46 and 48.

It will be seen from the photograph that the secondary cortical tissue has greatly increased in amount, yet in longitudinal section the same gradual transition from periphery to centre is still noticeable. The extent of the primary cortex now bears a very small proportion to that of the secondary, and the hyphae arise from the outer as well as the inner layers, thus apparently increasing the number of elements in each radial

row. In longitudinal section the outermost cells of each radial row are seen to be much elongated and are square-ended, but there is a very gradual transition between these and the inner elements, which have much swollen longitudinal walls, are dilated at the septa, and, in their turn, pass gradually into the medulla. The question of the physiological function of the outer cells of the primary cortex is a very interesting one, and, as it is impossible to separate them from the inner elements, which are certainly sieve-tubes, I will say at once that I propose to call them 'outer secondary sieve-tubes'. The use of this term will be seen to be justified by an investigation of their histology.

Though a sharp line cannot be drawn between primary and secondary cortex, there is no reason to suppose that elements of secondary origin ever become transformed into outer secondary sieve-tubes; on the other hand I believe that these are entirely derived from the primary cortex, and that, when that tissue has been used up, no more sieve-tubes are formed¹. The oldest specimen of *Laminaria saccharina* which I examined had a stem about three quarters of an inch in diameter, and, in a transverse section of this stem, there were found radial rows consisting of some ten or twelve elements surrounding the medulla. This number agrees with that observed in the radial rows of sieve-tubes in the plant figured, which had a diameter of less than half an inch.

The medulla is now a much more elongated oval in shape, and has considerably increased in size since the last stage, several of the inner secondary sieve-tubes from each radial row having become included in it.

A few elements, distinguished by their greater diameter and their position nearer the centre, probably represent the primary pith filaments. While these are always unbranched, the sieve-tubes formed from the primary cortex and added to the medulla often have lateral branches which in many cases arise at the level of the sieve-plate, recalling those cases in the young plant where some of the inner cortical cells gave rise to hyphae opposite their transverse septa. The three-armed sieve-plates thus produced are very striking (Fig. 36, Pl. XX).

The hyphae in the medulla have formed a much branched system, running in all directions in the jelly, and filling up the centre, but they have now no free ends.

Callus is largely developed in the primary pith filaments, and is also present in both the outer and inner secondary sieve-tubes, confirming the conclusion already arrived at that these elements are to be looked upon as true sieve-tubes. The extent of development of the callus was found to differ in winter and summer material, but reference to this point will be made later.

¹ Cf. *Macrocystis*, p. 300.

(b) HISTOLOGY.

i. *Nature of cell-walls.*

The cell-walls in *Laminaria saccharina* are of an even more mucilaginous character than those of *Macrocystis*, and hence they stain very strongly with such reagents as Methylene blue, but more faintly with Congo red; with Iodine and Sulphuric acid the outer layers of the cell-wall rapidly turn blue, but the middle lamella first takes a yellow tinge, and afterwards turns blue like the rest of the wall.

The pit-closing membranes, as in *Macrocystis*, differ in their reactions from the general cell-wall. They become yellow when treated with Iodine and Sulphuric acid, and do not stain at all with Methylene blue or Congo red. The distribution of the pits in the cortex has been described above, but a more minute investigation of their nature shows that each primary pit is made up of a number of secondary pits; Figs. 1 and 2, Pl. XX, represent transverse walls of cells of the secondary cortex, and are drawn from preparations stained with Methylene blue. The general wall is deeply stained, but the pits appear as rounded areas in which are blue lines on an unstained ground. The blue lines separate the secondary pits from one another, while the spaces between them, which are left unstained, represent the pit-closing membranes of the secondary pits. The distribution of the connecting-threads is found to be practically confined to these unstained areas.

The sieve-plates, like the pit-closing membranes of the cortical cells, are of a more resistant nature than the general cell-walls. They turn yellow when treated with Iodine and Sulphuric acid, and do not stain with Congo red. After remaining for a short time in a solution of Methylene blue they present the appearance of a delicate reticulum, the meshes of which are blue, while the spaces between, which correspond to the areas traversed by the threads, remain unstained. The transverse walls of the young primary cortex, from which some of the sieve-plates are derived, give a similar reaction.

ii. *Protoplasmic continuity in cortical cells and hyphae.*

a. *Cortex.* In the outer secondary cortex the connecting-threads are always arranged in definite groups, almost, if not entirely, confined to the pits. A surface view of the cross wall of a cortical cell shows five to eight of these groups arranged in the form of a ring near the periphery of the wall, as seen in Fig. 3, Pl. XX. Each pit in the longitudinal wall is traversed by threads which here also are arranged in clusters (Figs. 6 and 7, Pl. XX).

In the inner secondary cortex the pits often extend much further towards the centre of the cross wall, and, when such cases are examined for threads, it is seen that, while groups of threads are present corresponding to the pits, a few isolated threads are also found in the other parts of the wall

(Fig. 4, Pl. XX). On the longitudinal walls the threads are restricted to well-defined pits.

In the primary cortex of the young plant no threads could be demonstrated, owing to this material having been preserved in spirit. No definite pits can be recognized in this region, and it is to be supposed that the threads would not be sharply differentiated into groups as in the secondary cortex, but that they would be evenly distributed as in the young sieve-plates.

b. Hyphae. At the point of origin of a hypha the threads are arranged in four or five rather indefinite groups, as seen in surface view in Fig. 12, and in section in Fig. 14, Pl. XX. In the winter material, callus was found to be developed to a small extent at the origin of the hyphae, and, as described below in the sieve-plates, each thread produces its own callus-rod (Figs. 13, 15, Pl. XX).

Connecting-threads were also seen in the transverse septa of many of the hyphae of the medulla, and here also callus was often found in the winter material (Figs. 22, 24, 25, 27, Pl. XX).

iii. *Development of the sieve-plates and obliteration of the sieve-tubes.*

(a) In the square-ended *outer secondary sieve-tubes*, derived from the outer layers of the primary inner cortex, the cross walls were found, in longitudinal section, to be traversed by numerous small groups of threads, seen in surface view to be arranged in various ways to form a larger pattern (Fig. 10, Pl. XX). In the summer material these threads stain with Safranin, in exactly the same manner as do the cortical threads, but in the winter material a deeper stain is taken by the threads of some of the inner of these sieve-plates, and, when a section is placed in London blue, each thread is found to be enclosed in its own callus-rod. Cases of callus formation in these secondary sieve-tubes are shown in section in Figs. 17, 18, 20, Pl. XX. The heads of the separate callus-rods are generally fused to form a small patch over each group of rods, but as a rule there is no great accumulation of callus. Fig. 21, Pl. XX, is taken from a very exceptional sieve-plate, situated on the innermost edge of a row of the outer secondary sieve-tubes, and consequently next the sieve-tubes of the medulla; a large mass of callus has been deposited on one side of the plate. In summer material a small amount of callus was twice found in elements in a position similar to this last.

On the lateral walls of the outer secondary sieve-tubes, pits traversed by threads are sometimes found (Fig. 18 *a*, Pl. XX). Larger lateral plates are present on the longitudinal walls of the inner of these elements, and some of these at any rate represent the origin of hyphae, while others are found at the origin of the larger cross connexions (Fig. 11, Pl. XX).

(b) *Sieve-tubes of the medulla.* There is a gradual transition from the

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square-ended, more or less rudimentary outer sieve-tubes, to the elongated, swollen-walled sieve-tubes of the pith. In the younger sieve-plates belonging to the latter, the threads are rather difficult to demonstrate. In favourable material they were, however, well-stained, and surface views, showing their arrangement, are given in Figs. 28, 29, Pl. XX. Sometimes the threads are almost evenly distributed, sometimes they form small groups of ten or twenty, arranged in various ways, similar to the methods of distribution of the threads in the rudimentary sieve-tubes.

In a very few of the older sieve-plates these groups of threads have become converted into sieve-fields. Each group does not, as in *Macrocystis*, give rise to a single slime-string traversing a single hole in the sieve-plate, and enclosed in a callus-rod, but each thread of a group remains independent and forms a separate slime-string surrounded by its own callus-rod. The number and distribution of the slime-strings in such a stage as Fig. 32 *a*, Pl. XX, are found to be similar to those of the threads in Fig. 29, Pl. XX.

The first step towards the formation of the slime-strings is shown by the deeper staining power of the threads when treated by the Safranin method. At this stage, if the section be placed in London blue, a faint blue ring is seen, both in surface views and optical sections, to surround some of the threads (Fig. 30, Pl. XX). After this, perforation soon follows, and Figs. 31, 32, Pl. XX, represent surface views of sieve-plates riddled with holes. Each thread has become converted into a slime-string running through a hole in the plate, but in many cases the slime-strings have fallen out in the course of preparation of the section. Each hole is surrounded by callus which in surface view appears to spread over the sieve-field areas, but does not extend over the portions of the plate between the areas; Fig. 32 *b*, Pl. XX, is a small portion of Fig. 32 *a* more highly magnified, and showing the limits of the callus-patches. In optical section each slime-string can be seen in the thickness of the plate to be surrounded by a ring of callus, which is of course a tube in longitudinal section. The heads of the callus-rods soon fuse and form a small aggregation over the ends of each group (Figs. 36, 38, Pl. XX). Such aggregations are seen in surface view in three places in Fig. 31, Pl. XX.

At this stage the sieve-plate is at its fullest development. The sieve-tubes nearer the centre of the medulla, some of which represent the primary pith filaments, are in various stages of obliteration. Callus soon begins to accumulate over the original patches and from there spreads over the whole surface of the sieve-plate (Fig. 37, Pl. XX). A large mass of callus is found in the innermost elements and is generally much more developed on one side of the plate, probably, from analogy with *Macrocystis*¹, that side which is furthest from the apex (Fig. 39, Pl. XX). Not more than two sieve-tubes in this state are usually present in any radial section. In some of the

¹ p. 302.

oldest sieve-tubes, and also in a few of the hyphae, callus is formed throughout the length of the cell, and here, as in *Macrocystis*, it seems highly probable from its mode of formation that it is laid down directly by the protoplasm (Figs. 40, 41, Pl. XX).

All these stages, just described under the head of sieve-tubes of the medulla, were examined in winter material, but some obliterated sieve-tubes were found in summer material, and, after much swelling with various reagents, callus was also demonstrated in a few of the younger examples.

iv. *Nature of the callus.*

The callus of *Laminaria saccharina* does not appear to be all of the same kind. That found in the outer secondary sieve-tubes, in most of the hyphae, and in the young inner secondary sieve-tubes is only visible when stained with London blue, and it stains with that reagent in unswollen winter material, but gives no reaction with Water blue either in swollen or unswollen material.

It was stated above that in the summer little or no callus was to be seen. This statement applies to fresh material, but it was found that, after being left for a considerable time in some swelling agent, such as Potassium iodide and Iodine, a substance which stained with London blue was to be seen in several of the older sieve-tubes and plates, as well as in a few younger stages. In summer material rare examples of callus were found in the innermost sieve-tubes, which stained with Water blue or Russow's callus reagent, but in the winter material the callus present in all the older tubes, as well as that in such unusual cases as that figured in Fig. 21, Pl. XX, stains with these reagents.

It seems probable that callus in various states of hydration is present in *Laminaria saccharina*, for:—

(a) Most of that found in summer differs but little from the substance of the ordinary cell-wall, and only gives a characteristic callus reaction after further hydration with swelling reagents.

(b) In winter and in a few cases in summer the callus is sufficiently hydrated in the fresh material to stain with London blue. It becomes much more definite after swelling for some time, but it was never found to stain with Water blue.

(c) Finally, the most hydrated form of callus is similar to that found in *Macrocystis*, and stains with Water blue as well as with London blue. It was only found in the sieve-tubes of the medulla and in the exceptional case mentioned above¹, and shown in Fig. 21, Pl. XX. This fully hydrated callus is hardly ever formed in summer.

¹ p. 311.

V. SIEVE-PLATE DEVELOPMENT AND CALLUS FORMATION IN
MACROCYSTIS AND LAMINARIA.

In this section I wish first to point out how completely the observed series of changes in the development of the sieve-plate in these two plants harmonizes with the interpretation of sieve-plate development in *Pinus* given by Hill.¹ From these researches it appears probable that the boring-out of the protoplasmic threads to form slime-strings is due to ferment action. In *Macrocyctis* and *Laminaria*, after the manner described for *Pinus*, the passage of the ferment down the young threads may be traced by the alteration of the staining properties of the protoplasm and by the change of the cell-wall into callus. It appears in *Macrocyctis*², as in *Pinus*³, that the ferment produced in any given sieve-tube is only able to act as far as the middle lamella of the wall which separates this sieve-tube from an adjoining one, a fact which lends support to the view that the young threads are interrupted at the middle lamella.⁴ Structures which may be compared to the median node of the threads of *Pinus* and to the median nodules of the slime-strings in that plant⁵ were occasionally met with in *Macrocyctis*⁶, and might have been more distinctly seen in better preserved material.

The various states of callus found occurring in *Laminaria saccharina*⁷ confirm the idea that that substance is a hydrated form of cellulose, and it is suggested that it is in a different degree of hydration in each of the states described. In summer there is present in the sieve-tubes a substance which will not react to any known callus-stain, but by hydration with swelling reagents it is possible to induce it to stain with London blue. Further swelling of callus which stains naturally with London blue causes it to stain more deeply with that reagent, but can never induce it artificially to attain a sufficient degree of hydration to stain with Water blue, though sometimes it can be made to react when treated with Russow's callus reagent after swelling for a considerable period. In winter all degrees of hydration of the callus were found in the various elements of *Laminaria*.

It appears that in the Laminariaceae, as in Phanerogams,⁸ callus can originate either by a change in the already formed cell-wall or by deposition from the protoplasm; in the formation of callus-rods in the sieve-plate it seems highly probable that the callus is produced by a change in the cell-wall.

But with regard to the accumulation of callus in masses at the sieve-

¹ Hill, l. c., 1901, II, pp. 594-596.

² Cf. Figs. 14, 15, Pl. XIX.

³ Hill, l. c., 1901, II, Figs. 18, 23, Pl. XXXII.

⁴ Cf. Gardiner, Proc. Camb. Phil. Soc., xiv, 1907, p. 209.

⁵ Hill, 1901, Fig. 12, Pl. XXXII, and Fig. 20, Pl. XXXIII.

⁶ Cf. p. 304.

⁷ Cf. p. 313.

⁸ Hill, l. c., 1901; and Hill, Notes on the Histology of the Sieve-tubes in certain Angiosperms, Ann. of Bot., 1903, p. 267.

plates, and its formation throughout the length of all classes of sieve-tubes I have been unable to confirm Oliver's conclusion that here also callus is due to mucilaginous degeneration of the cell-wall. Oliver may have been led to this view by working on herbarium material, but in the course of the present research all the evidence obtained from *Macrocystis* and *Laminaria* has pointed to the conclusion that the callus is directly laid down by the protoplasm, and thus supports the views of Gardiner¹ and Hill². It is only necessary here to refer to the description on p. 302, and to Figs. 23, 27, and 17-20, Pl. XIX, Figs. 21 and 40-42, Pl. XX, in order to show how difficult it would be to harmonize such appearances with the view that the callus is produced from the already formed cell-wall. No cases were observed in which the callus was seen to fade gradually into the cell-wall, as described by Oliver³, and as also shown by Hill⁴ in an abnormal case in a sieve-tube in *Pinus*.

As in *Pinus*, the unaltered portions of the old cross wall are still visible in the oldest sieve-plate and are seen in longitudinal sections as beads, of a glistening appearance, which do not stain with callus-stains. The callus-cushion attains a very large size (Fig. 19, Pl. XIX), but the dimensions of the original cell-wall diminish very slightly, if at all, and it is impossible to believe that the minute layer of cellulose which could be thus accounted for can be responsible for the production of such a large mass of callus.⁵

VI. COMPARISON OF THE ANATOMY AND HISTOLOGY OF *MACROCYSTIS PYRIFERA* AND *LAMINARIA SACCHARINA*.

In the young plants of both *Laminaria* and *Macrocystis* the original central cells become stretched and give rise to elongated elements which have been described as 'trumpet hyphae' by other authors.⁶ As suggested above, it seems better to term them 'primary pith filaments'. They probably function for conduction in the young plants; in *Macrocystis* they are very early obliterated by callus, but in *Laminaria* this does not take place till a very much later stage.

The stretching process begun in the central cells of the thallus is later continued in the primary cortex. In *Laminaria* the cells of this tissue elongate and are transformed, without further cross division, into secondary sieve-tubes; in *Macrocystis* each cell divides transversely several times during the process of elongation, and here also all the primary cortex finally gives rise to secondary sieve-tubes. Thus in both plants the secondary

¹ Gardiner, Observations on constitution of callus, Proc. Camb. Phil. Soc., v, 1885, p. 230, and cf. Gardiner and Ito, Mucilage-secreting hairs in *Blechnum* and *Osmunda*, Annals of Botany, i, pp. 33 et seq., and p. 39 a, Figs. 34, 36, 41, 43.

² Hill, l. c., 1901, pp. 597-600, and cf. Figs. 22, 26, 27, Pl. XXXIII.

³ Oliver, l. c., 1887, Figs. 2, 10, 11, Pl. I and II.

⁴ Hill, l. c., 1901, p. 598, Fig. 25, Pl. XXXIII.

⁵ Ibid., p. 599.

⁶ Pp. 298 and 307.

sieve-tubes together with the primary pith filaments represent the whole of the young thallus, the entire cortex of the adult being of later origin.

Growth in thickness in *Macrocystis*, as in *Laminaria*, is due to the production of this late cortex by the divisions of an outer cortical meristem, and no other meristem is ever present. When the primary cortex has been entirely transformed to sieve-tubes, there is no reason to suppose that further sieve-tubes are produced from the secondary cortical cells in either plant. Hence the increase in size of the medulla, after additional sieve-tubes have ceased to be formed, must be ascribed to the swelling of the walls and the greater diameter of the elements already present, also to the numerous hyphae which grow in from the inner cortical layers and form an anastomosing system throughout the medulla.

In *Laminaria saccharina* the innermost secondary sieve-tubes, produced from the primary cortex, have much swollen walls, and resemble in every particular the primary pith filaments. On the other hand, even in the oldest stem examined, the outermost secondary sieve-tubes remain more or less rudimentary, but there can be no doubt that both kinds of elements are to be regarded as homologous with the well-known sieve-tubes of *Macrocystis*. In the latter, however, the secondary sieve-tubes have attained a far greater degree of development; they differ in size and general appearance from the primary pith filaments, and there is no gradual transition as in *Laminaria*. This difference in *Laminaria* may well be explained as due to the more gradual growth in the latter plant, there being no pause in the differentiation of the elements of the original thallus, but merely a slow diminution in amount of elongation, from the central cells to those of the periphery. It is probable that the greater development of the sieve-tubes in *Macrocystis* is due to its habit of growth and to the considerable length attained by the stem, as has already been suggested by Oliver¹ and Wille².

In longitudinal section it is of interest to compare the appearance of an adult stem of *Laminaria* with that of a young plant of *Macrocystis* in the stage described as *Stage ii*³. At that age it is found that there are only two or three layers of sieve-tubes as yet developed from the primary cortex, while the still rapidly elongating square-ended cells of the remainder of the cortex are not yet transformed into sieve-tubes. While the differentiated sieve-tubes are here to be compared with those of the adult *Laminaria* which become part of the medulla, the primary cortical cells are in appearance not unlike the outermost sieve-tubes in *Laminaria*, which always remain rudimentary.

The main points in connexion with the development of the sieve-plate in the two plants are strikingly similar, but some difference in detail is found in *Macrocystis* which may be largely due to the far greater size

¹ Oliver, l. c., 1887.

² Wille, l. c., 1897.

³ p. 299.

of the sieve-plate in that plant. In *Laminaria* the single threads traversing the young plate are arranged in more or less well-defined areas. Each thread develops its own callus-rod and gives rise to a separate slime-string, and, though the heads of a group of these rods are often united, the rods themselves always remain distinct throughout the thickness of the plate. In other words, each thread produces a separate perforation in the sieve-plate. In *Macrocystis* the threads are homogeneously distributed in the very young sieve-plate, and are not confined to any special areas. Each original thread soon divides to form a group, and in the first stage of callus formation each thread of such a group has its own callus-rod; but from each group of threads with their separate callus-rods is finally developed a single slime-string enclosed in one callus-rod. In other words, each thread of a young sieve-plate eventually gives rise to a separate perforation in the sieve-plate, but, in between the original and the final state, a stage is found in which a group of threads is present, derived by the division of the single thread, and giving rise by fusion to a single slime-string.

In *Macrocystis* and *Laminaria* the old sieve-tubes are obliterated in very much the same way by the deposition of callus. In the former plant the callus begins to accumulate at the centre of the sieve-plate in a single mass and spreads thence to the periphery; in the latter, callus begins to be formed over each area of threads in numerous places on the sieve-plate, and then spreads in every direction over its surface.

Rarely in the summer, more often in the winter, callus is found in *Laminaria* which has the same properties as that of *Macrocystis*, but callus in a lower state of hydration is more often present in *Laminaria*.

The structure of the general cell-walls and of the pit-closing membranes is essentially the same in the two plants.

VII. OTHER SPECIES EXAMINED.

I have examined various other species of the Laminariaceae, by way of supplementing the results obtained during the above research, and I propose to refer to them shortly here.

Sacchoriza (Laminaria) bulbosa, De la Pyl., has no distinct medulla and no sieve-tube-like elements. Some elongated cells, with living contents and very thick walls, are found in the central part of the stem; these do not appear to anastomose with each other or with other elements.

Laminaria digitata, Lamour., of which the material examined was preserved in summer, is very similar in structure to *Laminaria saccharina*. Its stem is differentiated into cortex and medulla, and numerous sieve-tubes are found in the medulla. The inner cortical layers, which resemble in position and appearance the outer secondary sieve-tubes of *L. saccharina*, give rise to hyphae which grow into the medulla. In the innermost cells of this tissue exceptional cases of callus formation were found; in two

of these the callus stained with Water blue or London blue, and in two only with London blue. In the sieve-tubes of the pith and in the cross walls of several of the hyphae numerous callus-rods were found, some of which stained only with London blue, while a few of the more central elements contained callus which gave the characteristic reaction with Water blue. Threads traversing the young sieve-plates, and slime-strings in the older sieve-plates, were satisfactorily demonstrated in a few cases, and several inner sieve-tubes, probably primary pith filaments, were found to be entirely filled with callus which stained with London blue and Russow's callus reagent, and sometimes with Water blue.

It is probable that, if well-preserved material were examined, *Laminaria digitata* would yield results similar to those obtained from *L. saccharina*.

Alaria esculentea, Grev. Some material pickled in Formalin was examined by the Safranin method. The results obtained were very similar to those of Wille¹: callus was seen in the sieve-tubes of the medulla, but no secondary sieve-tubes were demonstrated.

Nereocystis Luetkeana, P. and R. Some spirit material was investigated, and the conditions obtaining in the sieve-tubes appeared to be very similar to those present in *Macrocytis*. In the primary pith filaments callus was found in large quantities, often entirely obliterating the lumen. In the young secondary sieve-plates callus was seen in surface view to appear in patches as in *Macrocytis*; in older stages it was seen to accumulate in masses, at first as in *L. saccharina*. Soon, however, it became heaped up in the centre by deposition from the dense central mass of protoplasm, and then spread over the whole sieve-plate, forming a large cushion. In a few old sieve-tubes callus was seen to be formed throughout the length of the tube, and stages like that seen in Fig. 23, Pl. XIX, were observed, in which there could be no doubt that the callus was deposited by the protoplasm.

VIII. SUMMARY AND CONCLUSIONS.

I. There can be no doubt that the 'trumpet hyphae' in *Macrocytis pyrifera* and *Laminaria saccharina* are to be looked upon as true sieve-tubes. They represent the modified, original central cells of the thallus, and may be termed 'primary pith filaments'. Though they differ as to their degree of development, they are certainly homologous with the secondary sieve-tubes of *Macrocytis*, which are similarly derived from the modified primary cortex of the young thallus.

II. Secondary sieve-tubes have also been demonstrated in *Laminaria saccharina* and are probably present in *Laminaria digitata*. These, like the secondary sieve-tubes in *Macrocytis*, represent the whole of the original

¹ Wille, l. c., 1897.

primary cortex and, both in position and mode of development, are undoubtedly homologous with those well-known elements. The greater development of the conducting organs in *Macrocystis* and *Nereocystis* is probably due to the habit of those plants.

III. The histology of the sieve-plates, in the primary pith filaments and secondary sieve-tubes, is essentially the same. Threads are found traversing the young sieve-plate, and each gives rise in the older plates, apparently by means of ferment action, to a slime-string enclosed in a rod of callus. In *Macrocystis* each original thread first divides to form a group, and each thread of a group forms its own callus-rod, but finally, by fusion of these, only one slime-string is produced from each group. The older sieve-plates are obliterated by the deposition of callus in large masses over their surface, and callus is also formed throughout the length of the old sieve-tubes.

IV. Callus is to be looked upon as a hydrated form of cellulose, and is found in *Laminaria saccharina* and *L. digitata* in various states of hydration. It appears to be produced in the young sieve-plates by the action of a ferment on the already formed cell-wall, but is afterwards accumulated by deposition from the protoplasm, both on the surface of the sieve-plate and on the lateral walls of the tube.

V. It is interesting to note how fully the histology of the sieve-tubes agrees with that of the sieve-tubes of Phanerogams. It is observed that, at the advent of the callus, a simultaneous increase of staining capacity becomes noticeable in the threads, and, as in *Pinus*¹, it is suggested that the development of the sieve-plate is a function of ferment action. There is one point of contrast between the method of obliteration of the sieve-tubes in the Laminariaceae and *Pinus*. In *Pinus*² the heads of the slime-strings were found to be still visible on the free edge of the callus-cushions, and the path of the slime-strings could be traced throughout the callus-mass; but in *Macrocystis* and *Laminaria*, on the other hand, the callus is laid down by the protoplasm of the sieve-tube, over the heads of the slime-strings, so that they are buried by the overlying callus, and no perforations can be traced through the pad³.

VI. It has been shown that in young stages of *L. saccharina* the cells of the hyphae become secondarily attached to those of the primary cortex, and that this phenomenon also probably occurs in *Macrocystis*.

VII. Protoplasmic connecting-threads have been demonstrated throughout the tissues of *Macrocystis pyrifera* and *Laminaria saccharina*, but it is impossible to be certain of their formation in the case of secondary attachments. Their demonstration in cells not genetically connected would

¹ Hill, l. c., 1901.

² Ibid., 1901, II, Fig. 14, Pl. XXXII.

³ Fig. 19, Pl. XIX, Fig. 39, Pl. XX.

be interesting in connexion with recent theories of the development of connecting-threads¹.

The remarkably close resemblance found at every stage between the development of the sieve-tubes in the Laminariaceae and Phanerogams² would almost have sufficed some years ago to convince us of the truth of the homologous theory of alternation of generations, but, though it may still be interesting in connexion with that theory, the present knowledge of cases of homoplasy is so rapidly accumulating that such a resemblance can no longer be regarded as having much weight.

EXPLANATION OF FIGURES IN PLATES XIX-XXI.

Illustrating Miss Sykes's Paper on *Macrocystis pyrifera* and *Laminaria saccharina*.

PLATE XIX. *Macrocystis pyrifera*.

The lenses used were Swift's, Zeiss', and Watson's $\frac{1}{4}$ apoc. with six and eight compensating oculars. Unless otherwise specified, the preparations were stained by the Safranin method followed by London blue. The blue colour in the figures represents callus.

Fig. 1. Cross wall of a cortical cell in surface view, showing arrangement of pits; note curious striations in the wall itself. (Stained watery Methylene blue.) $\times 600$.

Fig. 2. Cross wall of a cortical cell in surface view, showing the ends of protoplasmic threads as dots on the pit-closing membranes. $\times 600$.

Fig. 3. Longitudinal section of a cell of the inner cortex. Pits are seen in section in the end-wall, and connecting-threads traverse the pit-closing membranes both there and in the solitary pit (x) found in the lateral wall. $\times 600$.

Fig. 4. Longitudinal section of part of the lateral wall of an inner cortical cell. An unusual case showing several lateral pits quite near together. $\times 750$.

Fig. 5. Surface view of a very young sieve-plate, from the outermost row of secondary sieve-tubes in an old stem. The ends of the threads are seen as fine dots and are arranged singly except on the periphery of the plate, where a few groups are seen. $\times 600$.

Fig. 6. Surface view of a slightly older sieve-plate, being the second element in a radial row, counting from the outer edge. The threads are arranged throughout in groups of four, five, and six. (Stained Safranin and Acid violet.) $\times 600$.

Fig. 7. Surface view of a sieve-plate, showing progressive differentiation from the centre to the periphery. A mass of deeply staining protoplasm hides the centre of the plate, where probably only single threads would have been found. A few single threads are still visible round this mass, but most of them have each given rise to groups of two and three threads. Nearer the outside, groups of five are present, and those represented as surrounded by a ring have already developed callus, while on the extreme periphery single slime-strings, each formed from one of these groups and enclosed each in a single callus-rod, are found. $\times 600$.

Fig. 8. Surface view of part of a sieve-plate; a slightly older stage than Fig. 7, with callus represented blue. In the centre are seen groups of threads (a) which have not yet developed callus. Around these are groups of more deeply staining threads each enclosed in a callus-spot (b); nearer the outside the callus-spots forming a group have united by their edges to produce a ring (c); d is a case in which the threads, callus, and portion of wall enclosed by such a ring have begun to break down, and have given rise to two slime-strings; on the periphery the process of boring out is complete and a single slime-string (e) has been produced from each original group and is surrounded by a thick ring of callus. In one place (f) the callus has begun to spread over the portions of the wall which lie between the perforations. $\times 600$.

¹ Gardiner, Roy. Soc. Proc., 1900. See also Meyer, A., Die Plasmaverbindungen und die Fusionen der Pilze der Florideenreihe, Bot. Zeit., 1902, p. 139.

² Hill, l. c., 1901 and 1903.

Fig. 9 is a diagrammatic representation of the changes undergone by a single group of threads. (Letters as in Fig. 8.)

Fig. 10 is a lateral sieve-plate seen partly in section, partly in surface view. Single callus-rods are seen traversing the sieve-plate. $\times 750$.

Fig. 11. Very young sieve-tube in longitudinal section showing single threads traversing the sieve-plate. $\times 600$.

Fig. 12. As Fig. 11, but the central portion of each thread more deeply stained. $\times 600$.

Fig. 13. Young sieve-plate, showing single threads in some places, and groups of twos and threes in others, in longitudinal section. $\times 750$.

Fig. 14. First step in callus formation, in a young sieve-plate in which the threads are arranged throughout in groups. Callus is found in three places, but in each case has only been formed on one side of the middle lamella. $\times 750$. At (a) two pairs of deeply staining threads are seen, in one of which the change has not yet reached across the middle lamella.

Fig. 15. An unusual case of a sieve-plate in which development has proceeded further on the centre than on the periphery. On the left of the section are seen groups of two threads, and in one of these groups a median node is visible on each thread. Next to this group comes one made up of three threads each of which has its own callus-rod; next to this, one in which two slime-strings are present, the formation of which is not yet complete across the middle lamella; in the centre is a case in which the single slime-string is nearly complete, but across the middle lamella two separate darkly staining threads still stretch. On the right of the plate the outermost pit but one shows a case in which callus and slime-string formation is complete on the upper side, while threads without callus are still present on the under side. $\times 1,000$.

Fig. 16. In this sieve-plate fully formed slime-strings, each enclosed in a callus-tube, are seen on the periphery in longitudinal section. In between the periphery and the centre all stages in their formation are present. At (a) is seen a very common phenomenon: the half-formed slime-strings on one side dragged out of the pits during the processes of staining, &c. In many places in this sieve-plate the callus has spread over the areas between the perforations. The protoplasm of the sieve-tube is massed in the centre. $\times 750$.

Fig. 17. First stage in the obliteration of a sieve-tube: an accumulation of callus has occurred at the centre of the plate. $\times 750$.

Fig. 18. An old sieve-tube in which is seen the commencement of callus deposition on the side-walls. This preparation was stained to show the much swollen inner layer of the wall. *ml* = middle lamella; *x* = lateral plate, finely perforated. (Stained Thionin and London blue.) \times about 500.

Fig. 19 represents one of the oldest sieve-tubes in longitudinal section. Obliteration of the sieve-plate is here complete and callus has also been laid down in considerable quantity on the lateral walls. At (a) a portion of protoplasm is seen entirely enclosed by callus, clearly showing that the callus cannot here be due to an alteration of the wall. \times about 450.

Fig. 20. *a, b, c, d*, are cross sections of sieve-tubes showing various stages in the blocking of their lumina with callus. $\times 600$.

Fig. 21. As Fig. 20, showing lumen almost obliterated. $\times 600$.

Fig. 22. *a, b, c, d*, are cross sections of sieve-tubes showing the formation of lateral pads of callus. The pad in 22 *d* is seen to surround a hole, and the edges of the hole are continuous with the inner edge of the sieve-tube wall. Fig. 22 *c* is one of these lateral pads very much enlarged, and an attempt is made to show the flocculent nature of the callus.

Fig. 23 is a longitudinal section of one of the innermost sieve-tubes and shows the unequal deposition of the callus on the lateral walls.

Fig. 24. A longitudinal section of the cross wall of one of the anastomosing hyphae of the medulla. The connecting-threads are shown as fine lines traversing the wall. $\times 600$.

Fig. 25. As Fig. 24, showing callus formation at a cross wall.

Figs. 26-32 are drawn from the young apex of *Macrocystis* preserved in spirit. Primary pith filaments.

Fig. 26. A longitudinal section of a young primary pith filament in which no callus has yet been formed. The knobs on either side of the sieve-plate probably represent developing slime-strings. $\times 600$.

Fig. 27. A slightly older element in which the development of callus has begun both at the sieve-plate and on the lateral walls of the sieve-tube. $\times 600$.

Fig. 28. A longitudinal section of a sieve-plate on which callus has begun to accumulate. (Thionin and London blue.) $\times 600$.

Fig. 29. A longitudinal section of a primary pith filament in which an aggregation of callus has been formed at the centre of the sieve-plate; cf. Fig. 17. $\times 600$.

Fig. 30. An older element in which a large mass of callus has been deposited on the lower side of the sieve-plate, that is the side furthest from the apex. $\times 600$.

Fig. 31 is taken from a case in which a considerable amount of callus has accumulated on both sides of the sieve-plate and has also been deposited down the sides of the tube; cf. Fig. 23. (Stained Thionin and London blue.) $\times 600$.

Fig. 32. A cross connexion between two primary pith filaments; callus has been formed at the cross walls and is greater in amount at the wall which is nearer to the centre of the stem. $\times 750$.

PLATE XX. *Laminaria saccharina* (adult preserved i winter).

Unless otherwise specified, the preparations from which the following figures are taken were stained by the Safranin method, followed by Aniline blue and London blue.

Fig. 1. Surface view of the cross wall of a cell of the middle cortex. Five large primary pits, each divided into a number of secondary pits, are present. The ordinary wall is stained deep blue, the pit-closing membranes left white. (Stained Methylene blue.) $\times 600$.

Fig. 2. As Fig. 1, from the inner edge of the middle cortex. $\times 600$.

Fig. 3. Surface view of the cross wall of an outer cortical cell, showing the ends of the connecting-threads arranged in groups, each group corresponding to one of the primary pits in Figs. 1 and 2. $\times 600$.

Fig. 4. Surface view of the cross wall of an inner cortical cell just outside the secondary sieve-tube layer. The threads here are arranged in groups mostly confined to the pits, but a few are also seen in the central part of the wall. $\times 600$, and drawing slightly enlarged.

Fig. 5. Longitudinal section of a cell of the outer cortex, showing four pits in an end-wall, and connecting-threads as fine lines traversing the pit-closing membranes. $\times 600$.

Fig. 6. Two pits in a longitudinal wall of a middle cortical cell, showing the threads in longitudinal section. $\times 750$.

Fig. 7. A single pit on the lateral wall of an inner cortical cell, in longitudinal section. $\times 750$, and drawing much enlarged.

Fig. 8. Longitudinal section of a cell of the middle cortex passing through two pits in an end-wall. $\times 600$.

Fig. 9. Longitudinal section of a cell of the inner cortex, situated just outside the secondary sieve-tube layer. The end-wall is traversed by threads which are arranged in rather indefinite groups. Note very shallow pits. $\times 750$, and drawing much enlarged.

Fig. 10. Surface view of the cross wall of a young outer secondary sieve-tube, showing the ends of the threads arranged in a star-like pattern. $\times 600$.

Fig. 11. Surface view of the point of origin of a hypha from an outer secondary sieve-tube. $\times 600$.

Fig. 12. As Fig. 11.

Fig. 13. Surface view of the point of origin of a hypha from an outer secondary sieve-tube, showing callus. $\times 600$.

Fig. 14. Surface view of the point of origin of a hypha after callus formation.

Fig. 15. Longitudinal section of an outer secondary sieve-tube, showing the cross wall, partly in section and partly turned up so as to appear in surface view. The threads are arranged in small groups. $\times 600$.

Fig. 16 as Fig. 15. $\times 600$.

Fig. 17. Longitudinal section of an outer secondary sieve-tube situated the third from the periphery in a radial row. Callus has been formed, and each thread has its own callus-rod, which can be seen traversing the thickness of the plate, but the heads of the threads are fused together to form a mass. (a) = origin of hypha. $\times 600$.

Figs. 18, 19, 20 are similar cases to Fig. 17. (a) = origin of hyphae at which callus formation has occurred. $\times 600$.

Fig. 21 is an unusual case in which a large accumulation of callus has taken place on one side of a sieve-plate in an outer secondary sieve-tube. The element from which this figure was taken was next to the medulla. $\times 600$.

Fig. 22. Surface view of a cross wall of an anastomosing hypha from the medulla. $\times 600$.

Fig. 23, as Fig. 22 displaced in longitudinal section.

Figs. 24, 25, 26, 27. Longitudinal sections of hyphae in the medulla, in the cross walls of most of which callus formation has occurred. $\times 600$.

Fig. 28. Surface view of a young inner secondary sieve-plate from the periphery of the medulla. Hyphae are seen cut during their course in the walls, and a small sieve-plate is also seen in surface view in the left-hand corner, and probably represents the cross wall of a large hypha. The threads seen in end view in the large sieve-plate are confined to certain areas in the wall and are arranged in a star-like pattern. $\times 750$.

Fig. 29. Surface view of a sieve-plate from an inner secondary sieve-tube. The ends of the threads are arranged in smaller groups than are those in Fig. 28. $\times 750$.

Fig. 30. Surface view of a slightly older sieve-plate from the medulla. A few of the threads stain more darkly than the rest, and are each enclosed in a ring of callus. $\times 600$.

Fig. 31. Surface view of a portion of a sieve-plate in which perforation is complete. Each thread has become transformed to a slime-string, and a faintly staining ring of callus was visible round each string, but is only indicated in the figure by a black line. The callus has already begun to accumulate in three places. $\times 600$.

Fig. 32 *a*. A slightly later stage than Fig. 31 in surface view. The callus has now spread over all the areas of the plate which were originally traversed by threads, but has not yet been formed in the other portions of the plate. $\times 600$.

Fig. 32 *b* is a very much enlarged representation of a small portion of the plate, in which the faintly staining callus is coloured blue, and a small area left unattacked is left white. (In Figs. 31 and 32 the slime-strings are seen to have fallen out of most of the holes during the process of preparing the sections.)

Fig. 33. Longitudinal section of a sieve-tube of the medulla, showing the sieve-plate traversed by groups of threads. $\times 600$.

Figs. 34 and 35 as Fig. 33.

Fig. 36. Longitudinal section of 'three-armed sieve-plate' from the medulla. Callus has been formed in both terminal and lateral plates, each thread being enclosed in its own callus-rod, and the heads of each group of threads being fused together. $\times 600$.

Figs. 37 and 38. Longitudinal sections of two sieve-plates from the medulla, showing callus formation in each group of threads and accumulation above the groups to form small cushions. Fig. 38 *b* shows one of these cushions much enlarged. $\times 600$.

Fig. 39. An old sieve-tube in longitudinal section ($\times 600$), showing a large accumulation of callus on one side of a sieve-plate. $\times 600$.

Fig. 40. A similar case in which callus formation has also commenced down the sides of the sieve-tube. $\times 600$. This figure should be compared with Figs. 23 and 31, Pl. I, and Fig. 41, Pl. II.

Fig. 41. Longitudinal section of a similar sieve-tube, showing callus formation on the lateral walls obliterating the lumen in some places. $\times 600$.

Fig. 42, *a, b, c, d*. Transverse sections of sieve-tubes and primary pith filaments from the medulla, showing the lumina in various stages of being blocked by callus. $\times 600$.

Fig. 43. Transverse section of a sieve-tube, showing origin of lateral branch or of a pulled-out cross connexion. Callus has been formed as on the end-walls of the sieve-tubes. $\times 750$.

PLATE XXI.

(From photographs, which are described in text.)

Fig. 44. Cross section of young stem of *Macrocystis pyrifera*.

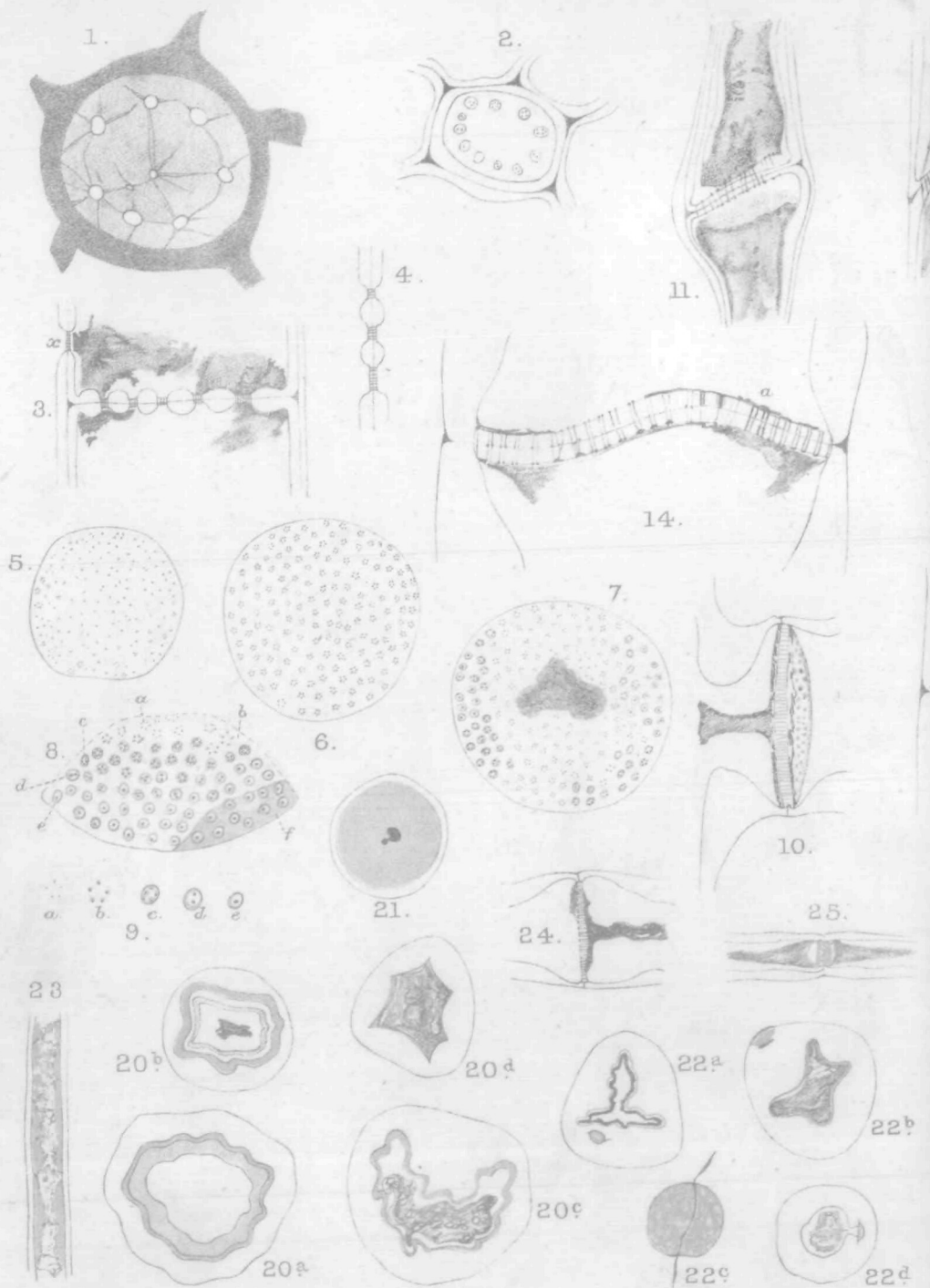
Fig. 45. Part of cross section of old stem of *Macrocystis pyrifera*. The radial rows of secondary sieve-tubes are seen on the periphery of the medulla.

Fig. 46. Cross section of very young plant of *Laminaria saccharina*.

Fig. 47. Central portion of Fig. 3, much more highly magnified.

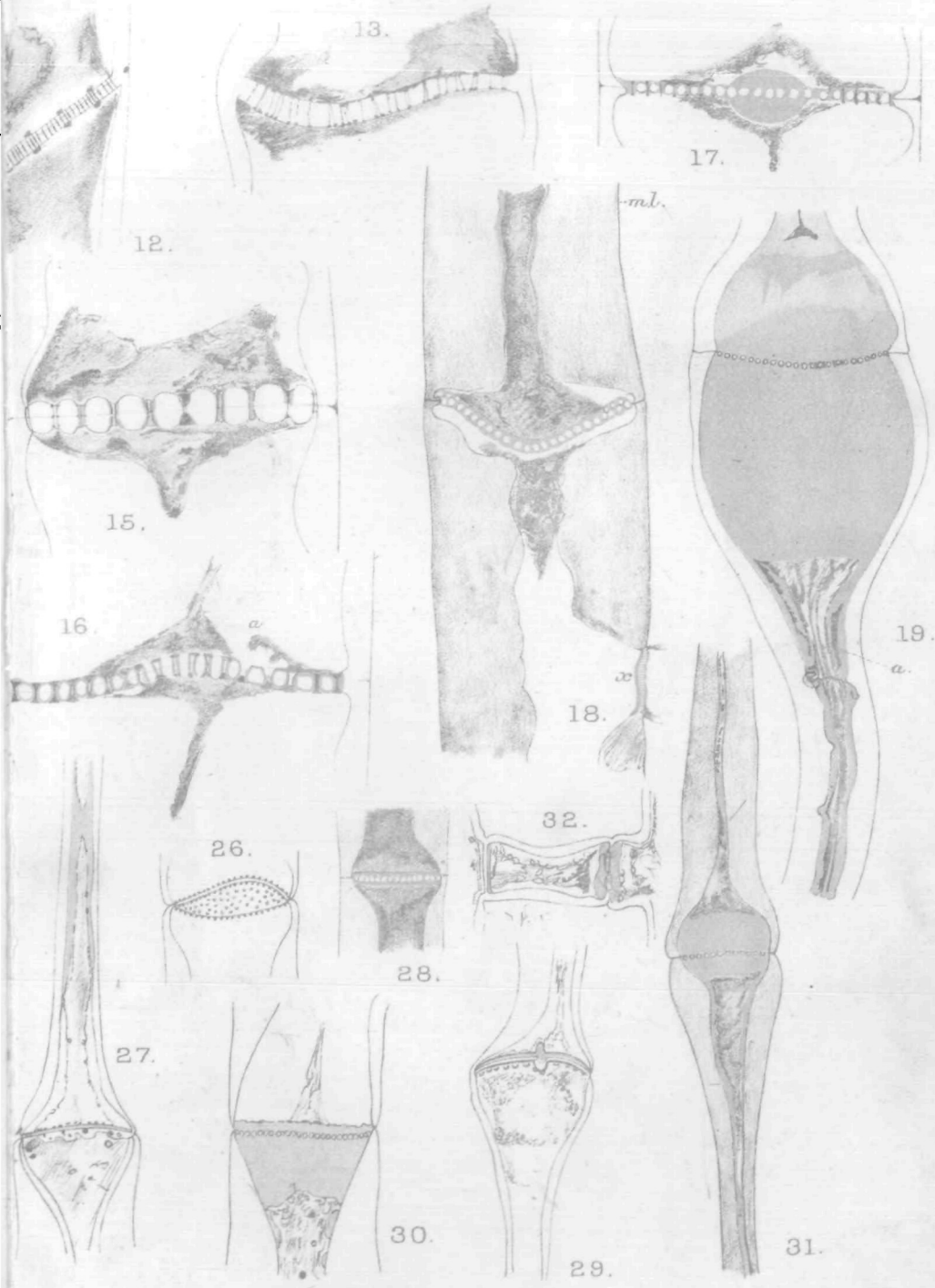
Fig. 48. Cross section of young plant of *Laminaria saccharina*.

Fig. 49. Cross section of old plant of *Laminaria saccharina*, showing radial rows of sieve-tubes on the periphery of the medulla, and a few large sieve-tubes nearer the centre of the medulla. Figs. 3, 5, and 6 are all magnified to the same extent, and illustrate the tremendous increase in amount of the secondary cortex.

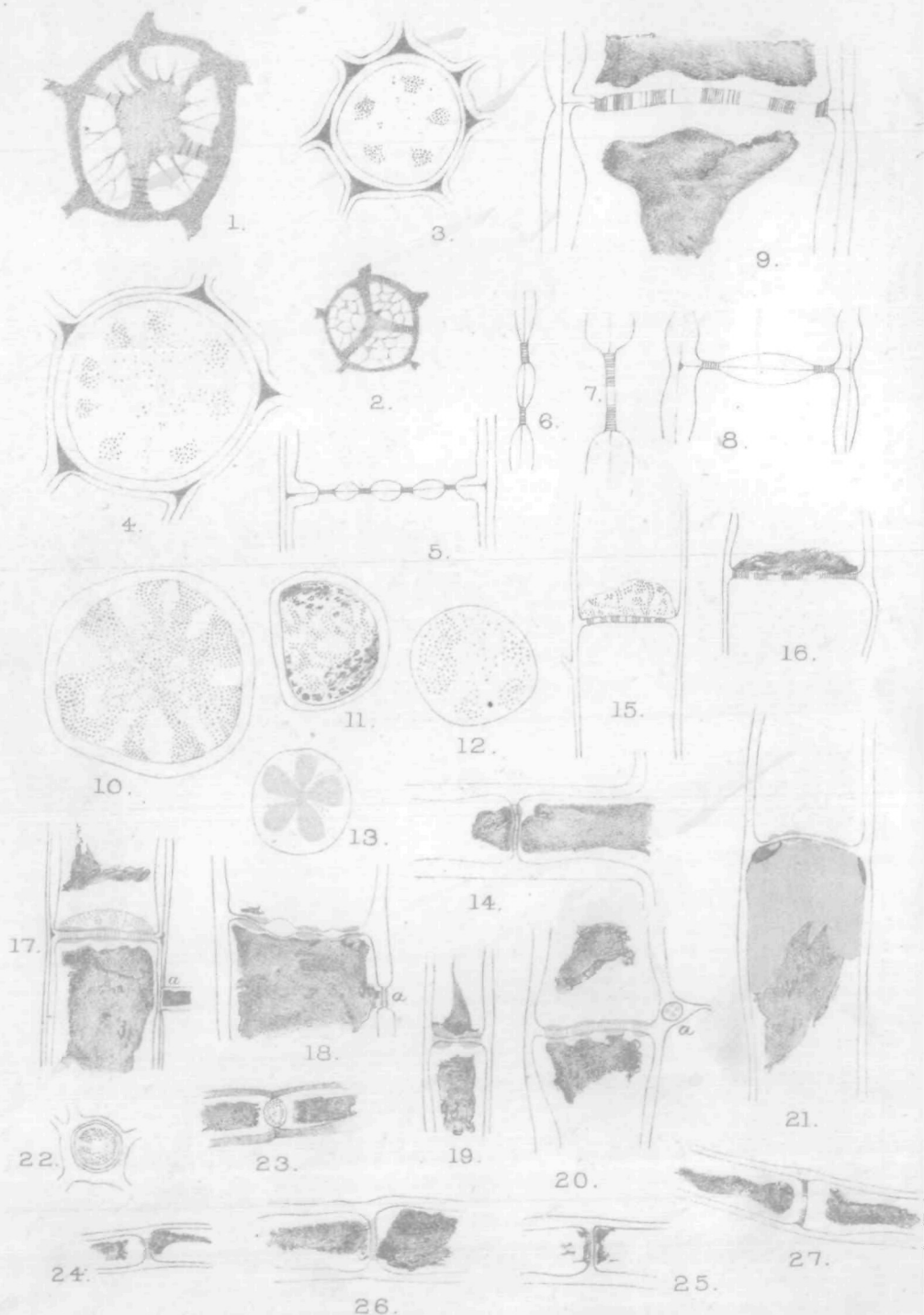


M. G. Sykes, del.

M. G. SYKES — MACROCYSTIS.

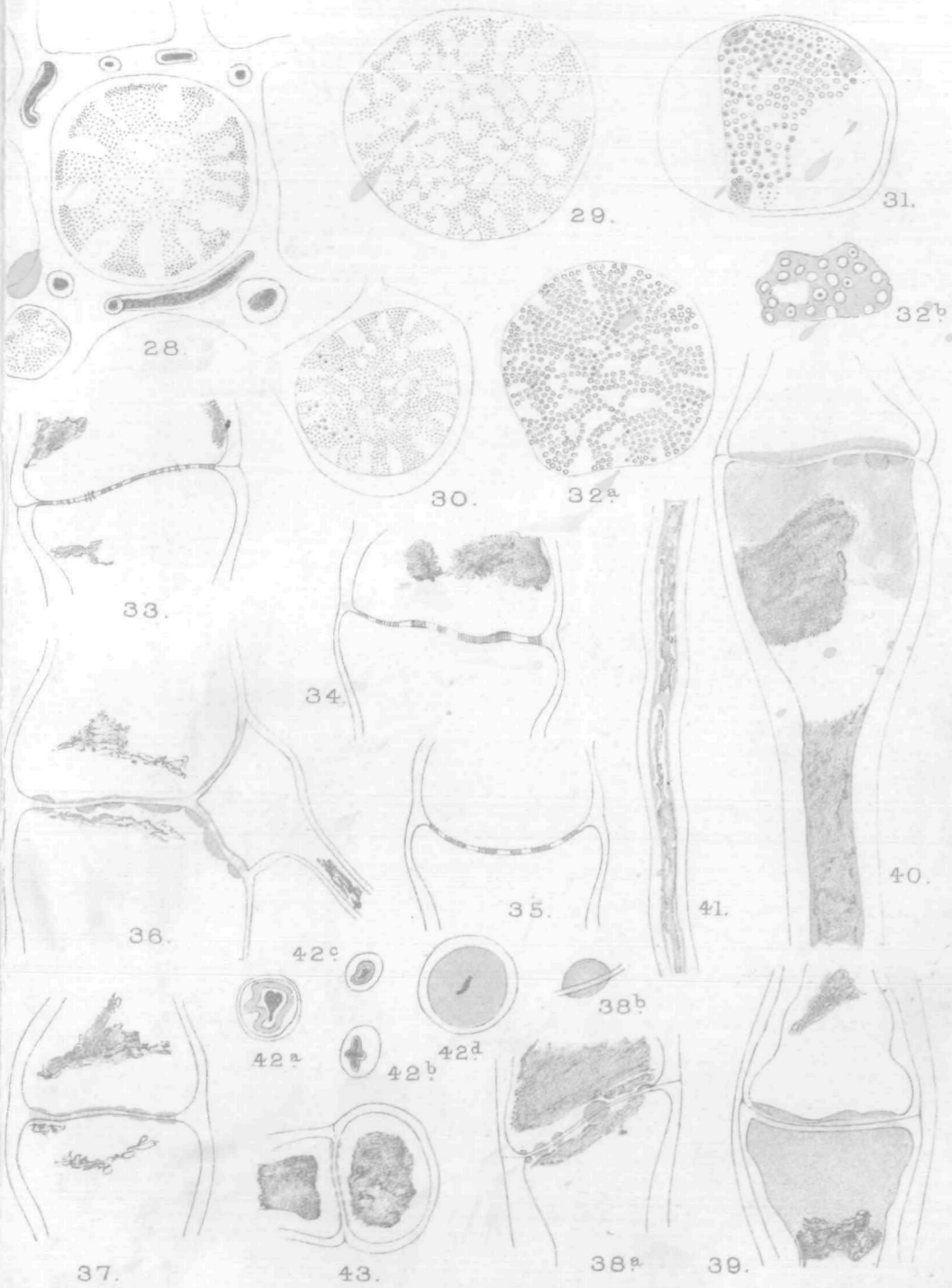


Huth, lith. et imp.

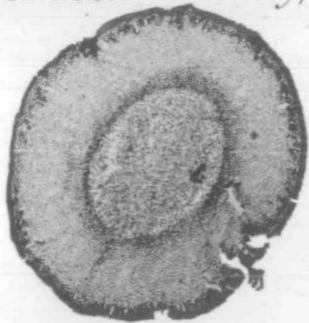


M.G. Sykes, del.

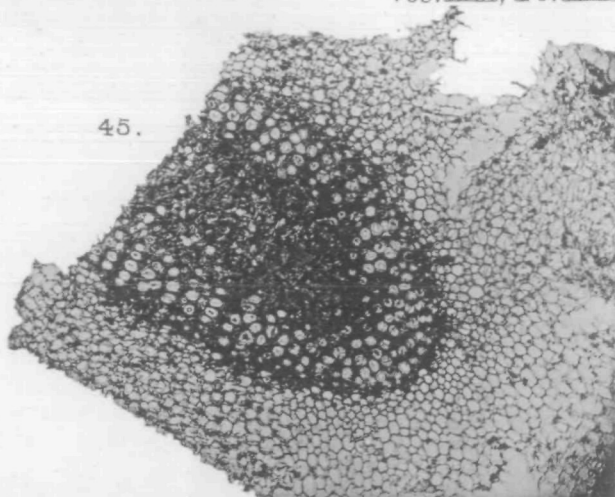
M.G. SYKES - LAMINARIA SACCHARINA.



*Huth, lith. et imp.



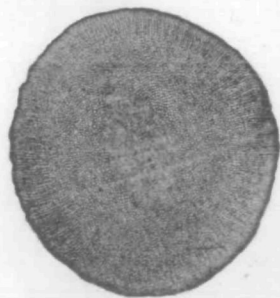
44.



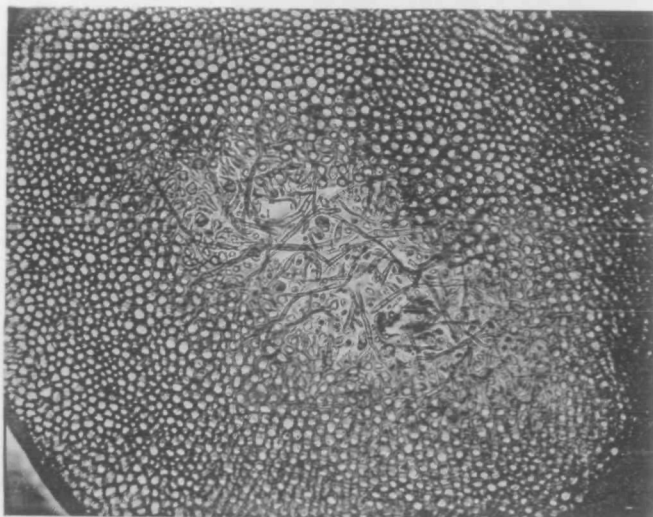
45.



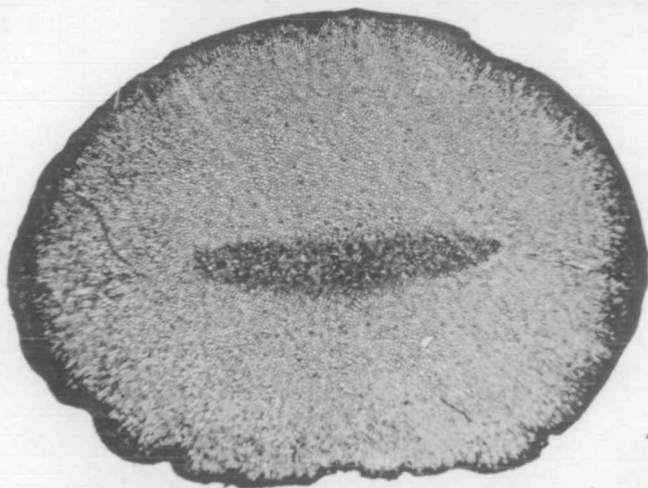
46.



48.



47.



49.

SYKES — MACROCYSTIS AND LAMINARIA.

Huth, coll.

