The Histology of the Sieve-Tubes of Angiosperms.

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With Plates XVII and XVIII and thirteen Figures in the Text.

PART I.

HISTORICAL.

NUMEROUS attempts have been made to arrive at a clear understanding of the histological structure of the sieve-tubes of Angiosperms, and of their relations to the other elements of the phloem.

Among the most important of the earlier works on this subject are the papers of Wilhelm, Russow, and Janczewski which appeared in the years 1880-3. These three investigators were concerned chiefly in examining the structure of the mature sieve-plate and its mode of formation, although Wilhelm's work in this direction occupies only a small space in his beautiful monograph on the phloem of *Vitis*.

Fischer's papers are the next in chronological order to which it is necessary to draw attention, and in them physiological rather than histological questions are considered and very little light is thrown on the origin of the sieve-plate. An interesting paper, from the point of view of sieve-tubes in general, appeared in 1887, by Oliver, which dealt with the formation of the callus in algal sieve-tubes, and reference will be made to it later.

Lecomte's elaborate thesis appeared in 1889 and is the next important paper on our subject, dealing entirely with angiospermous sieve-tubes. It is prefaced by a well-written historical summary, so that it is unnecessary to give more than a short critical account of earlier work by way of introduction to the present research.

Ten years later—in 1899—Perrot published a separate treatise, entitled 'Le Tissu criblé', the important part of which, from the point of view of this paper, is the excellent historical summary of previous research. Among the most recent attempts to solve the question of the structure and mode of origin of the angiospermous sieve-plate will be found the

paper by Strasburger on protoplasmic connexions, published in 1901; as this will be discussed critically in subsequent pages no account of his views need be given here. In the same year a paper on the sieve-tubes of *Pinus* appeared, by the author, which is intended to throw light not only on the phloem of Gymnosperms but also on the histology of sieve-tubes in general. This was followed by a preliminary note on the sieve-tubes of certain Angiosperms in 1903, of which the present long-delayed paper gives a more complete account.

In spite of Perrot's historical summary, it will help to a better understanding of the present position of sieve-tube research if the principal results of the various authorities are briefly stated and criticized. In particular the points of difference will be indicated in order that the nature and scope of the problems still outstanding may be clearly realized. Some of these it is hoped may have been solved by the present research.

Wilhelm worked especially with *Vitis* and to a smaller extent with *Cucurbita*, and his principal results from the histological point of view relate to the sieves and callus. With regard to the companion cells and the sieve-tubes, however, he has pointed out their origin from a common mother-cell, and he draws attention to the granular protoplasmic contents of the companion cells and to their nuclei. He noticed also the numerous corresponding pits in the cell-walls between these cells and their sieve-tubes and the almost complete absence of pits in the walls between companion and cambiform cells. The callus is considered to be formed from the cellulose of the sieve-plate, and its origin in the pits of the young sieve-plates and the subsequent perforation of these pits by the sieve-pores are described. In addition to this he shows that the development and character of the sieve-fields on the long walls of the sieve-tubes agree exactly with the sieve-plates of the cross walls. The reopening of the sieve-plate in *Vitis* after the winter rest is described with some figures, though the way in which this condition is brought about does not appear to have been properly understood.¹ The callus of old sieve-tubes which have ceased to function as such is considered by Wilhelm to serve as reserve material. The short sieve-tubes which cross the medullary rays and link up adjoining groups of sieve-tubes are also accurately described and figured, but very little information is given about the development of either sieve-plates or sieve-fields, though the early appearance of the callus was noticed.

This latter question is attacked more particularly by **Janczewski**, but his researches have not thrown very much light on the subject. It is rather to **Russow**,² whose work on the protoplasmic connexions was of so interesting a nature, that we are indebted for much valuable information on the structure of the sieves. Owing to the modification of some of his former

¹ Wilhelm, l. c., p. 37, Figs. 89 and 90, Pl. VII.

² Russow, in Sitzber. der Dorpater Nat. Ges., 1882; *Trans.* in *Ann. des Sc. Nat.*, 1882.

views in connexion with his researches on the continuity of protoplasm,¹ and also, no doubt, to the absence of figures, his conclusions have not received the recognition which they deserve. Though his methods were of a very drastic nature it is remarkable that they have yielded results which have been proved to be substantially correct by our modern histological technique. He distinguishes clearly between the callus and the slime-strings which perforate it, not only in the large sieve-plates but also in the sieve-fields, and shows that the strings in the canals of the sieve-tubes are smooth and homogeneous throughout. He considers therefore that they cannot be of a protoplasmic nature, though in his earlier paper these strings were regarded as being of the nature of callus, owing to the brown coloration they gave with his callus reagent. In sieves which function for more than one year Russow realized the important part played by such strings (or *Stifte*) in the reopening of the sieve-plate after the winter's rest, though he does not seem to have understood either the condition of the callus-plate during the dormant period or the method by which it again becomes functional.²

With regard to other connexions in the phloem Russow could get no proof; he was unable to see any threads between the sieve-tubes and companion cells, or between these latter and the bast parenchyma or the medullary ray cells. No doubt his failure to obtain results in these cases was largely due to the disorganization of the cell-walls by the reagent employed.³ His views on the early history of the sieve-plate differ from those of Wilhelm and Janczewski. These observers held the opinion that the callus arose in little spheres in pairs on either side of the young sieve-plate, owing to the transformation of the cellulose. These then fused together to form the stoppers of callus filling the pores of the sieve-plate, and finally the stoppers are pierced by the connecting filaments.

Russow, on the other hand, was of the opinion that the young sieve-plate is first pitted with corresponding pits on either side of the membrane; the callus is then formed in these little depressions by the protoplasm and not by a transformation of the cellulose.⁴ The callus appears in the form of little basins, with a polygonal outline, and Russow attaches considerable importance to its shape as proof that the callus substance is formed by the contents of the sieve-tube and deposited on the surface of the sieve; the gradual overlaying of the cellulose sieve and the great size to which the callus may subsequently attain afford further proof, he considers, of the correctness of these views.

¹ Russow, l. c. 1883, p. 570.

² According to Janczewski, l. c., p. 145, Figs. 10-12, Pl. VIII, the sieve is entirely shut by a homogeneous callus mass in *Vitis*, and is reopened with the formation of striae about mid-April.

³ Russow used about 81 per cent. sulphuric acid.

⁴ Gardiner, 'On the constitution of the cell-wall and middle lamella', in *Proc. Camb. Phil. Soc.*, vol. v, p. 15, *ibid.*, 'Observations on the constitution of callus', p. 230, agrees with the views put forward by Russow as to the protoplasmic derivation of the callus, and considers this substance to be of the nature of a starchy mucilage.

Neither the solution of the cellulose lamella between the two opposed callus-basins nor the formation of the connecting filaments was observed.

Both Janczewski and Russow have investigated the sieve-tubes of Gymnosperms also, but their results need not detain us here, since they have been discussed elsewhere.¹ The papers of Fischer,² which follow in chronological order, deal with the physiological rather than with the histological aspect of our subject, one of his objects being to show that the ordinary contracted appearance of the sieve-tube contents and the characteristic masses of slime (*Schleimkopf*) seen on one side of the sieve-plate have been artificially produced, and that in the normal condition the sieve-tube contents are homogeneous.

One of his papers is of some histological interest, since he employed Russow's method, and two of his figures probably show the sort of results which Russow must have obtained. In both cases the results are artificial and untrue, the cell-walls being enormously swollen. In the one case the wall between two sieve-tubes shows stratification with 1-4 threads in each pit, and, in the other, direct continuity by single hair threads³ is figured between a companion cell and a sieve-tube.

In the summary of his results he states that the sieve-tubes are in 'direct continuity' with one another through fine threads and with the companion cells,⁴ and that the cambiform cells are in continuity with one another, but not with either the sieve-tubes or the companion cells. Fischer's results seen by the light of his figures are so misleading that they cannot be considered as a very valuable contribution to the histology of the phloem tissues. The next work on this subject is Lecomte's careful study of the phloem of Angiosperms⁵, a good deal of which is occupied with questions of histology. His own work is collected into nine chapters, of which his chapters IV, on the Development of the Sieves,⁶ and V, on the Development, Form, Structure, and principal reactions of the Callus,⁷ concern us more particularly in this paper.

Lecomte, after a useful review of previous work, proceeds to give the results of his own researches, which in some points differ fundamentally from those to which allusion has already been made. According to him,⁸ the young thin membrane—the future sieve-plate—is at first composed of some nitrogenous body; this soon becomes coated with cellulose in such a way that the cellulose forms intersecting bands, leaving the meshes of the

¹ Hill, A. W., Histology of the sieve-tubes in *Pinus*, in *Ann. Bot.*, 1901, vol. xv, pp. 576-80.

² Fischer, *v.* list of Literature cited at end.

³ There is no doubt that the so-called threads are the pit fillings of paired pits so drawn out by the intense swelling of the wall that they appear like a fine continuous thread.

⁴ Fischer, A., *Neue Beiträge zur Kenntniss der Siebröhren*. Ber. über Verhandl. der kön. Sächs. Ges. d. Wissen. zu Leipzig, 1886, p. 297.

⁵ Lecomte, in *Ann. des Sc. Nat., Bot., Sér. vii*, 1889, vol. x, p. 193.

⁶ *Ibid.*, p. 244.

⁷ *Ibid.*, p. 258.

⁸ *Ibid.*, pp. 248 and 250, Fig. 9, Pl. XXI, Fig. 12, Pl. XXII.

original substance which will become the pits of the young sieve-plate; the meshwork thus tends to be of greater thickness than the rest of the plate. After this the substance occupying the meshes swells, forming projections on either side of the membrane, and stains with aniline blue, so that the contents of two tubes appear to be united by blue rods which occupy the axes of the meshes.¹ Gradually the whole of the substance filling the mesh takes on this blue coloration. Osmotic exchanges take place at first through the substance filling the meshes of the sieve-plate, but later communication between two adjoining tubes is effected by a definite mucilaginous filament traversing a canal or hole in the plate owing to the replacement of the original substance of the meshes by the invading filaments of the albuminous contents of the sieve-tubes.

The parietal protoplasm also accompanies these filaments, so that there is communication not only of the slime but also of the protoplasm of the sieve-tubes. The exact way in which the change from the embryonic to the mature mode of communication takes place is by no means clear from Lecomte's account. The development may not always go as far as this; in Gymnosperms, for instance, and probably in many Angiosperms also, the meshes, according to his view, may always be occupied by the primitive substance of the membrane and may only afford places more suitable for osmotic exchange than other parts of the sieve-plate.

Before criticizing these views of Lecomte on the development of the sieve-plate, it will be more convenient to give a brief résumé of his opinions on the formation of the callus. He criticizes Russow's results,² with which he entirely disagrees, and comes to the conclusion that the callus forming the callus-pads is due to the swelling of the thin layer of the substance which covers the cellulose network of the sieve-plate,³ but that the cellulose network itself never gives rise to any callus. The first callus layer is an integral part of the membrane of the sieve-plate, and then this layer thickens at the expense of the albuminous contents of the tube.

Thus this substance which fills the meshes of the young sieve-plate and also covers over the cellulose framework functions first of all—and in some cases always⁴—as the pathway for osmotic exchanges. The portions which are not transformed into mucous strings—i. e., the parts covering the cellulose network—then swell up to join the first layer of callus.

According to Perrot's account of the work of Lecomte and Léger,⁵ the

¹ Perrot, 'Le Tissu criblé', p. 38, and Fig. 19.

² Lecomte, l. c., p. 263.

³ Ibid., p. 261.

⁴ Ibid., p. 260. After the penetration of the meshes, the substance covering the cellulose network shrinks to an almost imperceptible layer, since communication is now direct, but in those Dicotyledons where he was unable to show the perforations he found the wall notably thicker than all the others. This is due to the fact that the osmotic changes take place exclusively here, and the non-cellulose portion retains the first stage of swelling seen in the young sieve-plates.

⁵ Cf. Perrot, l. c., p. 36; cf. Lecomte, pp. 248 and 260. It does not appear that Lecomte realized that the substance of his meshes which stains blue with aniline blue is really callus (*v.* Conclusion,

substance of the meshes must thus be transformed first into callus and afterwards progressively, in certain cases, into an albuminous substance.

Lecomte's results, which seem to have been generally accepted (certainly by French botanists), not only make the early history of the sieve-plate more complicated than really appears to be the case, but also include several erroneous conceptions as to the state of the active sieve-tubes in Gymnosperms and some Angiosperms. There seems every reason to suppose that the sieve-tubes in these two groups of plants are—at any rate in the active condition—always in communication with one another by means of mucilaginous slime-strings.¹ Further, the callus is always visible before any perforation of the membrane by the slime-string has taken place, as Russow and Wilhelm observed,² and Lecomte does not appear to have noticed the little paired basin-shaped patches of callus separated from each other by a thin intervening piece of the membrane, which were accurately described by Russow and form so marked a feature of the developing sieve-plate. Nor does he allude to the callus lining the holes of the sieve-plate, which has been well figured by Wilhelm.³ According to Lecomte the callus first arises as a transformation of the substance covering the cellulose network of the sieve, whilst according to Wilhelm, Janczewski, and Russow, with whom on this point the present researches are in accord, the callus makes its appearance in the meshes, depressions, or pits of the young sieve-plate, i. e., the spaces between Lecomte's hypothetical intersecting bands of cellulose. His theory of the origin of the perforations in the sieve-plate and of the value of the substance of the meshes as the pathway for diffusion will be referred to again later. It is enough to add here that the knowledge gained by recent histological work on the cell-walls and protoplasmic connexions makes this theory highly improbable. There are other points of which the explanations are by no means clear, such as the nature and function of the striae in old sieves and the way in which sieves may be reopened after the winter's rest. In concluding this criticism of Lecomte's work it must be remarked that his figures are not sufficiently clear in many cases to enable one fully to understand his meaning.⁴ The question of 'connecting-threads' in the phloem tissues does not appear to have engaged his attention.

A contribution to this subject of the sieve-plate was made by Léger,⁵

No. 16, p. 320). 'Le cal est dû au développement exagéré de la mince couche de la membrane qui recouvre les filaments de cellulose.'

¹ Hill, *Ann. Bot.*, vol. xv, p. 590, &c.

² Wilhelm, *Figs.* 12, 14, 15, Pl. II; *Fig.* 25, Pl. III; *Figs.* 114, &c., Pl. IX.

³ *Ibid.*

⁴ e. g., his figures of sieve-plates in section and surface view, *Figs.* 9 and 10, Pl. XXI, and *Figs.* 12, 21, and 22, Pl. XXII, do not correspond in an intelligible manner, and they are even less clear when copied by Perrot, l. c., p. 37, and the sieve-plates of *Figs.* 5 and 6, Pl. XXI, and *Figs.* 14 and 17, Pl. XXII, do not appear to represent real structure.

⁵ Léger, *Mém. de la Soc. Linn. de Normandie*, tome xix, 1897, p. 68.

who finds that it is pectic in composition, when deprived of its callus, and gives no cellulose reactions.

Such then was the condition of our knowledge of the sieve-tube and phloem histology in 1899 when **Perrot** wrote his memoir, in which he accepts nearly all **Lecomte's** results. The next contributions of a serious character are those of **Strasburger**. In his important paper on protoplasmic connexions,¹ published in 1901, he still held the view put forward in an earlier work² that with the formation of the callus-rod from the original connecting-threads the complete development of the sieve-plate has been reached. In his review³ of my paper on the sieve-tubes of *Pinus*,⁴ he accepts certain of the conclusions there put forward and agrees that it is the slime-string and not the callus-rod which plays the important rôle in the communication between two sieve-tubes.⁵

In the paper published in 1901 he also traces the history of the development of the sieve-plate and sieve-fields in *Wistaria chinensis* (*Kraunhia*), and other Angiosperms, and, since the results there stated differ considerably from those in general acceptance at that date, it will be necessary to give some account of them and also to add some criticisms.

According to **Strasburger**, the young sieve-plate membrane soon after its formation becomes covered by a network due to local thickening. The whole membrane is then overlaid by a substance which is characterized by the readiness with which it stains blue with aniline blue, and at this period the fine protoplasmic threads are seen in the sieve-fields.⁶ The threads, however, soon begin to take up aniline blue, and, in favourable cases, a surface view effect can be obtained of a fine network in a sieve-field corresponding to this perforation of the membrane (*v.* Fig. 30, Pl. XIV). So far this agrees with the course of development given by him for *Pinus*, to which reference has already been made. The further stages of the development, however, are different. The aniline blue staining substance which overlies the whole sieve-plate accentuates particularly the height of the network, and continuity across the unstained portion of the membrane is

¹ Strasburger, in Jahrb. f. wiss. Bot., Bd. xxxvi.

² Strasburger, Leitungsbahnen, 1891, pp. 60 et seq.; *v.* Hill, Histology of Sieve-tubes of *Pinus*, Ann. Bot., vol. xv, 1901, pp. 582-3.

³ Strasburger, in Bot. Zeit., vol. lx, 1902, p. 49.

⁴ Hill, Ann. Bot., vol. xv, p. 575.

⁵ *l. c.*, p. 47, Strasburger states that in his 'Kleines Practicum,' fourth edition, he had corrected the figure of the sieve-plate of *Pinus* from the figure given in his paper before my work was published; his figures are certainly somewhat altered, but in both cases they appear to be so badly reproduced as to be almost unintelligible. Moreover, in the 'Practicum,' fourth edition, there is no adequate explanation of the altered figures in the text; cf. p. 85, Fig. 49.

⁶ Cf. Strasburger, *l. c.*, Fig. 29, Pl. XIV. The sieve-fields in Strasburger's sense are the small sieve-areas or pits of the single sieve-plate, they thus correspond to **Lecomte's** meshes of the sieve-plate network. Other writers limit the term 'sieve-fields' to the thread groups on the *lateral walls* of the sieve-tubes; and it is so used in this present paper.

effected by stained callus-threads; a surface view may sometimes be seen showing separate blue dots which are his callus-threads, and Figs. 31 and 32, Pl. XIV, illustrate this stage in the development.¹ The network or framework of the sieve-plate continues to project still more above the general surface of the membrane owing to the progressive conversion of its substance into callus, and at the same time the perforation of the several sieve-fields takes place. The mucous contents of the sieve-tubes begin to bore through the centre of the membrane of each field from one side only; this boring is not due to the fusion of the callus-threads which traverse the membranes of the sieve-fields, for it commences with a small hole in the centre of the field, and this gradually increases in diameter until the whole of the field area is occupied by a slime-string. By means of these strings the slime masses of adjoining sieve-tubes are in communication (cf. Figs. 33–35, Pl. XIV). The conversion of the thickened portion, or network, of the sieve-plate into callus still continues, and there results eventually a narrowing of the several pores (Figs. 36–37, Pl. XIV).

It may be noted from the figures (Fig. 36, Pl. XIV; Figs. 38 and 39, Pl. XV) that during the progress of this narrowing the callus has increased in size, whilst the cellulose or substance of the original membrane has decreased. Finally the thickening callus masses of the framework fuse together and the shutting of the sieve-plate by a callus-rod is complete (Fig. 40, Pl. XV). In a longitudinal section of the callus-mass the remains of the original membrane can still be seen as a series of nodes, and in a surface view, on the solution of the callus, this same primary membrane is left behind as an open network (Fig. 42).²

The finer network seen in *Pinus*, representing the remains of the sieve-field membrane (cf. Figs. 26 and 27), is therefore absent in *Wistaria*, and the network of the latter and of Angiosperms generally is considered to be homologous with that larger network which encloses the sieve-fields in *Pinus*.

Strasburger regards the callus in *Pinus* as derived from the swellings of the ends of the changed protoplasmic threads, whilst in the sieve-plates of *Wistaria* and Angiosperms he considers that callus is formed by the transformation of the substances of the main network of the cell membrane. He thus agrees, in the main, with Lecomte as to the mode of origin of the callus-pads in Angiosperms, and alters the opinion previously advanced in the 'Leitungsbahnen', that the callus formation in Angiosperms and Gymnosperms must follow the same line of development. That a substance, so constant in its occurrence as the callus, and found in such diverse groups

¹ It seems necessary to point out here that, although his Figs. 29–32 are all drawn to the same magnification, the surface views Figs. 30 and 32 do not appear to correspond with their respective sections, Figs. 29 and 31. It is also difficult to understand Fig. 30, and in Fig. 31 the callus-rods, into which the protoplasmic threads are supposed to change, are not indicated.

² Fig. 42, Pl. XV, shows such an empty plate in surface view: but it is not easy to understand its correspondence with the section in Fig. 41.

of plants as the Brown Algae, Gymnosperms and Angiosperms, always in the same connexion with the perforations of the sieve-plates of the sieve-tubes, should have a different origin in the different groups, seems to be unlikely. My own results suggest that the mode of formation of the callus in connexion with the sieve-tubes takes place in the same way in the different groups of plants.¹ With regard to the smaller sieve-pits on the lateral walls of the sieve-tubes—the sieve-fields of Wilhelm² and others—Strasburger³ considers that they differ but little from the pits in the walls of ordinary parenchymatous cells, and do not therefore agree in structure with the terminal sieve-plates. They differ from ordinary pits, however, since their threads are said to be transformed into callus, whose ends then swell—as he has described in the Conifers—to form callus-heads and eventually fuse together to form the lateral callus-pads (*v.* Fig. 44, Pl. XV).

Thus it appears that in the same sieve-tube the callus-pads of the terminal and of the side walls have a different mode of formation. On the disappearance of the callus by solution there is no solution of the membrane separating the several callus-threads as was described in *Pinus*; the membrane thus retains its original thickness and is perforated by empty canals (*v.* Fig. 45, Pl. XV). In this respect, therefore, these lateral pits of Angiosperms differ from those of the Conifers in which, according to Strasburger,⁴ a fine network is seen in the sieve-fields left naked by the solution of the callus; for only the thickening layers have been dissolved and the middle lamella is left behind. The nodules therefore which he sees and figures in the middle of the sieves of young sieve-tubes are not swellings on the callus-threads, but represent the thicker parts of the middle lamella forming the fine meshwork. As this question has already been discussed in a former paper⁵ it is unnecessary to go into details again here. Suffice it to say that the condition of the lateral thread groups of Angiosperms, after the solution of the callus, appears to be essentially similar to the sieve-areas of Conifers. In both cases the membrane appears to be perforated by empty canals and the refractive nodules of the Conifers have vanished. Nothing⁶ has been found to necessitate in any way an alteration of the views already put forward which tend to unite the

¹ Cf. the paper by Miss Sykes in this present number of the *Annals*.

² Wilhelm, l. c., Figs. 34 and 35, Pl. IV.

³ Strasburger, l. c., p. 531, Figs. 43-45, Pl. XV.

⁴ *Ibid.*, p. 525, Figs. 26 and 27, Pl. XIV.

⁵ Cf. Hill, l. c., pp. 584, 592, Fig. 13, Pl. XXXI.

⁶ In the review of the paper on the sieve-tubes of *Pinus*, Strasburger, in *Bot. Zeit.*, vol. lx, p. 57, 1902, adheres firmly to his views about the median nodules of the sieve-plates. Material has been examined which has been fixed in various ways, including alcohol material, and no trace can be seen of the conspicuous highly refractive nodes situated at the middle lamella between the paired callus-rods, after the callus has disappeared; cf. Hill, l. c., pp. 587-8, Figs. 11 and 13, Pl. XXXI; and Fig. 21, Pl. XXXIII. In the explanation to Fig. 11, Pl. XXXI, p. 608, for nodules (*mn*) read (*m*), and for median nodes (*m*) read (*mn*).

phenomena attendant upon the development of the various types of sieve-tube connexions throughout the vegetable kingdom into one harmonious series of events.

It may be noted here that Strasburger says nothing further as to the character of the sieves in Conifers.¹ In the fourth edition of the 'Kleines Botanisches Practicum' the figures of the sieves of *Pinus*² have, as I have already stated, been altered, and the callus is represented as being pierced by fine threads, but whether he considers that the method of callus formation put forward in 1901 still holds, or whether the results of the paper on the histology of *Pinus* have altered his opinions, is not clear. With regard to the sieve-plate of Angiosperms the statement is made³ that in consequence of my results with *Pinus*⁴ a correction is necessary in the sense that slime-strings and not callus-rods are formed from the 'Plasmodesma' or connecting-threads. How the acceptance of these results affects his views on the boring of the fields of the sieve-plate by one large central slime-string, when according to his new point of view the young field should be occupied by a group of slime-strings,⁵ or how the callus which lines the pores of the sieve-plates is formed, is not stated. In the same way the character of the lateral thread groups is left in an unsatisfactory condition, for no explanation is given of the relation of the slime-strings to the callus, which was supposed to be formed by the swelling up of the free ends of the transformed protoplasmic threads⁶ as in Conifers.

Two other papers require a brief notice before closing this résumé. **Kuhla**,⁷ in his paper on the protoplasmic connexions in *Viscum album*, published in 1900, includes some account of the sieve-tubes of *Cucurbita* and of *Viscum*. He worked with alcohol material of *Cucurbita* fixed by boiling, according to Fischer's method⁸, and stained by Meyer's Pyoktanin method.⁹ The points which he wished to settle are clearly stated in his paper,¹⁰ but, though figures are given which purport to answer the questions propounded, there is reason for believing that the appearances figured do not, in most cases, really correspond to actual structures.

In the first place the callus is neither mentioned in the text nor referred to in the figures, which therefore renders Figs. 8, 17, and 35,

¹ Strasburger, Bot. Zeit., lx, pp. 49-53.

² Bot. Pract., ed. iv, 1902, p. 86, Fig. 49 *a* and *b*, p. 85. In these two figures fine threads each with a dark median node are shown. In Fig. *b* there seems to be a suggestion of a median nodule enclosing the nodes, which does not appear in *a*. The figures, however, are so vague and indefinite, and there is so little allusion to them in the text, or to the fact that they differ from those previously published, that it is difficult to be certain about them. There is no proper explanation of Fig. 49 *c*, and it does not apparently harmonize with either *a* or *b*.

³ Strasburger, B. Z., l. c., p. 52.

⁴ Hill, Ann. Bot., vol. xv, 1901.

⁵ Cf. Strasburger, Pringsh. Jahrb., 1901, Fig. 32, Pl. XIV, in the light of B. Z., 1902, p. 52, § 2.

⁶ Strasburger, 1901, pp. 531, 532.

⁷ Kuhla, F., Bot. Zeit., 1900, p. 29.

⁸ Fischer, 1886, Beit. zur Kennt. der Siebröhren, Leipzig.

⁹ The *Viscum* material appears to have been fixed in 1 per cent. Osmic acid.

¹⁰ Kuhla, l. c., p. 39.

Pl. III, inaccurate. His figure (Fig. 10) of the threads between a sieve-tube and a cambiform cell in *Cucurbita* is also inaccurate for the same reason, since callus is always present in the sieve-tube side of these pit-closing membranes. Connexions between the sieve-tubes and the companion cells of *Cucurbita* are figured in Figs. 7 and 9, but these two figures are very different in character, and Fig. 7 corresponds very closely with his Fig. 8, which represents a sieve-plate¹. It seems possible, therefore, that these thick threads may really be the stained pit fillings in the narrow paired pits of the companion cell-wall which have become narrowed by the swelling of the wall owing to the nature of the treatment to which the tissues have been subjected.² Even if the threads in Fig. 9 are real threads, the swollen state of the wall has produced a highly artificial effect. With regard to the presence of threads between the other tissues of the phloem, there is no doubt that they exist; Kuhla's figures of these are, however, unsatisfactory, and this is largely due to the great swelling of the wall and to the unsatisfactory and misleading nature of Meyer's method as a method for research.³

Fig. 35 appears to have no adequate reference in the text⁴; although it purports to be a very young sieve-plate, it is, I think, without doubt an old and empty plate with the sides of the pores stained by Pyoktanin.

Kuhla's results with the sieve-tubes of *Viscum* are worthy of more serious consideration and will be referred to again later, but it is as well here to point out certain errors of observation. In the first place the end-wall or sieve-plate of *Viscum*⁵ is provided with callus, like that of the sieve-plates of other plants, and is pierced by slime-strings. Also, owing to the swelling of the walls, the very numerous pits in the side walls of the sieve-tubes have been entirely overlooked, and the threads are drawn with a regularity foreign to their normal distribution.⁶ Nevertheless it should in all fairness be stated that the long walls between the sieve-tubes and the companion cells and between two adjoining sieve-tubes are filled with threads somewhat after the manner he has indicated.

A few remarks about sieve-tubes are made by Kienitz Gerloff⁷ in his recent paper, and a figure of a sieve-plate of *Gagea lutea* is given, but no callus is indicated, and there is nothing further on this subject in his paper which need claim our attention.

It is possible that certain general statements made in the preliminary

¹ Cf. Kuhla, Bot. Zeit., 1900, p. 40.

² Ibid., p. 40; Eau de Javelle and Chlor-Zinc-Iod. appear to have also been used on the same sections; cf. Fischer's results, v. p. 248.

³ For the above reasons and from my own results with *Viscum* obtained by other methods I am inclined to think that Kuhla's elaborate numerical calculations may not be thoroughly reliable.

⁴ Perhaps p. 41 at the foot refers to this Figure.

⁵ Cf. Kuhla, l. c., Fig. 17.

⁶ Kuhla, l. c., pp. 42 and 43, Figs. 12-14; cf. Hill, Fig. 26, Pl. XVII.

⁷ Kienitz Gerloff, Ber. der Deut. Bot. Ges., xx, 1902, p. 108.

account¹ of the present research with reference to the structure of the young sieve-fields may require modification, but all such questions will be referred to in the course of this paper.

Summary :—From the review of the literature presented in the previous pages it will be seen that more than one account of the developmental history of the sieve-plate and other questions of phloem histology has been given during the last thirty years. Wilhelm's work, as far as it goes, agrees with the results of the present research, and had he been in command of a better technique there can be little doubt that he would have been able to work out the finer histological details. Nearly all his work can be accepted as it stands, and serves as the foundation on which the complete history may be built up.

The importance of Russow's work, taken in conjunction with Wilhelm's results, lies in the fact that the relation of the slime-strings to the callus-rods both in adult sieve-plates and in sieve-fields (lateral pits) is definitely established. Russow also contributed to the early history of the sieve-plate, and his description of the first appearance of the callus is apparently in exact agreement with the results of the present research.

The opposed views of the formation and development of the callus by these two botanists are probably both partly true, for, as will be shown later, it seems likely that the earliest formation of the callus is due to a local transformation of the cell-wall, whilst the activity of the protoplasm is responsible for its subsequent increase.

Lecomte, influenced no doubt by Mangin's work on the cell-wall, produced a totally different theory of the origin of the sieve-plate of a complicated character, since the young end-wall is considered as being composed of two different substances forming a meshwork and interstitial matter respectively. The latter substance is considered in some cases to be gradually transformed into slime-strings, whilst in other plants there is said to be no open communication. Lecomte's views on callus formation are not very clear, but he agrees rather with Wilhelm in supposing that it is due to a change of part of the pit-closing membrane. On the whole Lecomte's work, from the histological point of view, does not advance our knowledge of the sieve-tubes to any very valuable extent, nor has he thrown any light on the presence of connecting-threads in the tissues of the phloem.

To Kuhla and Fischer we owe some knowledge about protoplasmic connexions between sieve-tubes and other phloem tissues, &c., but, beyond the fact that they have demonstrated the existence of such threads, their work cannot be considered as very trustworthy, owing to the nature of the methods which they employed.

Strasburger has attempted to carry on the investigation of the histology of the sieve-tubes from the point where it was left by Wilhelm

¹ Hill, *Ann. Bot.*, vol. xvii, 1903, pp. 265-7.

and Russow, and has thrown light on the early history of the sieve-plate, but owing to his confusion of slime-strings and callus-rods, which is not thoroughly cleared up by his review of 1902,¹ his results are shorn of some of their value.

That he is in error about the two different methods of callus formation, whether found on the end or on the lateral walls of the sieve-tubes, will, it is hoped, be proved in the course of this paper. He also has had nothing to say about the possibility of the existence of connecting-threads between sieve-tubes and other tissues of the phloem.

Such then is the state of our knowledge of the histological details of the sieve-tubes and phloem of Angiosperms at the present time. It is to the general elucidation of the development and character of connecting-threads and subsidiary structures in these tissues that the present research—commenced in the spring of 1901—has been directed. A short preliminary note has already appeared, but owing to a journey abroad the completion of the work has been considerably delayed. Even now there are many points which must be subjected to future research, but it has seemed advisable to publish the results which have been obtained and to give a general account of phloem histology without waiting for the solution of certain small outstanding difficulties.

PART II.

THE PRESENT RESEARCH.

A short preliminary note on the present research was published in the *Annals of Botany*² in 1903, and some account of the results was given at the Southport meeting of the British Association³ in the same year. The research has been carried out mainly on material of *Vitis vinifera* and *Wistaria chinensis*, whilst *Cucurbita pepo*, *Tilia vulgaris*, *Viscum album*, and *Phaseolus vulgaris* and *multiflorus* have also been studied in greater or less detail.

As in the case of *Pinus*, it was found that the nature of the material employed was a matter of extreme importance for the study of the development of the sieve-tubes, and it is advisable that the pieces of tissue for preservation shall be taken only from the main stems of strong and vigorous plants. The young shoots of woody plants in their first or second year of growth are seldom favourable. In order to obtain the best results it is necessary to cut a piece of the phloem out of a fairly old stem, and care must be exercised in order to insure that the cambium is removed at the same time. In *Vitis*, *Wistaria*, and *Tilia* the pieces of tissue so removed will

¹ Strasburger, Bot. Zeit., vol. lx, 1902, p. 50.

² Hill, Ann. Bot., vol. xvii, 1903, p. 265.

³ Brit. Assn. Report, Southport, 1903, p. 854.

show a broad band of phloem with sieve-tubes in all stages of development, from their earliest formation to their disused and collapsed condition.

In material of this kind the sieve-tubes are larger and have thicker walls than those of the young shoots; moreover, the developmental changes appear to proceed more slowly, and are more easily observed in tissues gouged out of the main stems.

The preservation of the material is probably the most important matter in connexion with this kind of histological research, and the sieve-tubes of Angiosperms appear to offer much greater difficulties than those of the Gymnosperms. This seems to be due mainly to the character of the sieve-plate, which, owing to the size of its pores, allows a shrinkage of the sieve-tube contents to take place on cutting into a stem, and produces the characteristically contracted appearance which is nearly always seen in fixed sieve-tubes. The same kind of difficulty is experienced with developing sieve-tubes, since their watery contents are enclosed in only a thin protoplasmic sac; on fixation plasmolysis readily takes place, and the contraction of the protoplasm is liable to distort the true structure of the sieve-plate with its developing slime-strings.

The artificial appearance of the fixed sieve-tube was pointed out by Fischer,¹ but his method of fixation by boiling tends to produce effects which do not represent faithfully the living structure.

Certain experiments, which have been carried out on living plants of *Cucurbita* with the intention of fixing the sieve-tubes in an uninjured condition, with the usual reagents, were to a certain extent successful, for they show a more or less uniform distribution of the slimy contents within the protoplasmic sac, and in this respect show some agreement with Fischer's results.

METHODS.

The methods employed in this investigation were based on those published by Gardiner² in 1898. They were on the whole similar to those employed in the examination of *Pinus*, but certain modifications in detail have been introduced as occasion has required.

The material was killed either in very small pieces, consisting of cambium and phloem only, or in the form of sections cut from fresh frozen material.

All killing agents and fixatives were in aqueous solution, and comprised solutions of Iodine in Iodides, Picric acid, and mixtures of such solutions with other reagents. Solutions containing corrosive sublimate and Chromic, Acetic or Osmic acids were not found useful as killing agents.

It has been found necessary, as in the demonstration of ordinary connecting-threads, to induce a certain amount of swelling of the killed tissues in order to obtain satisfactory staining reactions, and for this reason

¹ Cf. p. 248.

² Gardiner, Proc. Camb. Phil. Soc., 1898, p. 508.

it is as well to omit Osmic acid from the killing solutions. The requisite amount of swelling, which often need only be very slight, can be brought about by a solution of Iodine in an Iodide, or by Picric acid or by Picro-Sulphuric acid, for two or three days or longer as circumstances may require, and the material may be swollen either in bulk or after it has been cut into sections.

The material may be preserved in Thymol water either before or after the swelling of the tissues has been effected, provided the killing, fixing, and swelling reagents have been thoroughly washed out. It is also important that the material in Thymol water should be kept in glass or india-rubber stoppered bottles, as, with ordinary corks, moulds are very liable to appear in the bottles.

STAINING.

The same principles underlie the methods employed in staining as have already been published in brief by Gardiner. Certain modifications have been introduced to suit special cases, and some new methods have been devised, without, however, departing from the general principle which appears to govern the method as a whole.

Staining is effected in all cases after previous mordanting of the protoplasm. If Safranin is to be the stain, then the mordant may be a salt of Uranium or Platinum, Potassium permanganate, and also to a certain extent Iodine. Uranium and Platinum salts are usually used in conjunction with Osmic acid according to a modified formula of either Kolossow or Hermann. These latter mordants require careful washing out before the staining can take place, and they should be used for pieces of tissue rather than for sections. To overcome this difficulty, and also to shorten the time taken over the mordanting process, a modification has been introduced which is found to be of considerable value in many cases. Since both Iodine in Potassium Iodide and Uranium Nitrate are mordants for protoplasm, and the Iodine has rapid penetrating powers, it seemed likely that Uranium Iodide, should such a compound exist, would be a very useful reagent. Dr. Fenton suggested that if equivalent quantities of solutions of Uranium Nitrate and Potassium Iodide were mixed together, when required for use, the desired compound might be formed which would mordant the protoplasm of the sections placed therein. This has been tried with quite successful results; it is especially useful for dealing with sections, and, should the staining be not sufficiently heavy at the first attempt, it is quite easy to repeat the whole process one or more times.

The Safranin method of staining is the most useful for sieve-tube research, since it is possible to stain the callus with Water Blue without interfering with the stained protoplasm, and it is very important for a right understanding of the structure of the sieve-plate that staining of both

callus and protoplasm shall be possible in the same section. With the Safranin method it is possible, if deeper staining is required, either to remordant with any of the proper mordants and restain with Safranin or with Gentian Violet, or else to use the Acid Blue method after the first staining with Safranin.

The Acid Blue method, which is sometimes a useful alternative to the Safranin staining, may be used on sections cut from fixed or fresh material, but cannot be used directly with any success on material which has been mordanted for Safranin; intensification can be brought about by repetition of the process. By this method results are somewhat artificial; it is not always easy to be certain whether the effects produced are due to precipitation between the Iodine and the dye taking place in empty spaces, or whether they represent the staining of actual structure. In addition to this it is not possible to stain the callus properly with Water Blue or other reagents, since the action of the acid distorts the callus, and the action of the Water Blue tends to upset the protoplasmic staining. The unchecked use of this method has led Kuhla¹ into error with regard to the structure of the sieve-plates, &c. of *Cucurbita*, and though the modification of Meyer's Acid Violet (Pyoktanin) method, by which staining takes place in watch-glasses and not on the slide, gives fairly reliable results, it can only be used usefully in comparison with the Safranin method.

It may be as well to remark here that the success of the modification of the Acid-Violet method is due to the employment of Benzyl Blue², a peculiar impure dye made some twenty-five years ago by the Actien-Gesellschaft, &c. of Berlin. A dye is still sold under the same name, but it is now made by a different process, with the result that it is practically useless for the method.

A third method which has recently been devised is, in principle, like the Safranin method, over which, in some cases, it has certain advantages. Sections of fixed material may be mordanted with a weak solution of Iron Alum, and after a brief washing staining is effected by an Aniline water solution of Water Blue made up after the manner of the Safranin solution. By this method the cell-walls are not stained at all, which is an advantage over the Safranin method, and the Water Blue in this case does not stain the callus. Intensification of the staining is possible by repetition of the process, and a very dark staining of protoplasm and threads is obtained with scarcely any coloration of the cell-wall, and without any precipitation effects.

The stained sections are mounted for observation either in Glycerine with Iodine, or a mixture of Glycerine and Zinc Chloride with Iodine, or in Glycerine Jelly, and there is nothing to add to what has been already published. Preparations so mounted are found to be quite clear at the end of

¹ Kuhla, Bot. Zeit., 1900, pp. 38 et seq.

² The value of this dye was discovered by Gardiner.

four or five years, and if remounted in a medium containing Iodine they will regain their former sharpness of detail. As in the case of *Pinus*, the material has been studied as much as possible by the use of transverse and radial sections. By this means the various stages in the development of the sieve-plate can be more readily followed, and mistakes can be avoided such as may easily be made when examining tangential sections of unknown age by the Acid Blue method.

THE ACTIVE SIEVE-TUBE.

So much has been written about the structure of sieve-tubes, with so many conflicting details, that it may help to make what is to follow more clear if a short account of the structure of the active sieve-tube is given by way of preface.

The active sieve-tube may be regarded from two points of view, namely, with reference to the structure of the walls of the tube, and also as to the nature of the contents. Without going into details it may be stated that the contents consist of a hollow sac-like protoplasmic body in intimate communication with the protoplasmic bodies of neighbouring sieve-tubes, in the vertical direction by means of the large pores in the sieve-plates, and laterally by means of the finer connecting-threads or strings, and occasionally also by lateral sieve-plates. The protoplasmic body encloses the peculiar slimy contents, consisting of metabolized material in a state of flux, which give the sieve-tubes their characteristic appearance in fixed material. The slimy contents pass through the pores of the sieve-plate from one sieve-tube to the next—the slime-strings—but they do not come into contact with the cell-wall, owing to their enclosure in protoplasmic tubules which pass with them through the holes in the sieve-plate. A similar structure, but on a much smaller scale, appears to be shown by the groups of lateral connexions.

FIG. 1. A young sieve-tube (s.t.) in longitudinal section, showing the sieve-plates (s.p.) crossed by slime-strings. The tube shows the slimy contents (s.) within the protoplasmic sac (p.s.). On one side a companion cell (c.c.) is seen. (Copied from Wilhelm, Pl. II, Fig. 13, *Vitis vinifera*.)

The cell-walls of the sieve-tubes—at least in *Vitis* and *Wistaria*—do not appear to be sharply differentiated in their chemical composition from other cell-walls of the phloem, though according to their staining reactions

they seem to possess a somewhat higher percentage of cellulose. The sieve-plates, or rather the sieve-plate reticulum, at first shows a composition similar to that of the lateral walls of the tube, but in the mature condition

FIG. 2. Diagrammatic longitudinal section of an active sieve-tube of *Vitis*, showing the cellulose wall and meshwork of the sieve-plate in section (cl.). The protoplasmic bag (p.) encloses the mucilage or slime (s.); the sieve-plate meshwork is coated with a layer of callus (c.), and this in its turn is separated from the sieve-tube contents by the protoplasm. Cf. the surface view of a similar sieve-plate (Fig. 3).

its free surface has become converted into callus, so that in a transverse section of the plate there is seen to be a central core of cellulose with a border of callus, whilst in a surface view the pores of the sieve-plate show a callus lining (cf. Text-figs. 2 and 3). A somewhat similarly complex structure obtains in the lateral thread groups, for callus is nearly always to be found in association with the connexions between two adjoining sieve-tubes.

A transverse section then of a sieve-plate with its slime-strings would show a highly complex structure, for, taking a single string and its pores,

FIG. 3. Diagrammatic view of a sieve-plate similar to that of Fig. 1, in surface view. The mesh of the plate (cl.), with the pores lined by callus (c.), are shown, through which the slime-strings (s.) pass, ensheathed by protoplasm (p.).

there would be in the centre the slime itself, around which is the protoplasmic tubule, and immediately outside comes the ring of callus due to the peripheral alteration of the cellulose membrane of the sieve-plate itself. Such a structure appears to be typical for the sieve-plates of all Dicotyledons, including *Viscum*, though according to Kuhla's figures¹ it possesses no callus in its sieve-plate.

With regard to the position of the sieve-plate, enough has been written by Wilhelm and Lecomte to render any lengthy description unnecessary.

The sieve-plates are commonly placed transversely like ordinary end-walls, and they occur in this manner in *Wistaria*, *Cucurbita*, *Viscum*, and in the

¹ Kuhla, l. c., Pl. III, Fig. 17.

sieve-tubes of the protophloem of *Vitis*. In the secondary phloem of *Vitis* and many other plants the end-walls are inclined and bear several sieve-plates,¹ so that in a transverse section the sieve-plates appear to be borne on the radial walls of the tubes.

PHLOEM : GENERAL ANATOMY.

The general anatomy of the phloem varies considerably in different plants. In *Vitis*, which has been so carefully studied by Wilhelm, and also in *Tilia* (cf. Perrot, pp. 127, 128), the phloem is composed of alternating bands of sieve-tubes, together with their companion cells and parenchyma, and of bast fibres.² In *Wistaria* the alternation of fibres amongst the

FIG. 4. *Vitis*. Phloem in transverse section, showing the alternating bands (*s.*) of the sieve-tubes (*s. t.*) and companion cells (*c. c.*), and the bast fibres (*f.*); the edge of a medullary ray is seen on the right. (Copied from Wilhelm, Pl. I, Fig. 1.)

sieve-tubes is less regular, but the latter show an interesting feature in always having their sieve-plates at about the same horizontal levels. This characteristic makes *Wistaria* a useful plant for research, since a single favourable transverse section will show sieve-plates in every stage of development. *Viscum* is peculiar in that its sieve-tubes and companion cells are grouped in lenticular areas, as seen in a transverse section, which

¹ Wilhelm, l. c., Pl. II, Fig. 16, &c.; Perrot, l. c., Fig. 39.

² Ibid., Pl. I, Fig. 1.

are enclosed like small islands in the general parenchymatous tissue (Fig. 27, Pl. XVII). *Viscum* also differs markedly from the other plants which have been examined, both in the form and position of the companion cells, and also in the distribution and character of the connecting-threads between them and sieve-tubes, and in the lateral walls between adjoining sieve-tubes.

THE COMPANION CELLS.

The anatomical relation of the companion cell to the sieve-tube is too well known to need any remark. In the case of *Vitis*, excellent figures are

given by Wilhelm¹ (cf. Text-figs. 1 and 4), and there is also a short résumé of the subject by Perrot.² Two of Wilhelm's figures of longitudinal sections are of especial interest (viz., Figs. 26 and 35), for the numerous pits in the wall between the companion cell and its sieve-tube are very faithfully drawn, and the addition of connecting-threads in the pits is only needed to make his picture complete. In longitudinal section the pits in these walls appear to be very narrow, but when seen in surface view they are found to be extended transversely almost across the whole face of the companion cell to form narrow fusiform areas, over which the short threads are distributed either uniformly or aggregated more or less into small secondary groups. The histology of the connecting-threads will be referred to later.³

FIG. 5. *Vitis*. A sieve-tube (*S.T.*) and companion cell (*c.c.*) in tangential section. The radial wall of the sieve-tube shows several sieve-fields (*s.f.*) in the winter condition, with callus-rods crossing the wall, and small callus-cushions. (Copied from Wilhelm, Pl. IV, Fig. 35.)

THE BAST PARENCHYMA CELLS.

Between the bast parenchyma cells and both the sieve-tubes and the companion cells there are small pits of connecting-threads similar in character to those which connect the cells of the bast parenchyma themselves. The pits are more or less circular in outline

¹ Wilhelm, l. c., Pls. I and II, Figs. 13, 16, 18-20; Pl. III, Fig. 26; Pl. IV, Fig. 35.

² Cf. Perrot, l. c., p. 62.

³ Cf. p. 279.

and fairly deep, and the pit-closing membrane is pierced by a small group of some five to seven threads, as seen in section, which usually stain darkly.

Between a sieve-tube and an adjoining bast parenchyma cell only a few such pits will be seen, though in a single sieve-tube of *Vitis* as many as ten little pits have been counted. In winter material small hemispherical pads of callus are found covering these pits on the sieve-tube side, and with appropriate treatment the threads or their remains can be seen fanning out to the margins of these little callus cushions (v. Fig. 49, Pl. XVIII).

Between the bast parenchyma cells and companion cells small pits of threads are fairly numerous, and appear to be similar in every respect to those between two parenchymatous cells,¹ and no callus is formed over them in winter material. As was noticed in the case of *Pinus*,² callus is formed normally only in the sieve-tubes themselves.

The pits in the walls of the phloem medullary ray cells are very numerous, and are occupied by small groups of threads similar to those of the phloem parenchyma and of parenchymatous tissue generally.

BAST FIBRES.

Bast Fibres are a further constituent of the phloem commonly to be found, and may occur in definite bands alternating with the bands of sieve-tubes, as in *Vitis* (cf. Text-fig. 4) and in *Tilia*, or with a somewhat irregular distribution as in *Wistaria*. In *Viscum* and *Cucurbita* bast fibres are apparently absent.³

When young their protoplasmic contents are in communication by means of connecting-threads, both amongst themselves and also with adjoining parenchymatous tissue. In *Vitis* the gradual development of the thick cell-walls has been followed; the final result is the formation of deep funnel-shaped pits with thin pit-closing membranes, which are traversed by a number of short and delicate threads (Fig. 53, Pl. XVIII). As long as the walls of these sclerous cells are increasing in thickness they retain their protoplasmic contents and their connecting-threads, but on the completion of the secondary changes they become dead and empty, and the perforations of the membranes either remain as empty holes or, more probably, are obliterated.

THE DEVELOPMENT OF THE SIEVE-FIELDS AND OF THE SIEVE-PLATES.

THE SIEVE-FIELDS.

The term 'Sieve Fields', in the sense in which it is used in this paper,⁴ denotes the groups of fine connecting-threads or strings which are found normally on the lateral walls, and which serve as a means of communication

¹ Wilhelm, l. c., Pl. IV, Fig. 36.

² Hill, Ann. Bot., vol. xv, p. 597, &c.

³ v. Perrot, pp. 76 et seq., and Figs. 59 and 60.

⁴ v. p. 251, footnote 6.

between adjoining sieve-tubes. The sieve-plates occur on the horizontal or oblique end-walls of the sieve-tubes, and occasionally on the lateral walls also, but their slime-strings are readily distinguished from those of the sieve-fields owing to their larger size. As the developmental history of the fields appears to be simpler than that of the plates, it may help towards a better understanding of the latter structures if the details of sieve-field development are first considered.

Great difficulty has been experienced in trying to demonstrate threads in tissues arising from the cambium or in any embryonic tissue, and the youngest sieve-tube in which threads can be seen in the lateral walls is at some distance from the actual cambium, and removed, in the radial direction, by not more than two or three cells from the youngest active sieve-tube. That threads have not been successfully seen as yet in the youngest cell-walls is due probably to the limitations of our methods rather than to their absence. The walls of the very young elements cut off from the cambium are extremely thin, and of course contain a high percentage of water, so that it seems likely that they undergo relatively violent alterations on fixation, and that the demonstration of connecting-threads is thereby prevented. Similar distortion of cell-wall structure appears to take place with the fixation of mature tissues in Alcohol, since if this reagent is used even with full-grown walls it is impossible as a rule to see anything of the finer histological details.

It is not until the walls of the young elements show a distinct and definite increase in thickness, somewhat like that described for the phloem of *Pinus*,¹ that it is possible to stain the fine protoplasmic threads. In the case of *Vitis* a differentiation of the lateral walls of the sieve-tubes is soon noticeable, giving rise to shallow pits separated by thicker portions of the cell-wall. In a transverse section these pits are often seen to be considerably elongated in the transverse direction, and in a surface view they are more or less elliptical or fusiform in outline, and are closely crowded over the surface of the wall.² The thin pit-closing membranes are provided with delicate, faintly-staining threads, which in a surface view are seen to be distributed more or less evenly over the surface. The threads appear to be continuous across the membrane; and there is no evidence of any break or node at the middle lamella (Fig. 37, Pl. XVIII).

In a slightly older sieve-tube there is a noticeable increase in the thickness of the wall, leading to a greater distinction of the pitted areas; the threads, which still present an unbroken appearance, are somewhat longer and more prominent than before, but show a tendency to become arranged in small secondary groups within the limits of the original pit. On examining a pit such as that just described, after the action of Water

¹ Hill, Histology of Sieve-Tubes of *Pinus*. Ann. Bot., vol. xv, p. 587, 'The boundary layer.'

² Cf. Wilhelm, l. c., Figs. 29 and 38.

Blue, tiny specks or dots of callus will probably be seen at the end of each thread on one side of the membrane, or possibly on both surfaces (Fig. 38, Pl. XVIII). With increasing age the thickened portions of the wall become more conspicuous, and the smaller groups of threads also tend to become arranged in small secondary pits (Fig. 39, Pl. XVIII). Whilst this change in the general configuration of the cell-walls has been taking place, more remarkable changes are taking place in the character of the threads, and in the wall in their immediate environment. The callus has now reached the middle lamella, usually at first from one side only (Fig. 39, Pl. XVIII), and then shortly afterwards from the adjoining element, so that each thread shows a callus coating (appearing as a blue rod with Water Blue alone), which has been formed by an alteration of the cell-walls of the two sieve-tubes concerned, proceeding inwards as far as the middle lamella (Fig. 40, Pl. XVIII).

By slightly swelling the walls and treating the section with either Russow's Callus Reagent or Water Blue, the callus-rods, which appear at first sight to be continuous across the membrane, are seen to be made up of two distinct portions or pegs belonging to the walls of their respective sieve-tubes, and apparently there is no fusion or actual contact between them (cf. Figs. 12 and 13, Pl. XVII).

With the appearance of the callus-rods a further structure becomes visible, namely, a median node or dot, situated between the two opposed pegs of the callus-rod, which is stained with protoplasmic dyes (Fig. 16, Pl. XVII). The formation of the callus-rods is, however, only the obvious sign of a more important change, which has taken place in the character of the threads themselves. Ferments—which, as will be shown later, also determine the structure of the developing sieve-plate—appear to attack the fine threads of these lateral sieve-fields, and bore out the holes or capillaries in the cell-wall through which they pass from cell to cell.¹ So then not only do the ferments, which pass in from each sieve-tube, enlarge the thread canals, but they also alter the nature of the wall in the immediate neighbourhood, by converting it into callus as far as the middle lamella. The contents of these fine tubes are found to react in a different manner to stains after the appearance of the callus, for the protoplasmic threads of the embryonic condition have now become the more important slime-strings of the mature sieve-tube, and in addition to this the small darkly staining node has been formed at the middle lamella. Each slime-string is thus seen to be contained in its own callus-rod or tube, and there is no such inclusion of several strings in a single callus 'cork' as has been described for *Pinus*² and other Gymnosperms. In *Wistaria* the development of the

¹ Cf. Hill, l. c., p. 588. The probable rôle of ferment action in the production of the slime-strings and callus-rods was suggested by Gardiner, and he drew my attention to the analogous appearances presented by the endosperm walls of *Tamus communis* during germination.

² Ibid., p. 590.

fields is easier to study than in *Vitis*, since the threads are fewer, larger, and well separated from each other. In a surface view of the wall of a young sieve-tube, the individual strings, each enclosed in its callus-sheath, can be clearly seen (Fig. 15, Pl. XVII), whilst in a section the paired callus-rods with their contained slime-string, together with the node at the middle lamella, are no less conspicuous after treatment with Safranin and Water Blue (Fig. 14, Pl. XVII).

Precisely similar courses of development appear to obtain for the lateral sieves of *Tilia*, *Cucurbita*, and other Angiosperms. In *Cucurbita* the development of the callus-rods and the appearance of the median node are very clear, for the young sieve-field, viewed in section, is seen to be studded with little knobs of callus at the surface of each fine thread (Fig. 25, Pl. XVII).

Lateral sieve-fields may be found both in the radial and the tangential walls, and are usually distributed in a similar manner in either wall, but in *Tilia*, and probably in some other plants, the threads are far more abundant in the tangential than in the radial walls (Fig. 59, Pl. XVIII), in some cases being so numerous that a surface view shows the tangential wall covered with dots (Fig. 61, Pl. XVIII). In general structure these threads appear to be similar to those of the smaller groups in the radial walls, but owing to their close crowding it is not easy to determine the exact relations of the callus to the individual threads.

Viscum differs from what appears to be the normal arrangement in some interesting details; the threads in the walls between two sieve-tubes are seen in surface view to be grouped in narrow fusiform areas, with which the walls are closely packed, and the wall appears to be full of threads. In section the threads are seen to be in small pits in groups of three to five, and neither in summer nor in winter can any callus be demonstrated in these lateral walls. The threads do not stain very deeply, and there is no appearance of a node at the middle lamella¹ (Figs. 26 and 27, Pl. XVII).

The Median Node.

If these observations are correct, it seems probable that the conspicuous node at the lamella is in some way a function of the slime-string and callus-rod, since in *Viscum*, where the lateral threads do not become converted into slime-strings, the node is apparently absent.

A slight enlargement at the middle lamella is often noticeable on the threads of ordinary cortical cells, e.g. in the cortex of *Cucurbita*, or on the threads of the thick cell-walls of Endosperms,² but in such cases the staining of the thread throughout is of the same character, and the minute

¹ Kuhlmann figures a node on all these threads. Bot. Zeit., lviii, 1900, Pl. III, Figs. 12-14 and 17.

² Gardiner and Hill, Histology of Endosperms; Proc. Camb. Phil. Soc., xi, 1902, p. 449, Pl. V, Figs. 13, 15, &c.

nodal swelling appears to belong definitely to the thread itself.¹ The darkly-staining median nodes of the sieve-field slime-strings appear, therefore, to be similar to those described for *Pinus*,² and may be due to the action of the ferment on the small portion of the middle lamella between the two opposed callus-rods. It has been noticed that these nodes can be stained with Safranin without previous mordanting, and that the threads themselves remain unstained by this treatment; also after the action of Russow's Iodine and 75 per cent. Sulphuric acid the median nodes stand out as highly refractive granules, arranged along the middle of the middle lamella, when all other structures have vanished (Fig. 43, Pl. XVIII). After two days in 10 per cent. Potash, and subsequent washing in water and weak Hydrochloric acid, the middle lamella nodes had apparently vanished from the sieve-threads in sections of *Vitis*, since they could not be seen after treatment with Russow's Iodine and 75 per cent. Sulphuric acid. With the 'Acid Violet' method of staining, however, the nodes reappear, and it is probable that in this latter case we have to deal simply with a precipitation effect in the empty spaces once occupied by the substance of the nodes themselves. The callus also disappears with the Potash treatment, and the split middle lamella with the thread-tubes in the two separated portions of the wall can be clearly seen (Fig. 42, Pl. XVIII).

Owing to the very small size of the median node, it is by no means easy to obtain a clear idea of its nature, but from the experiments which have been made it appears that this conspicuous structure is a kind of connecting-link between the two callus-rods of a single string containing or enclosing the median portion of the string itself (Fig. 41, Pl. XVIII).

THE SIEVE-PLATES.

The study of the development of the sieve-plates is a matter of greater difficulty than that of the sieve-fields, owing to the extremely delicate character of the membrane which is to become the sieve-plate.

The most common stage of development met with in surface views of properly preserved material shows the young sieve-plate furnished with more or less polygonal areas, which give the callus reaction, separated from each other by narrow bands of cellulose. These cellulose bands, which will constitute the mesh of the adult sieve-plate, appear in section as ridges on either side of the membrane, and it is owing to their elevation above its general surface that the young sieve-plate membrane assumes its deeply pitted character.

The pits are lined by callus in the form of little basins³ (Figs. 1 and 3, Pl. XVII), but the actual pit-closing membranes, together with the ridges or mesh of the plate, are formed of cellulose (Figs. 1 and 2, Pl. XVII). Younger stages have been observed in which, owing to the shallow pits, the cellulose

¹ Cf. Gardiner on the mode of formation of the initial cell-wall; Proc. Camb. Phil. Soc. xiv, 209.

² Hill, l. c., p. 587, Pl. XXXI, Fig. 11.

³ Cf. Russow, Sitz. d. Nat. Ges. Dorpat, 1882, p. 304.

membrane shows a definitely reticulated structure in surface view before there is any trace of callus formation.

In all cases the protoplasm is seen to have pit processes which occupy each depression of the young sieve-plate (Figs. 2 and 3, Pl. XVII) in a manner precisely similar to that of the pit-fillings of normally pitted tissues. Very rarely traces of delicate connecting-threads have been seen crossing the thin pit-closing membranes of these youngest sieve-plates, and there seems little reason to doubt that they are traversed by groups of fine threads like those seen crossing the pit membranes between other forms of tissue (cf. Text-fig. 6, p. 271). It seems probable that the development of the sieve-plate may not take place along quite the same lines either in different plants or in the same plant at different times of the year.

WISTARIA.

Before discussing the nature or cause of this variation, a description of the mode of formation of the sieve-plate in *Wistaria* will be given, since some of the clearest and most definite results have been obtained from the study of this plant. The general appearance of the horizontally placed end-wall of the young sieve-tube—the embryonic sieve-plate—has already been noticed (cf. Fig. 1, Pl. XVII, and Text-fig. 6).

Protoplasmic threads have not been successfully demonstrated in the very youngest stages, and even when the pits of the sieve-plate show their callus linings no certain proof of threads crossing the cellulose membrane has been obtained. There is very good reason, however, to believe that there is a group of protoplasmic threads in each of these membranes, but definite proof of their existence does not seem possible by the methods at present at our command.¹

In sieve-plates showing the paired callus-basins, the protoplasm of the sieve-tube may be seen forming little rounded pit processes or fillings which fit into the pits of the young plate, and a close examination of these processes when seen in section reveals the presence of little papillae at their base (Figs. 2 and 3, Pl. XVII). The protoplasm of the sieve-tube is always slightly contracted by fixation, and so also are the pit fillings; the appearance just described appears to be due therefore to the retraction of the little papillae from definite holes in the floor of the callus-basins. This view is confirmed by a surface view of a young plate, for a group of small round holes may be seen occupying the central portion of each polygonal callus-area

¹ Strasburger (Jahrb. f. wiss. Bot., Bd. xxxvi), working with Alcohol material, states that he has seen the fine protoplasmic threads in the pit membranes of the very young sieve-plates, and he gives two figures (v. Pl. XIV, Figs. 29 and 30) in illustration of his views. It is hardly possible, however, to trace any correspondence between his figures of the surface view and the section of similar sieve-plates, nor do his figures of the next older stage in section and surface view (Figs. 31 and 32) enable one to arrive at a clear idea of his views as to the construction of the young sieve-plate.

(Fig. 4, Pl. XVII, and Text-fig. 7). Into these holes or tubes the papillae of the pit processes fit, and in some instances portions of these papillae may be seen in the actual holes in a surface view of such a plate. Owing to the necessary thickness of the sections it is practically impossible to distinguish these little pores in a transverse section of a sieve-plate, for there must be a certain amount of (blue staining) callus either at the back or the front of a pore by which it is hidden from view. In the next stage the paired pit processes on either side of the thin membrane are found to be united by a group of short darkly-staining threads or slime-strings, and the callus extends across the membrane. In some cases the small strings are very short and

FIG. 6.

FIG. 7.

Diagrammatic sketch to show the development of the slime-strings of the sieve-plate from groups of threads:—

FIG. 6. Shows the deeply pitted plate or end-wall, with connecting-threads (*p. t.*) crossing the pit-closing membrane (*c.l.*) and uniting the protoplasm (*p.*) of adjoining sieve-tubes.

FIG. 7. A later stage; ferment action has commenced, the threads are being bored out to become small slime-strings (*s. s.*), and this is accompanied by the alteration of the surface layers of the membrane (*c.l.*) to callus (*c.*). The final stage is the fusion of the small slime-strings to one large string (cf. Figs. 2-4, Pl. XVII).

FIG. 8. The active sieve-plate almost completely developed at *a*; a group of slime-strings is seen crossing the pit-closing membrane, which is now converted entirely into callus (*c.*), whilst at the other end of the plate single thick slime-strings are seen, due to the confluence of the group of strings, each consisting of a mucous string ensheathed in its protoplasmic tube; protoplasm and slime are not differentiated in this figure (cf. Figs. 2 and 3, p. 262, and Figs. 5-9, Pl. XVII).

FIG. 8.

difficult to distinguish (Fig. 8, Pl. XVII), whilst in others they are of a fair length and the whole group can be seen to be definitely ensheathed in callus (Figs. 6 and 7, Pl. XVII). The mesh of the sieve-plate retains its cellulose character, but is everywhere coated with callus.

These groups of small slime-strings, which on the analogy of the sieve-fields must be due to the boring out and enlargement of pre-existing connecting-threads, have as a rule only an ephemeral existence; for the little membrane, now converted into callus—through which each group passes—is rapidly disorganized, with the result that a large open hole is formed

through the sieve-plate in the position of the original pit, and the group of fine threads is supplanted by the single large slime-string of the active sieve-tube (Text-fig. 8). The characteristic appearance of a sieve, with its more or less polygonal holes of varying size, is thus produced. Each hole has a thin lining of callus, and in section it is seen that the whole of the free surface of the sieve-plate mesh is coated with a thin film of callus (Figs. 8 and 9, Pl. XVII).¹

By means of the short, thick slime-strings, with their accompanying protoplasm, the sieve-tubes are placed in direct continuity throughout the length of the plant, and may now be considered to be in the active condition (Text-fig. 8). Owing to the rapidity with which the developmental changes proceed, and to our ignorance of the periodicity of any given plant with regard to such changes, the earliest stages of the sieve-plate are unfortunately very rarely met with.

Throughout the summer the holes in the sieve-plate remain fully open, but towards autumn, and occasionally at an earlier period of the year, they

FIG. 9. An old sieve-plate of an empty sieve-tube, in surface view, to compare with Fig. 2. The protoplasm, slime, and callus have vanished, and a cellulose mesh-work (*cl.*) with empty holes (*m.*) is left.

become constricted owing to the enlargement of the callus. This may be due partly to the swelling of the callus lining of the tubes, but mainly it is caused by the deposition of fresh callus by the protoplasm on the surface of the plate and of its pores. The slime-strings are thus narrowed and lengthened, with the result that the translocation current through the sieve-plate is gradually inhibited (Fig. 10, Pl. XVII). This process may continue until a considerable accumulation of callus has been formed on either side of the sieve-plate.² After a

time the slime-strings become disorganized, owing to the accumulation of callus, and the communication between the sieve-tubes ceases, but the much attenuated pores or canals may still be seen crossing the callus-pads, often enclosing disintegrated portions of the slime-strings, whilst at the middle lamella the original cellulose membrane is clearly visible (Fig. 11, Pl. XVII).

In *Wistaria* the sieve-tubes function for one year only, so that the further history of the sieve-plate simply concerns its degradation. The

¹ Contrast Strasburger, l. c., Pl. XIV, Figs. 33 and 34. According to Strasburger the boring of the sieve-areas commences by a small hole after the fusion together of the callus-rods which have arisen by the transformation of the protoplasmic threads.

The view here put forward, however, entirely differs from this, since there seems good evidence to prove that the largest possible hole is formed in each area immediately on the fusion or boring out of the group of small slime-strings, which are the transformed protoplasmic threads.

² As has been noticed by Lecomte, l. c., p. 265, the callus-cushion may be unequally developed on the two sides of a sieve-plate.

callus is dissolved away by ferments, and only the cellulose mesh or reticulum of the plate is left (Text-fig. 9).¹

CUCURBITA.

The course of sieve-plate development described for *Wistaria* appears to obtain in *Cucurbita* also. The sieve-areas are here very large, and when young and only lightly covered by callus show little holes as though they had been pierced by pins. In some cases the pit-processes of adjoining sieve-tubes were united by definite threads or fine strings which appeared to pass through the holes (cf. Text-fig. 6).

It is important to point out that the size of the individual sieve-areas or pits varies considerably in any sieve-plate; those near the centre being larger as a rule than those near the periphery. It naturally follows that the smaller the area the fewer will be the threads which it contains, and in fact in the case of some of the smallest of these areas there seems to be only a single thread. Such cases as these afford a link between the ordinary sieve-fields and the larger sieve-plates, and, moreover, in some plants it appears probable that each area of a sieve-plate is provided with but one thread. The further history of the sieve-plate agrees closely with *Wistaria*. Large holes are formed in the meshes of the plate and there is a thin callus lining to each hole. After a period of full activity callus is gradually laid down over the sieve-plate to form a conspicuous pad, and the slime-strings, though they still remain thick, are considerably lengthened (Fig. 19, Pl. XVII). With the approach of autumn the callus masses are more pronounced, and the slime-strings become attenuated towards the periphery of the callus-cushions, so that each string is somewhat fusiform in shape and a surface view shows a small string in the middle of each callused sieve-area (Figs. 20 and 22, Pl. XVII). Finally, the slime-strings become broken up and cease to function as connexions, though the holes through which they passed still exist as such in the old callus-pads, and with appropriate methods their paths may be seen either as clear lines or more commonly marked by lines of dark granules, representing the remains of the slime-strings (Fig. 21, Pl. XVII).

The presence of pores in the old callus-masses appears to have escaped general notice,² for, according to the usually accepted view, the callus covers over and obliterates all trace of the slime-strings on the approach of winter.³ There is, however, no doubt that the holes or tubes do persist in the oldest plates, and their gradual attenuation seems to form quite a sufficient obstacle to the translocation currents. The importance to the plant of the

¹ Cf. Strasburger in Jahrb. für wiss. Bot., xxxvi, 1901, Pl. XV, Figs. 41 and 42. It is unfortunate that in these, as in some other figures, the proper correspondence between the surface views and sections is not clearly shown.

² Cf., however, Lecomte, l. c., p. 266, and Figs. 5, 6, and 13.

³ Wilhelm, Beit. z. Kennt. d. Siebröhren, Leipzig, 1880, Pl. VII, Figs. 80, 81, 85 &c.; Strasburger, l. c., Pl. XV, Fig. 40.

persistence of these pores in the callus-pads will be the more evident when the question of the re-opening of the sieves after the winter's rest is considered.

VITIS.

The large inclined sieve-plates of *Vitis* which next claim attention are really compound structures, since they are made up of several small plates (Fig. 47, Pl. XVIII), each of which is constituted like the horizontal plates of the sieve-tubes of other plants. Two methods of sieve-plate development appear to obtain in *Vitis*; in the one, the course of events seems to be closely similar to that already described, and distinct indications of little groups of four to five threads have been seen in each sieve-area (Fig. 30, Pl. XVIII). In one instance there was fairly clear evidence of threads in the walls between the paired callus-basins (Fig. 31, Pl. XVIII). In other cases, however, there can be little doubt that there is never more than one thread to each sieve-area, so that each small sieve-plate is quite comparable in its general structure to a sieve-field. Paired knobs of callus, owing to ferment

FIG. 10.

FIG. 11.

FIG. 12.

Diagrammatic sketches to show the development of the slime-strings from single threads:—

FIG. 10. A young pitted sieve-plate (*cl.*), with single threads (*pt.*) in each pit.

FIG. 11. The commencement of ferment action, showing the enlargement of the ends of the threads to form slime-strings (*s. s.*) and the formation of the callus basins (*c.*).

Fig. 12. The final shape, in which the thread has been bored out and enlarged, and the callus change has proceeded right across the membrane and forms a coating (*c.*) to the cellulose sieve-plate (*cl.*).

action, are now formed on either side of the pitted membrane at the ends of each thread, and the change gradually spreads across the membrane until a slime-string in its callus-rod or sheath is formed (Figs. 32 and 33, Pl. XVIII, and Text-figs. 10 and 11). Several cases of a dark dot between the two opposed callus-knobs have been noticed (Fig. 32, Pl. XVIII), which may represent the node of the original thread in course of alteration,¹ the pieces of the slime-string itself having been retracted from the callus owing to imperfect preservation of the material. In surface view such sieves show a small dot in the centre of a small callus-area (cf. Fig. 33, Pl. XVIII, with the sieve-fields of *Wistaria*, Fig. 15, Pl. XVII). The slime-strings so formed soon begin to enlarge, but a swollen node is often seen at the centre of each string, and in such cases the callus does not appear to be con-

¹ Cf. with the node on the threads of the sieve-fields (Figs. 14, 16, 18, Pl. XVII, and Figs. 45 (*b*), 59, Pl. XVIII).

tinuous across the membrane, thus suggesting a further analogy with the sieve-fields (Fig. 34, Pl. XVIII). After a short time, however, the boring out appears to be complete, and a string of equal breadth enclosed by its sheath of callus unites the contents of the adjoining tubes (cf. Figs. 36 and 47, Pl. XVIII). It has been frequently noticed that the pit areas at the extremities of the large inclined sieve-plates of *Vitis* are occupied by threads of the definite sieve-field type, having median nodes and small callus-rods exactly like those on the lateral walls (Fig. 46, Pl. XVIII). Such observations are not without interest in connexion with the analogy which has been shown to exist between these two forms of connexions.

Further, the sieve-fields may often undergo a subsequent boring after they have reached their normal stage of development. The pores are widened apparently by renewed ferment activity and the median nodal structure vanishes (Figs. 45 and 48, Pl. XVIII), so that to all intents and purposes a small sieve-plate has been developed as a secondary formation from a sieve-field. All these cases lend colour to the view that the sieve-fields and sieve-plates undergo similar developmental stages, but that in the case of the latter structures the changes have been more far-reaching.

The gradual accumulation of callus on both sides of the sieve-plates in *Vitis* takes place in the manner already described for *Wistaria* or *Cucurbita*, and the paths of the slime-strings are left as fine tubes after the disorganization of the strings themselves has taken place, i.e., when the slime disappears from the tubes on the approach of the resting period (Fig. 54, Pl. XVIII).

Good figures of the sieve-plates in the winter condition are given by Wilhelm,¹ and in two cases (Pl. VII, Figs. 89 and 90) he shows lines crossing the callus-masses. As the material was collected on April 27, these lines may represent the new slime-strings in the old pores of the callus-masses.²

RE-OPENING OF THE SIEVE-PLATES IN *VITIS*.

Vitis differs from the plants already mentioned in that its sieve-tubes are functional for more than one year. The sieve-plates, therefore, which become blocked by the callus in the ordinary manner in the autumn have to be reopened in the course of the coming spring. In order to obtain evidence of the time and mode of reopening of the plates, two plants of *Vitis* of different varieties were examined by means of sections taken at short intervals of time from February until the end of May in 1901, and some similar observations were made in 1904. The plants were grown in the open at the Cambridge Botanic Garden. It was noticed that at the beginning of April the buds began to swell, and on cutting into the stem the vine bled. The sieve-tubes and callus-cushions, however, appeared to be in the

¹ Wilhelm, l. c., p. 37.

² Perrot, *Le Tissu criblé*, 1899, Figs. 20, 21, and 32, copied from Lecomte.

same condition as in February and March, for the callus showed sharp contours, and no slime-strings were present. The pores of the old strings were seen only with difficulty and often appeared as dotted lines crossing the callus owing to the remains of the disintegrated portion of the slime-strings (Fig. 54, Pl. XVIII). By about April 20 the phloem again became active and the slime reappeared in the sieve-tubes and began to force its way through the old pores in the callus-masses. The callus had as yet undergone scarcely any change and preserved a firm and definite outline (Figs. 55 and 56, Pl. XVIII). By the beginning of May a great change was found to have taken place: the slime-strings had all been re-established and the callus had lost its sharp outlines and would not stain very deeply with the usual reagents, owing no doubt to the action of the ferments, which were actively engaged in its solution (Fig. 57, Pl. XVIII). At the end of May the sieve-plates were found to be practically in the normal summer condition, with the callus forming a delicate lining to the pores through which the short thick slime-strings passed (cf. Fig. 9, Pl. XVII, and Text-fig. 8, s.s).¹

SIEVE-TUBES CROSSING THE MEDULLARY RAYS.

Wilhelm² was the first to describe and figure the short cross-connecting sieve-tubes in *Vitis vinifera*, which traverse the medullary rays and join one group of sieve-tubes with another.

Similar anastomosing strings of sieve-tubes were noticed by Russow in *Quercus pedunculata*. Those of *Vitis* are again described and figured by Lecomte,³ whose figure is reproduced in Perrot's treatise.⁴

These cross-connecting bands or strings of sieve-tubes are composed of small elements, each being about the size of the adjacent parenchymatous cells, and there seems every reason to believe that they represent ordinary medullary-ray cells, which have undergone special development (Text-fig. 13).

It is unfortunate that, owing to the difficulty of finding these cross-connexions in sections of young material, their development has not been able to be studied in detail; for it seems probable that interesting information might be obtained as to the formation of slime-strings from the original thread-groups of the parenchymatous cells which serve as the mother-cells of these short sieve-tubes and companion cells.

The sieve-plates of these sieve-cells are essentially similar to those of the ordinary sieve-tubes, and show well-developed callus-pads.

¹ At the beginning of April the winter buds had begun to swell, and the Vine bled on being cut. Towards the end of the month the buds were bursting, and by May 9 the young shoots were one to two inches in length, and there was no 'bleeding' of the Vine on cutting. On May 16 one of the leaves of the young shoot had unfolded. Root pressure becomes active some three or four weeks before any visible changes take place in the phloem.

² Wilhelm, l. c., Pl. VI, Fig. 69.

³ Lecomte, l. c., Pl. XXIII, Fig. 40.

⁴ Perrot, *Le Tissu criblé*, p. 42, Fig. 24.

Companion cells, either one or two, accompany each sieve-cell and illustrate clearly the way in which both they and the sieve-tube are formed from the same mother cell. The wall between sieve-tube and companion cell is crowded with threads as is the case in the phloem proper, and their distribution and character in such walls suggest that the threads are closely connected with the processes of cell division.¹

FIG. 13. *Vitis*. Tangential section showing a string of sieve-tubes crossing a medullary ray. The connecting slime-strings crossing the callused sieve-plates are well seen. The short sieve-tubes are accompanied by their companion cells. (Copied from Wilhelm, Pl. VI, Fig. 69.)

THE CALLUS.

An examination of a callus-pad which is being reopened, and from which the slime-strings have been removed, throws some light on the way in which these masses are built up. For owing to the swelling action of the ferment a very regular and definite stratification is disclosed, which lends support to the view that the callus-cushions or pads are laid down by the protoplasm in definite layers in a manner similar to that of the formation by apposition of ordinary cell-walls. It seems highly probable, therefore, that the protoplasm has the power to deposit callus as it would cellulose in accordance with the demands of the plant (Fig. 58, Pl. XVIII). Additional evidence is afforded by those rare cases in which a layer of callus is laid down all over the wall of the sieve-tube apparently in place of a layer of cellulose such as that seen and figured in *Phaseolus* (Fig. 28, Pl. XVII).

¹ Cf. Gardiner, Roy. Soc. Proc., 1900, p. 186, and Proc. Camb. Phil. Soc., xiv, 1907, p. 209.

As the question of callus formation has been discussed somewhat fully in an earlier paper¹, there is no need to enter upon the subject again in any great detail. This research on the Angiosperms has tended to confirm the results already obtained, and there seems now little reason to doubt that the earliest formation of callus, which is coincident with the development of the slime-strings, is due to the alteration of pre-existing cellulose, whilst the superficial pads or cushions of the later-formed or secondary callus are gradually deposited by the protoplasm.

Some account of the diverse views on callus formation has also been given in the introductory portion of this paper, and Russow's interpretation of the shape of the callus-basins was mentioned. He considered that the polygonal outline of these basins or pits—as seen in a surface view of a young plate—afforded definite proof that the callus is laid down entirely by the protoplasm. There seems, however, very little reason for believing that the shape of these areas necessarily throws any light on their mode of formation. The pits of the young sieve-plate are at first more or less circular, then, as the whole membrane grows, they increase considerably in size, until finally the mesh or framework of the sieve-plate represents only a small proportion of the total area. As these changes proceed, the concomitant growth naturally causes the pitted areas to assume an angular outline in consequence of their mutual pressure (cf. Figs. 3, 4, 9, and 22, Pl. XVII).

It is at this stage, when the ground plan of the young plate has become fixed, that the callus makes its first appearance, and it appears to be due to the alteration of the surface layers of these polygonal pit membranes rather than to deposition by the protoplasm (cf. Figs. 1 and 2, Pl. XVII, and Text-figs. 7 and II).

This view of the primary formation of the callus is also supported by the developmental history of the sieve-fields, where the callus appears as little dots or spherical heads round about the end of each connecting-thread, which, when closely crowded, often assume an angular outline. There seems, therefore, to be little reason to doubt that in all cases the first formation of callus is due to the alteration of cellulose, by the action of ferments, in the immediate vicinity of the connecting-threads (Figs. 38–40, Pl. XVIII).

THE CONNEXIONS BETWEEN SIEVE-TUBES AND OTHER TISSUES IN THE PHLOEM.

Reference has already been made to the companion cells² in their relation to the sieve-tubes, but nothing has been said about the character of the threads connecting these two elements.

The simplest case is afforded by *Viscum*, where the connecting-threads in the walls between companion cells and sieve-tubes appear to be exactly similar to those in the lateral walls between two sieve-tubes. In both cases

¹ Hill, Ann. Bot., vol. xv, pp. 597–602, 605–606.

² Cf. p. 264.

the threads are very numerous and are arranged in small groups in pits distributed all over the wall, but at no time does there appear to be any callus associated with the threads. The cell-walls, however, are somewhat mucilaginous and respond readily to swelling agents (Fig. 27, Pl. XVII).

In the case of the more typical Angiosperms the connexions between a sieve-tube and a companion cell are very different in character to those of the sieve-fields. In the latter, the connexions are fine slime-strings ensheathed in callus-rods, and each half of the common cell-wall between the two tubes is of the same nature (Fig. 14, Pl. XVII). But in the case of the wall between a companion cell and a sieve-tube the two portions of the dividing wall show a different composition. On the sieve-tube side of the lamella the wall is fairly broad, whilst on the companion-cell side it is narrow and appears to be somewhat different chemically from the walls of the sieve-tubes. The middle lamella thus comes to occupy an unsymmetrical or extra-median position (Fig. 60, Pl. XVIII).

The connexions between sieve-tubes and companion cells are in consequence also unsymmetrical in appearance, and moreover are found to be composed of two distinct portions. In the sieve-tube 'half' of the wall the threads appear to be like those of the sieve-fields, being accompanied by callus, which reaches as far as the lamella; whilst in the companion-cell 'half' of the wall the threads are necessarily very short and are more like those in the walls of ordinary parenchyma cells, and there is no callus. The condition of affairs is thus analogous to that described for the Albuminous cells of *Pinus*,¹ where each individual 'thread' is similarly made up of two distinct portions. The character of the connexions between sieve-tubes and companion cells in Angiosperms is not nearly so easy of elucidation as is that of the similar connexions between the sieve-tubes and albuminous cells of the Gymnosperms, owing to the thinness of the walls in Angiosperms, and some difficulty exists as to the exact nature of the relation of the callus to the threads. During the spring and summer it is not possible in many cases to demonstrate callus at all in such a position, but in some plants a definite occurrence of callus in the sieve-tube portion of the common wall cannot be doubted (Fig. 29, Pl. XVII, Figs. 51, 52, and 60, Pl. XVIII).

Callus has been seen to extend as far as the middle lamella in winter material of *Vitis* (Fig. 52, Pl. XVIII), but owing to the delicacy of the walls it is very difficult to make out its precise relation to the threads. It seems probable, however, that both in *Vitis* and *Tilia*, and perhaps also in most of the Angiosperms, each whole 'connecting-thread' in the mature condition consists of a slime-string ensheathed in its callus-rod in the sieve-tube portion of the wall, which is then continued as, or rather is in contact with, an ordinary

¹ Cf. Hill, Ann. Bot., vol. xv, 1901, p. 600, Fig. 12, Pl. XXXI; Fig. 12, Pl. XXXII; Fig. 23, Pl. XXXIII.

protoplasmic thread in the wall of the companion cell (cf. Fig. 29, Pl. XVII, and Fig. 60, Pl. XVIII). Some uncertainty also exists as to the presence or absence of a definite median node.

The case of the thread groups between sieve-tubes and bast parenchyma cells appears to be similar to that of the companion cells just described. Here the thread groups show definite callus-cushions in the winter material on the sieve-tube side of the wall (Fig. 49, Pl. XVIII), and in some cases there is clear indication that callus-rods extend through the sieve-tube portion of the wall as far as the lamella (Fig. 50, Pl. XVIII). No callus is formed in the parenchymatous cell, so that here again the threads which appear homogeneous are compounded of two distinct portions—the slime-string in its callus-rod in the sieve-tube portion of the wall, and the protoplasmic thread in the wall of the parenchymatous cell.

At first, no doubt, the threads were protoplasmic throughout, as is the case with the connexions of the sieve-fields, but owing to ferment action the halves of the threads in the sieve-tube portions of the walls undergo secondary modifications and are converted into slime-strings enclosed in callus-rods.

Owing to the difficulty experienced in the staining of the slime-strings in the sieve-tube side of the wall, it is not easy to determine the exact structure of the connexions between the sieve-tubes and these other elements of the phloem; in fact, in most cases the slime-strings appear to have been retracted from the sieve-tube portion of the wall in consequence of the shrinkage on fixation of the general protoplasm. Empty holes are thus left, and it is for this reason, no doubt, that the threads between sieve-tubes and companion cells usually appear to be so short and darkly stained, since, as a rule, only the portions of the threads in the companion-cell half of the wall are visible (Figs. 50–52, Pl. XVIII).

The development of callus in connexion with companion-cell threads is nearly always very slight and difficult of observation, but conspicuous, though small callus-pads are frequently seen over the groups of threads between the sieve-tubes and bast parenchyma cells (Figs. 49 and 52, Pl. XVIII).

The presence of nodes at the lamella, comparable to those found in the sieve-fields, has not been satisfactorily proved, though they appear to be present in the walls between sieve-tubes and bast parenchyma cells (Fig. 50, Pl. XVIII).

GENERAL CONSIDERATIONS.

These curious, unsymmetrical thread groups, considered in connexion with the stages of development passed through by the threads of the sieve-fields, suggest questions of considerable interest, both as to the chemical composition of the cell-walls in which they are found and as to the physiological

processes which bring about the secondary modifications of the threads themselves. The real nature of continuity also requires further consideration in the light of the facts which have been detailed.

It is important, in the first place, to notice that the callus in the phloem is found only in the sieve-tubes, and that its inception appears to be due to the action of ferments on the cellulose walls in the immediate neighbourhood of the threads. From what has been said above (cf. pp. 267 and 279), it is clear that the influence of any developing sieve-tube does not extend beyond the actual limits of that element, that is as far as the middle lamella in any direction, for the callus formation accompanying the boring out of the threads always commences at the inner surface of the wall and proceeds outwards as far as the lamella. No cases have been observed of the ferment action from one tube passing across the lamella into the next, but each element behaves as though it is an isolated and independent organism, and the formation of the active slime-strings of a sieve-field is due to influences emanating from two different and distinct sieve-tubes. The unsymmetrical thread-groups between the sieve-tubes and the companion cells and parenchyma cells respectively are thus easy of explanation. For since the sieve-tube influence cannot be exerted beyond the lamella, the original protoplasmic threads remain more or less unaltered in the walls of the cells (companion or bast-parenchyma cells) to which they belong.

What, then, is the nature of continuity of protoplasm? Does the middle lamella, representing the original wall laid down after nuclear division, form a delicate imperforate membrane against which the ends of the threads impinge coincidently from the cells on either side,¹ so that, with Strasburger,² continuity must be considered as rather apparent than real? Or are the threads really continuous across the lamella from cell to cell?

The facts of slime-string development appear to lend support to the idea of discontinuity, and it might be held that it is owing to the need of establishing some form of actual continuity that the complex changes, already described, have been evolved.

If this be the case, then true continuity exists only in the phloem, between the sieve-tubes, and possibly between sieve-tubes and the other elements, both companion cells and bast parenchyma cells, with which there are connecting-threads. The connecting-threads in all other—i.e. extra phloem—tissues would, on this view, be little more than very fine pit fillings, separated by a pit-closing membrane formed by the middle lamella.

It is unfortunate that the origin of the threads and their relation to the

¹ The idea of discontinuity was first suggested by Gardiner, v. Roy. Soc. Proc., lxvi, 1900, p. 187. Also cf. Proc. Camb. Phil. Soc., xiv, 1907, p. 209.

² Strasburger, Jahrb. f. wiss. Bot., Bd. xxxvi, 1901, pp. 502, 503. It is of interest to notice that A. Meyer, in his review of this paper of Strasburger's, Bot. Zeit., 1902, pp. 102–106, is unable to accept his views on the formation of the connecting-threads. His objections, however, are based on points different to those which have influenced Gardiner in arriving at his conclusions.

cell-plate have not yet been studied in detail, but there seems very good reason for maintaining the views put forward by Gardiner¹ as to the genesis of the connecting-threads. On this hypothesis continuity exists *ab initio*, and at the middle lamella are to be found the first beginnings of the threads, which eventually may become conspicuous as median dots. Certainly the median dot shows the same staining capacity as the rest of the thread, and in many cases it is a scarcely noticeable feature.² But whether these nodes of the protoplasmic threads are entire or are bisected by a delicate membrane is somewhat uncertain.

At present, therefore, there seems to be some ground for doubting whether protoplasmic continuity—in the sense of a continuous filament of living protoplasm—exists between vegetable cells.

With regard to the question of the genesis of the connecting-threads, some support to Gardiner's view appears to be given by the distribution and character of the threads in the wall between a sieve-tube and its companion cell. As is well known, these two cells arise by division from a common mother cell, and the wall between them is crowded with threads. When the elements are young, the threads are distributed more or less uniformly throughout the wall, and their arrangement suggests strongly that they have originated in connexion with the processes of nuclear division,³ and may represent the nodes of the spindle fibres around which it seems probable that the primitive cell-wall is laid down.⁴

With regard to the functions of connecting-threads generally, it seems highly unlikely that they serve for anything more than the conveyance of impulses from cell to cell. Each cell therefore retains its individuality and is master of its own domain, even though it is in sympathetic communication with its neighbours. It is thus conceivable that even if a membrane of extreme tenuity should be found to exist separating the threads of one cell from actual contact with those of another, with which they appear to be in contact, our conception of the efficacy of the threads, as paths for the transmission of stimuli, would not be seriously impaired.⁵

Gardiner's view of the origin of the threads, however, though it has not received definite proof, deserves very careful consideration, since it affords a simple solution of the question of the mode of origin of the threads and one with which it is easy to accommodate the observed facts both as to the

¹ Gardiner, Roy. Soc. Proc., lxvi, 1900, p. 187; Proc. Camb. Phil. Soc., xiv, 1907, p. 209.

² Kuhla, Bot. Zeit., 1900, Pl. III. A conspicuous median dot is figured on nearly all the threads. In *Viscum* examined by our methods this dot is seldom seen, and its prominence is probably due to the method employed by Kuhla.

³ Cf. Hill, Phil. Trans. Roy. Soc., 1901.

⁴ Cf. Gardiner, Roy. Soc. Proc., 1900.

⁵ Cf. Hill, Ann. Bot., vol. xv, pp. 603-605, where the views of Pfeffer and of Brown and Escombe are discussed.

growth and obliteration of the threads¹ and also as to the secondary changes which they may undergo. It is not easy to understand how Strasburger's plasmodesmic pseudopodia² could be formed coincidentally on either side of a separating membrane with the regularity with which the connecting-threads are found in the two halves of the common wall.

SUMMARY.

1. The young cell-wall, which will develop into the sieve-plate, is at first a homogeneous, pitted membrane.

2. The pit-closing membranes of the young sieve-plate are crossed either by small groups of fine protoplasmic threads, e. g. *Wistaria chinensis* and *Cucurbita pepo*, or in some cases apparently by only a single thread, e. g. *Vitis vinifera*.

3. Callus makes its appearance in the form of little basins lining the pits of the developing sieve-plate. It appears to be due to the alteration of the superficial layers of the cellulose membrane at these points by ferment action. Eventually this change extends over the surface of the whole membrane.

4. With the inception of the callus-change the fine thread or threads of the young sieve-plate, as the case may be, begin to be bored out to form slime-strings apparently by a ferment, which at the same time affects the pit-closing membrane in the immediate vicinity of the threads and converts it into callus.

5. The enlargement of the slime-strings thus formed continues, with the final result that a single large slime-string is found occupying the place of each pit of the young sieve-plate. In the one case the single string is due to the enlargement and fusion of a group of strings, in the other to the enlargement of a single string.

6. The slime-string is in all cases enclosed in a protoplasmic tube, which passes through the callus-lined pore of the sieve-plate.

7. Subsequent formation of callus appears to be due to protoplasmic activity and not to cellulose changes. The callus is laid down over the sieve-plate by the protoplasm, apparently in definite layers, after the manner of cellulose, and at length so constricts the pores of the sieve-plate that translocation is prevented.

8. The sieve-pores in a large callus-mass are not obliterated, though much attenuated. In the plants examined, whose sieve-tubes function for

¹ Gardiner, Proc. Roy. Soc., 1900, p. 187. Also Hill, Phil. Trans. Roy. Soc., cf. Pl. XXXI, Figs. 1 and 7; Pl. XXXIII, Figs. 21 and 23. Gardiner, Proc. Camb. Phil. Soc., 1907, p. 209.

² Strasburger, l. c., p. 503, considers that this meeting of the plasmodesma is no more strange than the formation of corresponding pits. But the formation of the corresponding pits is surely due to the fact that there are already groups of threads at these spots, and that it is necessary for the greater efficiency of the threads that the pit-closing membrane should be thin.

more than one year, the slime-strings are re-formed in the spring along the old paths by renewed ferment activity. The accumulated callus is dissolved away and the plate resumes its normally active appearance.

9. With the disuse and death of the sieve-tube the whole of the callus is dissolved and an open sieve remains which represents the cellulose framework or non-pitted portion of the embryonic sieve-plate.

10. The sieve-fields in the lateral walls between two sieve-tubes show a similar origin to that of the sieve-plates. In the adult condition fine slime-strings, each in a callus-rod, cross the pit-closing membrane. In these cases it is probable that ferments from the sieve-tubes on either side of the common membrane, acting along the original protoplasmic threads, convert them into slime-strings and at the same time produce the callus-change in the cellulose immediately contiguous to the threads. In this way the single string in each callus-rod, characteristic of the Angiospermous sieve-fields examined, is produced. In the Gymnosperms examined a group of fine slime-strings was found in each callus-rod.

11. The activities of the sieve-tube contents appear to be limited by the outer boundary of the cell-wall, i. e., the middle lamella, since ferment action is never seen to cross the lamella, but always to proceed as far as this point from each individual tube. It seems likely that similar restrictions may apply to all cells.

12. The callus-rods are not continuous across the common membrane, but are formed of two distinct half-rods, separated by a median node.

13. The median nodes, which are found at the middle lamella separating the opposed halves of the callus-rods of the sieve-fields, appear to be the product of ferment action on the substance of the middle lamella. In *Viscum album*, where in the sieve-fields no callus is formed, the median nodes are also absent.

14. These median nodes enclose the nodules or nodal swellings which represent the points of origin of the protoplasmic threads; but whether the nodal points are halved by a fine separating lamella (thus preventing actual continuity between cells) is uncertain.

15. Callus-pads may be formed over the lateral sieve-fields, apparently by protoplasmic activity, in the same way as over the sieve-plates, and may be re-opened in a similar manner.

16. Callus does not always accompany the threads or strings of the sieve-fields; it is absent from the sieve-fields of the lateral walls in *Viscum album*. An analogous case may be afforded by the albuminous cells in the leaf of *Pinus sylvestris*.

17. Between sieve-tubes and companion cells, thread-groups, comparable to those described between the albuminous cells and the sieve-tubes of the stem of certain Gymnosperms, appear to exist. On the sieve-tube side of the common membrane the threads, at first protoplasmic, are con-

verted into slime-strings associated with callus-rods, but those of the companion-cell wall are continued as ordinary protoplasmic threads.

18. Similar thread-groups are found in the walls between sieve-tubes and bast-parenchyma cells; they are covered by definite callus-pads during the winter, and median nodes appear to be present.

19. The young bast fibres in the phloem of *Vitis vinifera* are in connexion by means of groups of fine protoplasmic threads.

20. The details of sieve-tube histology appear to be in general harmony in the material examined. The formation of slime-strings from protoplasmic threads and their association with callus-rods, as well as the origin of the callus both by transformation of the cellulose and by protoplasmic deposition, are found to proceed along similar lines in representatives of both Angiosperms and Gymnosperms.

21. The secondary changes which take place in the connexions between the sieve-tubes themselves and between sieve-tubes and other elements of the phloem may be explained as follows. In the sieve-tubes there is the need of the formation of definite holes for the translocation of material, and the connecting-threads serve as useful paths along which to work in order to produce tubes of sufficient diameter to enable an actual flow of slime to take place. Then, since the large holes produced are likely to be a source of danger to the plant when translocation diminishes, the callus is utilized to regulate the bore of the tubes, and finally appears to put a stop to translocatory processes altogether.

22. In the case of the threads between the sieve-tubes and other phloem elements, it is no doubt essential that the means of communication should be enlarged to enable the normal physiological processes to take place. And it seems likely that the numerous enlarged communicating channels allow of the passage of secretions into the sieve-tubes from the companion cells.

23. With regard to the physiological function of the threads it appears that they serve primarily for the transmission of stimuli, but that, owing to subsequent modifications in the special cases of the sieve-tubes, they become enlarged and are able to serve secondarily for purposes of translocation.

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EXPLANATION OF FIGURES IN PLATES XVII AND XVIII.

Illustrating Mr. A. W. Hill's paper on the Sieve-tubes of Angiosperms.

The lenses used were Swift's $\frac{1}{8}$ and $\frac{1}{12}$ apoc., Zeiss' $\frac{1}{12}$ apoc., with 6, 8, and 12 compensating oculars.

The following lettering is used in the figures: *c* = callus; *p* = protoplasm; *m* = median node; *cl* = cellulose; *bp* = bast parenchyma cell; *st* = sieve-tube; *cc* = companion cell; *r* = radial; *t* = tangential wall.

PLATE XVII (Figs. 1-17).

Wistaria chinensis.

Fig. 1. A young sieve-plate showing the deep pits lined by callus in the form of paired basins. $\times 1,000$.

Fig. 2. A slightly older plate with pit fillings. The papillae, retracted from the holes in the callus, are seen on the lower edge of the pit fillings. $\times 1,000$. (Cf. Text-fig. 7, p. 27).

Fig. 3. A similar plate to Fig. 2 in surface view; the protoplasmic processes filling the pits show traces of the papillae. $\times 1,000$.

Fig. 4. Surface view of part of a young sieve-plate showing a group of holes in each callus-area. In many cases remains of protoplasmic or slime strings are seen in the holes. $\times 1,000$.

Fig. 5. A slightly older plate, in which the central part of the callus-area appears to be undergoing solution to form the hole for the single slime-string. $\times 1,000$.

Fig. 6. A young sieve-plate in section, with groups of slime-strings surrounded by callus. The original cellulose network of the plate is seen. $\times 1,000$.

Fig. 7. A similar plate to Fig. 6, in surface view. The slime has been removed, and empty holes are seen in each callus-area through which the slime-strings passed; one of the plates shows the formation of a single pore from the original group. The cellulose network is deeply shaded. $\times 1,000$. (Cf. Text-fig. 8, p. 271.)

Fig. 8. A sieve-plate showing the formation of single large slime-strings. On the left a large pore has been formed. In the other cases the small strings are on the point of being broken down. The callus extends across the pit-closing membrane. $\times 1,000$.

Fig. 9. Portion of a sieve-plate, which has just been opened, in surface view. The pores have a narrow lining of callus which also spreads all over the free surface of the cellulose meshwork. $\times 1,000$. (Cf. Figs. 4 and 8.)

Fig. 10. An old sieve-plate in section. The callus has increased, and is blocking the sieves by narrowing the pores; the cellulose meshwork is seen at the middle lamella. $\times 750$.

Fig. 11. An old sieve-plate with callus-pad. The callus stains very faintly with water blue, and the pores are marked by dark dots—the remains of the strings. On the lateral wall are two sieve-fields with callus-cushions traversed by slime-string canals. $\times 750$.

Fig. 12. Young sieve-fields in transverse section; the paired callus-rods reach as far as the middle lamella, but do not cross it to come into actual contact with one another. Stained with Russow's callus-reagent. $\times 1,400$.

Fig. 13. A young lateral wall over-swollen to show the callus-rods abutting against, but not crossing, the split middle lamella. Stained with methylene blue and water blue. $\times 1,000$.

Fig. 14. An older lateral wall between two sieve-tubes in longitudinal section, showing three sieve-fields and a single string of another; each callus-rod encloses a slime-string, and there is a conspicuous node at the middle lamella. $\times 750$.

Fig. 15. A similar wall in surface view with five sieve-fields. The fields have only a few slime-strings, and each has its own callus-rod; the fields are in slight pits. $\times 1,000$.

Fig. 16. A slightly older sieve-field showing the slime-strings with conspicuous median nodes. The callus is not indicated. $\times 1,000$.

Fig. 17. An old lateral wall between two sieve-tubes, partly in surface view and partly in optical section. The callus has formed cushions over the fields, and the groups of median nodes are shown. $\times 1,000$.

PLATE XVII (Figs. 18–25).

Cucurbita maxima.

Fig. 18. A transverse section through the phloem of a root, showing two sieve-tubes with sieve-fields in the radial and tangential walls, and companion cells with threads to the sieve-tubes and to the bast parenchyma cells; sieve-tube wall over-swollen. The callus is not indicated. $\times 800$.

Fig. 19. A sieve-plate cut obliquely with a considerable accumulation of callus. The slime-strings, though thick, have been drawn out to some length. $\times 750$.

Fig. 20. An older sieve-plate, almost at the close of its active period; the slime-strings are fusiform in shape, having been attenuated owing to the deposition of the callus. $\times 500$.

Fig. 21. An old sieve-plate with a large callus-cushion. The slime-strings have broken down, and lines of dots mark their paths through the callus; only protoplasm is seen in the sieve-tube. $\times 500$.

Fig. 22. An old sieve-plate in optical section. The meshwork of the plate can be seen through the overlying callus. The slime-strings have become very small owing to the filling of the pores with callus. (Cf. Fig. 9.) $\times 750$.

Fig. 23. A very young lateral field with protoplasmic threads. Callus is beginning to be formed on one side and appears as little globular heads. (Somewhat over-swollen.) $\times 750$.

Fig. 24. A field slightly older, seen in section and partly in surface view. The callus-change has proceeded further along the threads towards the lamella from the one side, giving the appearance of short rods. Note also the protoplasmic pit-filling, *p.* $\times 750$.

Fig. 25. A young sieve-field, showing callus-formation on both sides of the wall. The median dots are clearly seen at the middle lamella. $\times 750$.

PLATE XVII (Figs. 26-29).

Viscum album.

Fig. 26. A piece of a sieve-tube in longitudinal section, with a sieve-plate crossed by slime-strings. The callus, which is present in the sieve-plate, is not indicated. The lateral wall shows protoplasmic threads in pits connecting two sieve-tubes. There is no callus associated with these threads. $\times 1,000$.

Fig. 27. A patch of sieve-tubes and companion cells in transverse section. The lateral walls are crowded with threads in shallow pits. Groups of threads between two parenchyma cells and between parenchyma cells and a companion cell (*b.p.c.*) are also seen. $\times 750$.

Phaseolus multiflorus.

Fig. 28. A sieve-tube in transverse section showing a layer of callus laid down all over the wall; also a portion of a sieve-field with slime-strings and callus-pads. $\times 750$.

Wistaria chinensis.

Fig. 29. A sieve-tube in transverse section showing a large pad of callus against the wall between the sieve-tube and the companion cell. Winter condition. $\times 750$.

PLATE XVIII (Figs. 30-58).

Vitis vinifera.

Fig. 30. A young sieve-plate in longitudinal section, showing the paired callus-basins almost in contact and traces of threads at their bases. In some cases the large slime-strings have just been formed. An optical section of one of these callus-basins is figured in which four threads are seen. $\times 1,000$.

Fig. 31. A young sieve-plate showing the paired callus-basins with groups of darkly staining threads crossing the membrane between them. $\times 1,000$. (Cf. Text-fig. 7, p. 27.)

Fig. 32. A young sieve-plate with paired callus-basins. There is only a single thread in each pit, which is converted into a slime-string. $\times 1,000$. (Cf. Text-fig. 11, p. 30.)

Fig. 33. A similar plate in surface view, showing a small slime-string in the centre of each callus-area. $\times 750$.

Fig. 34. A young plate of the 'single-thread' type just bored through. The callus of opposed basins is scarcely in contact, and a large median swelling is seen on each string. $\times 1,000$.

Fig. 35. A plate in a slightly older condition; the callus now forms continuous rods across the plate, and the slime-strings are of the same diameter throughout. $\times 1,000$. (Cf. Text-fig. 12, p. 30.)

Fig. 36. A portion of a similar plate in surface view, showing a fairly large slime-string in the centre of each polygonal callus-area. $\times 1,000$.

Fig. 37. A very young lateral thread group in section in the tangential wall of a sieve-tube just outside the cambium. There is no trace of any callus. The lower side is towards the cambium. $\times 1,000$.

Fig. 38. A similar thread-group in a young radial wall. The pit membrane shows slight secondary pitting, and callus-heads have been formed at the ends of the threads on one side of the membrane. $\times 1,000$.

Fig. 39. A tangential wall slightly older than Fig. 38. The callus has extended almost to the middle lamella from one margin of the membrane, and is just commencing on the other margin. The median dots have made their appearance at the lamella. $\times 1,000$.

Fig. 40. A young sieve-field in section, somewhat overswollen on one side. One-half of the wall shows slime-strings in their callus-rods, and nodes at the lamella, whilst the other, younger half shows threads and heads of callus with the commencement of slime-strings. $\times 1,000$.

Fig. 41. A sieve-field in the winter condition after treatment with Acid alcohol and Ammonia, and staining by the ordinary Safranin, Water-blue method. Only callus and median nodes are left. The callus-rods reach to the nodes, but do not touch each other. $\times 1,000$.

Fig. 42. A field from the same material as Fig. 41 after treatment with 10 per cent. Potash for two days, and washing in weak Hydrochloric acid; callus and nodes have vanished and empty tubes are seen in the two portions of the split membrane. $\times 750$.

Fig. 43. A transverse section of a sieve-tube after the action of Russow's Iodine solution and 75 per cent. Sulphuric acid. The walls have turned deep blue and have swollen greatly. Slime-strings and protoplasmic structures have vanished, and only the median nodes are seen as highly refractive bodies. $\times 1,000$.

Fig. 44. A lateral wall of a sieve-tube in surface view. The sieve-tubes are composed of small groups of strings, and each group is covered by a head of callus formed by the union of the heads of the callus-rods which enclose each slime-string. $\times 750$.

Fig. 45. A transverse section of the phloem, showing sieve-tubes and companion cells. One of the sieve-fields (*a*) in the radial wall has undergone further boring out to give a normal sieve-plate with thick slime-strings, and the field (*b*) shows an enlargement of some of its slime-strings. Small thread-groups are seen in the walls between companion cells and both sieve-tubes and bast parenchyma cells. $\times 1,000$.

Fig. 46. A sieve-plate in section. The pit nearest the side-wall is furnished with strings of the sieve-field type. The others are ordinary sieve-plates. This sieve-plate belongs to one of the short sieve-tubes crossing a medullary ray. $\times 750$.

Fig. 47. A piece of the phloem in tangential section, showing sieve-tubes with an inclined compound sieve-plate, a companion cell with its threads, bast parenchyma cells, and a group of threads between one such cell and a sieve-tube with the callus only on the sieve-tube side. Sieve-fields in surface view, &c. $\times 750$.

Fig. 48. A piece of a radial wall of a sieve-tube in transverse section. The slime-strings of the sieve-field have been bored out to form definite continuous connexions between the two tubes. $\times 1,000$.

Fig. 49. Wall between a bast parenchyma cell and a sieve-tube, showing groups of threads with callus-cushions on the sieve-tube side through which the threads are continued. $\times 750$.

Fig. 50. A transverse section showing the connexions between two sieve-tubes and a bast parenchyma cell. The callus-rods reach as far as the middle lamella on the sieve-tube side only. The sieve-tube portion of the wall is the broader. Slime-strings are seen in the callus-rods and there are nodes at the lamella. Thread-groups between a companion cell and a sieve-tube are also seen, but callus is not indicated. $\times 1,000$.

Fig. 51. A companion cell in longitudinal section. The threads in the walls between the cell and the sieve-tube do not appear to be homogeneous. On the cell side of the wall they are short and darkly staining, whilst on the sieve-tube side they are faint and longer and a trace of callus is seen on the surface. They are rather of the nature of fine slime-strings. A thread-group between the companion cell and a bast parenchyma cell is also shown. $\times 1,000$.

Fig. 52. Wall between a companion cell and a sieve-tube in winter material. Callus is seen in the sieve-tube half of the wall. $\times 750$.

Fig. 53. Young bast-fibres in transverse section, showing the funnel-like pits with groups of fine threads crossing the pit-closing membrane. A pit between a bast-fibre and a medullary-ray cell is also shown. Note the position of the lamella and the character of the pits. $\times 1,000$.

Fig. 54. A compound sieve-plate in the winter condition in longitudinal section. The large callus-cushions are traversed by dotted lines, which are the remains of the slime-strings. Material preserved April 4, 1901. $\times 750$.

Fig. 55. A sieve-plate in section, showing the recommencement of activity. Slime is beginning to accumulate in the sieve-tube and to bore its way along the old pores in the callus-cushions. Various stages are seen in the figure. Material preserved April 22, 1901. $\times 750$.

Fig. 56. A callus-cushion in transverse section, showing the formation of new slime-strings in the old pores of the cushion on the reopening of the sieve-plates in spring. The strings are thicker than those seen in Fig. 55. Material preserved April 24, 1901. $\times 750$.

Fig. 57. A sieve-plate in longitudinal section from material preserved May 9, 1901. The slime-strings are completely re-established and the callus is being rapidly dissolved. $\times 750$.

Fig. 58. A callus-pad, in transverse section, which is undergoing solution. The pores are well seen and the whole cushion shows a stratified structure, which is revealed by the swelling due to the ferment action. $\times 700$.

PLATE XVIII (Figs. 59-62).

Tilia vulgaris.

Fig. 59. A sieve-tube in transverse section, showing the difference between the sieve-fields in the tangential and radial walls. In both cases they show median nodes, but in the tangential walls the connexions are very numerous and delicate. Callus is found in both cases, but is not indicated in the figure. $\times 1,000$.

Fig. 60. A sieve-tube with two companion cells in transverse section. With Russow's callus-reagent, small callus-rods are seen in the sieve-tube half of the wall between companion cells and sieve-tubes. $\times 1,000$.

Fig. 61. A tangential wall between two sieve-tubes in surface view, showing the vast numbers of threads or fine slime-strings arranged in little groups. $\times 750$.

Fig. 62. A wall between a sieve-tube and a bast parenchyma cell in longitudinal section. A callus-pad is seen on the sieve-tube side over the groups of threads, but it could not be seen whether the callus extended to the lamella. Small median dots are seen on the threads. Winter material. $\times 1,000$.

A.W.H. del.

AW. HILL, — SIEVE-TUBES OF ANGIOSPERMS.

A.W.H. del.

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