XXV. *On the Continuity of the Protoplasm through the Walls of Vegetable Cells.*

By WALTER GARDINER, *B.A., late Scholar of Clare College, Cambridge.*

Communicated by W. T. THISELTON DYER, *C.M.G., F.R.S.*

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[PLATES 68–70.]

In Professor SACHS' latest publication the following remarkable passage occurs:* "Every plant, however highly organised, is fundamentally a protoplasmic body forming a connected whole, which as it grows on, is externally clothed by a cell membrane, and internally traversed by innumerable transverse and longitudinal walls." The above statement, both as being the outcome of pure physiological thought, and invested as it is with the authority of so distinguished a botanist, cannot fail to be very striking, on account of its forcible suggestiveness, and any observations which demonstrate an actual continuity in organs of large extent, must be of interest to show the truth of SACHS' remarks in a sense somewhat more literal than his own.

At the time of writing, the instances of the existence of any such continuity of the protoplasm were but few. SACHS† himself in 1863, and HANSTEIN‡ in the following year, had proved that in sieve-tubes an actual perforation of the sieve plate did take place, and that by means of the sieve-pores a connexion between the contents of neighbouring cells was established. Their results in this direction were fully confirmed by WILHELM,§ JANCZEWSKI,‖ and RUSSOW.¶

But it was not until the year 1880 that any further steps were made, when TANGL** demonstrated that in the ripe endosperm cells of *Strychnos Nux-vomica*, *Phœnix dactylifera*, and *Euterpe oleracea* the cell-walls were perforated by fine protoplasmic threads. His observations were in the main confirmed by STRASBURGER,††

* 'Vorlesungen über Pflanzen-Physiologie,' p. 102.

† SACHS' 'Flora,' 1863, p. 68.

‡ HANSTEIN, 'Die Milchsaftgefässe.' Berlin, 1864, p. 23.

§ 'Zur Kentniss des Siebröhrenapparates Dicotyler Pflanzen.' Leipzig, 1880.

‖ 'Études comparées sur les tubes cribreux.' Cherbourg, 1881.

¶ 'Sitzber. Dorpater Naturf. Ges.,' April 23. Also in the same journal, 1882, pp. 257–327.

** "Ueber offene Communication zwischen Zellen des Endosperms." PRINGSHEIM's 'Jahrbücher für Wiss. Bot.,' vol. xii., 1880.

†† 'Bau und Wachsthum,' p. 23, *et seq.*

whose general results in connexion with the mode of formation of the cell-wall had impressed him so strongly that the relations existing between the protoplasm and the cell-wall were of the most intimate kind, that he had devoted a special chapter in his work to the consideration of the probability of the perforation of the cell-wall by protoplasmic threads.* In this chapter he distinctly states that although he had not himself been able to establish the existence of any general continuity between vegetable cells, yet that he had but little doubt that such a relation did actually occur.

In a preliminary note published in the 'Quarterly Journal of Microscopical Science' for October, 1882,† I stated that I had succeeded in demonstrating that the continuity of the protoplasm of adjacent cells in the pelvinus of *Mimosa pudica* was maintained by protoplasmic filaments which passed through pits in the cell wall, and later on‡ I showed that the same occurs in *Robinia* and *Amicia*.

Subsequent to the publication of my first results, and previous to the present communication, appeared a most important paper by Russow.§ In this paper the author states that in the bast-parenchyma cells, and in the phlœm medullary-ray cells of many of the Amentaceæ, *e.g.*, *Populus*, *Salix*, *Quercus*, *Betula*, *Corylus*; in *Fraxinus*, *Syringa*, *Olea*, *Æsculus*, *Acer*; in the *Abietineæ*, and further in *Cucurbita pepo* and *Lappa tomentosa*, a treatment of thin sections with Chlor. Zinc Iod. demonstrates that a communication between adjacent cells is established by means of pits, the pit membrane being perforated by fine protoplasmic threads.‖

In the following paper I propose to deal more fully than I have hitherto done with my researches upon pulvini; to treat of the methods I employed, and also to give an account of my investigations as to the structure of endosperm cells, which were undertaken with the view of controlling my results with pulvini. I think that these investigations will succeed in proving not only that perforation of the cell-wall by protoplasmic threads does actually take place, but also that such perforation is of very frequent occurrence.

Methods.

Preservation of material.—As it was a point of primary importance that the material for an investigation of this kind should be preserved with the least possible change, I instituted a number of experiments with the view of ascertaining which of the various reagents commonly in use was the most reliable and what precautions were necessary to insure the most successful result.

* *Loc. cit.*, 'Die Wegsamkeit der Zellhaüte,' p. 246, *et seq.*

† Gardiner, "Open Communication between the Cells in the Pulvinus of Mimosa pudica."

‡ Proc. Roy. Soc., November 11, 1882.

§ 'Sitzber. d. Dorpater Nat Gesellsh.,' 1882, p. 350. See Strasburger's remarks, 'Sitzb. d. Niedenrrh Ges.,' December 4, 1882. I now find (Jan. 16th, 1884), that Russow's paper was read at the January meeting of the Dorpat Society.

‖ With Fromman's and Elsberg's results I have already dealt. See 'Quart. Jour. Micr. Sci.,' April, 1883. Gardiner "On some Recent Researches on the Continuity of the Protoplasm through the Walls of Vegetable C lls."

In my paper "On the Continuity of the Protoplasm in the Motile Organs of Leaves"* I stated that when the plasmolytic condition is induced in a cell, the contracted primordial utricle does not lie free in the cell cavity, but is connected to the cell-wall by numerous fine threads of protoplasm. Since these threads are exceedingly thin and easily ruptured, the value of a preservative agent can be readily tested by observing with what degree of success it can fix the protoplasm of such a cell, and can preserve unbroken the delicate threads. (See Plate 70, figs. 34, 35, 36, and 37.)

For this purpose thin transverse sections of the pulvinus of *Robinia pseudacacia* were rapidly cut in water, and treated for about five minutes with a 10 per cent. solution of sodium chloride. The excess of salt was quickly washed out with water, and the sections were exposed in a watch-glass with frequent stirring to the action of the fluid to be experimented upon, mounted and examined.

The following are the principal results of those experiments:—

With absolute alcohol all the threads were broken, great contraction taking place, attended by great alteration in the shape of the rounded central mass of protoplasm, which now assumed an irregular as opposed to a regular spherical form with a smooth contour.

With 1 per cent. osmic acid in the same way the sharply rounded contour gave place to an irregular, uneven outline, and general swelling of the protoplasm occurred. All the strings were broken. The nucleus, however, was well preserved, though somewhat swollen. It is possible that either a stronger solution of the acid or osmic acid vapour would be more successful.

One per cent. chromic acid, with the exception perhaps of an alcoholic solution of corrosive sublimate, gave the least satisfactory results. None of the threads were preserved, and the nucleus and protoplasm had undergone great alteration of form.

A saturated watery solution of picric acid, on the other hand, gave very satisfactory results indeed. With this reagent the nucleus was especially prominently brought into view, and the protoplasm had undergone the least change. Though in many cases obvious shrinking was produced, yet as a rule the rounded contour was well preserved, and many threads remained unbroken (see Plate 70, fig. 38). Silver nitrate after plasmolysis with nitre, and gold chloride were also tried, but with little success.

As a result of these experiments it would appear that none of these reagents are entirely successful. In every case the protoplasm, even if killed at once, undergoes more or less shrinking, attended with great alteration of form. My results as to absolute alcohol agree with those of FLEMMING, who also finds that saturated picric acid, and 1 per cent. chromic acid, are preferable fixing agents for nuclear investigation. As to chromic acid our results differ. But whatever the reagent used, it is quite apparent that it is easier to deal with young cells, full of protoplasm, with very

* Proc. Roy. Soc., November 11, 1882.

small vacuoles, or no vacuoles at all, than with large full-grown cells when large vacuoles are present.

In the latter case there is every opportunity for contraction, and there is moreover always a tendency to a dilution of the fixing fluid by the cell-sap. This being the case, any successful results with full-grown cells may be regarded as very favourable evidence for the efficacy of the reagent employed. In order to eliminate any doubt as to whether the salt solution influenced the result, analogous experiments were made with fresh tissue. The great drawback to the thorough efficiency of picric acid is that it wets the tissue with some difficulty and only penetrates after some time. This fact becomes very apparent when large pieces of tissue are used at any time of difficult permeability attendant on peculiar histological structure. A saturated solution of picric acid in absolute alcohol to some extent obviates this difficulty, but it is not so successful as a saturated watery solution, although it appears to be a valuable reagent for ordinary work.

With regard to other manipulative details, it is, as mentioned above, important to cut up the material into small pieces, and also to place it at once upon cutting in the preservative medium. My usual plan, in fact, was to cut off the pulvini and allow them to drop, then and there, into picric acid, in order to avoid any loss of water due to evaporation, which as far as delicate investigations are concerned will soon very gravely affect the whole cell-equilibrium. After treatment with picric acid for about 24 hours the material is removed, rapidly washed with water, and placed in alcohol, the latter being changed until the yellow coloration of the picric acid is no longer obtained. Any method of preservation is, however, very imperfect. Not only is appreciable contraction produced, but a great amount of rigidity of the protoplasm occurs due to coagulation and death. These considerations and results determined me to use fresh material, which I employed afterwards all through the investigation.*

* In connexion with the experiments upon fresh material the results obtained with Spirogyra are of ome interest. They confirm those alluded to in the text. Absolute alcohol was shown to be an utter ailure. Watery picric acid was the best reagent, preserving the lenticular form of the nucleus, and demostrating the threads going to the chlorophyll bands with great success. A saturated solution of picric acid in absolnte alcohol is to be preferred next, but it causes definite shrinking. The great point, however, that these experiments made evident was that throughout the entire process of preservation and staining it is necessary to keep all the solutions as nearly as possible of the same density and to avoid any rapid diffusion. Thus if it be required to put up a preparation of Spirogyra, one can first fix the cell with saturated watery picric acid. Then wash in dilute alcohol and stain with either dilute ammonia-hæmatoxylin or a dilute alcoholic solution of one of the aniline dyes. Any dense staining solution will at once cause shrinking. But after this point comes the difficulty. Dilute or strong glycerine will at once cause great shrinking, whatever be the precautions employed, and the only way which is apparently left open to adopt is to mount in such a medium as camphor water, which will cause swelling, in a dilute solution of potassium acetate or calcium chloride, or, still better, in dilute alcohol. To the latter there is the obvious objection that it will act upon most of the varnishes that are used to surround the cover glass and so work its way out. I should suggest as a varnish in this case, a strong solution of gelatine in glacial acetic acid, but hitherto I have not been able to try whether it would work. These results are, however, worth consideration.

Method of preparation.—Experiments have shown that in order to demonstrate in the most satisfactory manner the perforation of the cell wall by protoplasmic threads, it is usually necessary that the wall should be either swollen or dissolved.

Both these methods have already been successfully made use of in the case of sieve tubes by SACHS, who employed, as his reagent, strong sulphuric acid; and by HANSTEIN, who used Chlor. Zinc Iod. In both cases iodine served as a stain for the protoplasm. In investigating the subject of protoplasmic continuity, I have made use of both these methods, but with important modifications. Sulphuric acid is naturally by far the more powerful: strongly swelling or dissolving the cell wall, and laying bare, as it were, the protoplasm to the action of staining reagents, while Chlor. Zinc Iod., on the other hand, when possible, is always preferable, on account of its less vigorous action, attended with less distortion of relative arrangement.

The method used by SACHS for demonstrating the actual perforation of the sieve-plate is essentially based upon the difference of reaction of strong sulphuric acid towards the cell-wall and the protoplasm. The former is partially dissolved, or excessively swollen, while the latter remains but little acted upon, and can be readily stained and examined. The usual plan has been to mount a thin section of tissue in dilute iodine solution, and when sufficiently stained, strong sulphuric acid was run in, and the observation was made. Or the section was first stained with iodine, and then mounted in sulphuric acid. But there are some objections to this method. First, the sulphuric acid is run in, once for all, and thus its action cannot be regulated. Secondly, the iodine from its very colour is not a sufficiently deep stain. Further, the cellulose blue produced; the precipitation of the iodine; and the rapid disintegration of the tissue due to the powerful action of the acid; cause the method to be only satisfactory in such cases as sieve-tubes, where the continuity is pronounced and the material favourable, for here the cell-walls easily dissolve, and the middle lamella is but little developed.

The modification I have adopted has been to divide the process into two parts, and to substitute aniline colours for the iodine. I propose to give a detailed account of the whole process.

A thin section of fresh material is taken up on a platinum spatula; the water is removed with blotting-paper, and a drop of strong sulphuric acid is dropped upon it by means of a glass rod. When the acid has been allowed to act for a determinate time (some seconds), depending on the nature of the tissue and the extent to which the action is required to be carried, the section is rapidly washed by immersing the spatula in a quantity of water contained in a large watch-glass, at the same time stirring, so as to wash out the acid as quickly as possible, and stop its action. Thus the sulphuric acid can be kept entirely under control. After about two changes of water the section may be at once stained, or put into alcohol for future use.

The length of time that the acid requires to act naturally varies with the nature of the material used. Thick-walled tissue requires longer treatment than thin-walled,

and the permeability and peculiar characteristics of the cell-wall in question must be taken into consideration; the difference of reaction being in different cases very great. If, however, the action be properly regulated, the cell-wall will be much swollen; the protoplasm will undergo a certain amount of contraction, but, at the same time, will not be withdrawn from the cell wall at those points where any intimate union exists between the wall and the protoplasm. The middle lamella will, of course, not be destroyed. If the action be allowed to proceed further, the protoplasm itself will be attacked, the cell-wall will begin to dissolve, the middle lamella will also swell; and when in this condition will stain very deeply with reagents, thus making any satisfactory observation impossible.

Experiment shows that unless the action is decidedly forced, the cell wall, though apparently dissolved, does not in reality undergo complete solution, but is only swollen and diffluent. That this is the case may be proved by treating a washed out section with Chlor. Zinc Iod., when the ordinary blue cellulose reaction will be obtained.

The probable action of the sulphuric acid upon sections of the fresh material may now be dealt with. In the first place, the protoplasm is apparently at once killed, although, at the same time, decided shrinking occurs, owing to the great dehydrating power of the reagent. This shrunken appearance is, however, somewhat magnified, because, in addition to the contraction produced by the rapid abstraction of water, the protoplasm has also been squeezed and pressed upon, on all sides, by the swelling cell-wall. But the point which must be especially strongly brought into prominence, is the fact that during the swelling any close relation which may exist between the protoplasm and the cell-wall appears to be maintained, at least where such relation is at all pronounced. Thus in cases where reactions with Chlor. Zinc Iod. and iodine show that the closing membrane of a pitted cell is perforated by fine protoplasmic threads, it will be found that when such a cell is treated with sulphuric acid, the protoplasm projecting into the pit, and especially that portion of it abutting on to the closing membrane, will firmly adhere to the latter, and will resist, without rupturing, a very considerable strain; and even if rupture should at length take place, it will seldom, if ever, occur close to the pit membrane. In attempting to explain the appearances produced by the action of strong sulphuric acid, one must clearly bear in mind that there are two factors to be considered, viz.: the rôle of the protoplasm and the rôle of the cell wall. At the same time there is going on, not only a shrinking of the one, but a swelling of the other. Two principal objections may be very reasonably brought forward to explain the fact, that the protoplasm adheres to the pit. First, it may be said, that the protoplasm is retained and even injected into the pit by the pressure of the swelling wall. That this objection will not hold is apparent from the fact that the same phenomenon occurs in the case of cells which have been cut into. Furthermore, the swollen wall frequently does not abut directly on to the protoplasm, but a considerable space intervenes between the two. Again, by the action of strong

dehydrating agents a further shrinking of the protoplasm may be induced. The second and more important objection is, that the narrowing of the pit, on account of the swelling, imprisons and firmly embraces the protoplasm in the pit cavity. In answer to this, it may be urged that the shrinking of the protoplasm takes place more quickly than the swelling of the wall, and that the protoplasm projecting into the pit would have time to withdraw before being imprisoned. In deep pits of small diameter, it is indeed possible that the narrowing of the cavity does play some definite part, but whether this be so or not, experiment proves that the protoplasm also adheres to pits which are shallow, and moreover possess sloping sides. In this case, any such explanation could hardly be brought forward. Lastly, it must be remembered that the action of the sulphuric acid is carefully regulated, and is not carried to an extreme limit, and that the results obtained with this reagent have been fully confirmed with Chlor. Zinc Iod. All the preceding remarks as to the action of sulphuric acid apply only to the cases in which fresh material is used, since here the protoplasm has not been rendered brittle by any preliminary treatment with reagents, and consequently has undergone as little alteration as possible, and will not break when any slight tension is set up.

After treatment with sulphuric acid, and washing out with water, the section may be stained with iodine, as in the usual process; but I used in preference, and with greater success, analine dyes, especially the violet and blue.

In my earlier experiments I used HOFMANN'S violet (*Trimethyl rosanilin*) as the staining reagent. In the first place, HOFMANN'S violet is a dye which, of all others, is extremely rapid in its action, quickly and thoroughly permeating the tissues. Again, it works extremely well with sulphuric acid, being soluble in, and hardly affected by this reagent, as far as all its staining properties are concerned. Thus one need not take such care to wash out the acid before staining; for, although when the proportion of acid is large the HOFMANN'S violet is temporarily turned green, yet on subsequently washing with water before mounting in glycerine, the violet colour is restored. The whole process may, indeed, be done in one operation, for the solid dye may be dissolved in strong sulphuric acid; the mixture furnishes a dark brown-yellow solution. The section is now simply treated with the mixture, and then washed well with water. The above method gives extremely satisfactory results with sieve-tube preparations; and, moreover, any lignified tissue which happens to be present is coloured gold yellow, as in the ordinary aniline sulphate reaction.

To the use of HOFMANN'S violet there is, however, the great objection that the whole of the tissue—protoplasm, cell-wall, middle lamella, and pit-membrane—is stained. If, however, the stained section be treated for some long time (three to four days), with dilute glycerine the dye in the cell-wall, middle lamella, and pit-membrane dissolves out, whereas that staining the protoplasm remains but little acted upon. This lengthy manipulation is an obvious objection, but nevertheless HOFMANN'S violet often gives extremely satisfactory preparations, and by mounting the section in

strong glycerine, the middle lamella may be made almost transparent; and when in such a condition will no longer present any hindrance to successful observation.

But the better and more reliable reagent is HOFMANN'S blue.* As a result of numerous experiments I am able to state that this dye is a particularly satisfactory reagent for staining the protoplasm alone, and as such is of extreme value for botanical research, and supplies a long-felt want. I find that it works best after treatment with picric acid, and that unless the solution in alcohol be too strong or the staining be decidedly forced, there will be little if any coloration of structures other than protoplasm. But when HOFMANN'S blue is used, the washing-out of the sulphuric acid must be carefully attended to, for the two will not work together as in the case of HOFMANN'S violet. After staining, the section is well washed with water and mounted in dilute glycerine. Such was the method I used in my investigation upon the structure of pulvini. Having thus dealt at some length with sulphuric acid, I must now proceed to describe in the same way Chlor. Zinc Iod.

The action of this reagent is well known. It causes a swelling up of the cell-wall, and at the same time colours the cellulose blue. It is, however, much less violent in its action than sulphuric acid, causing but little distortion of form or displacement of relative arrangement. There is simply a slow and regulated swelling. Sections may be at once treated with Chlor. Zinc Iod., or may be first stained with iodine which helps as it were to accentuate its differentiating powers. The easy manipulation attending the use of Chlor. Zinc Iod., its high refractive index, and the satisfactory manner in which its gradual action may be observed, cause it to be one of the most valuable reagents employed in botanical research. For the demonstration of the presence of protoplasmic threads running through the thickness of the cell wall. TANGL† first used Chlor. Zinc Iod. in his investigation upon the endosperm cells of *Strychnos, Phœnix,* and *Areca.* The sections were first stained with iodine and then

* Under the somewhat loose term aniline blue, are frequently included and described by writers a number of salts which are obviously perfectly distinct, both as regards chemical and physical characters. For example, there is soluble or water blue, insoluble blue, gentiana blue, phenylene blue, benzyl blue, methylene blue, cyanine or chinoline blue, HOFMANN'S blue, besides others, Bavarian blue, Capri blue, &c., some of which are patented products of the various aniline-dye manufacturers. Frequently any given dye obtained from one maker will absolutely differ in staining properties from that of the same name obtained elsewhere. This being so, it is necessary to state very clearly the exact name and maker of any of the so-called aniline blues that may be made use of. I first used HOFMANN'S blue at the Würzburg laboratory, and to the kindness of Professor SACHS I am indebted for the information that it is known as HOFMANN'S blau (anilin blau), and may be obtained from MORELLI, Druggist, &c., Semmel Strasse, Würzburg. A tolerably strong solution is made in 50 per cent. alcohol, to which is added a drop or two of acetic acid. After staining, the section is washed with water and mounted in dilute glycerine or glycerine jelly. I find that when dyes are dissolved in solutions containing a higher percentage of alcohol than that named above, most dyes will lose their selective power for particular structures, and will begin to stain all tissues alike. These results are confirmed as regards animal tissues by Dr. MAYER, of the Naples Zoological Station. See Mt. Zool. Stat., Neapel ii. (1880), pp. 1–27.

† *Loc. cit.*

mounted in Chlor. Zinc Iod. The cell-wall is coloured yellow; the protoplasm and the protoplasmic threads a dark brown. The success of the reaction depends upon the fact that when cellulose has experienced a loss of water, and has become dry as in the case of ripe endosperms and other dry tissues, the Chlor. Zinc Iod. will not at first give the usual blue coloration. It is only after some considerable time that the section will begin to turn blue, or if the cell-wall be very thick or very dry, the blue colour may not be produced at all, but only a yellow brown, which is frequently increased in depth by the precipitation of iodine attending such lengthy action. Sections of a germinating seed of *Phytelephas* furnish an excellent proof that the assumption of the blue colour on treatment with Chlor. Zinc Iod. depends upon hydration, for whereas the normal cells will give a yellow colour, those which are being encroached upon by the absorbent foot of the growing embryo, and are being broken down and at the same time thoroughly wetted, will give in a peculiarly characteristic manner the customary blue cellulose reaction.

But usually in sections of ripe endosperms the cell walls become yellow, and the protoplasm colours dark brown. In most cases nothing can be seen at first of any threads in the cell-wall, but after some time (varying from a quarter of an hour to one hour) they gradually come into view and are moreover apparently increased in size by the gradual precipitation of iodine upon them due to the action of the Chlor. Zinc Iod.

One great objection to this method is that when fresh tissue or thin walled tissue is used, the ordinary blue cellulose reaction occurs which totally obscures the threads from view, and makes all observation of no avail. Moreover, no permanent preparations can be made. My first idea was to employ the same modification as I had adopted in the case of sulphuric acid and use HOFMANN'S blue instead of iodine. With this, however, I at first experienced some difficulty. TANGL* found in *Strychnos* that when he had swollen the walls with water and could see the threads with dilute iodine solution, he was unable to stain them with any ordinary dye, such as hæmatoxylin or carmine. In the same way I found that after the action of iodine and Chlor. Zinc Iod. I could not succeed in staining the threads with any solution of aniline colours. In consequence of this I made a number of experiments with *Strychnos*. I found that although no dyes would demonstrate the threads, yet with solutions of such coloured bodies as gold chloride, picric acid, chromic acid, and iodine, they became more or less clearly apparent. It will be noticed that all these substances are well-defined crystalloids, whereas most of the aniline colours are inclined to be colloidal or, at least, are crystallised with some difficulty. This suggested that the whole phenomenon was simply a matter of diffusion, the solutions of crystalline bodies apparently permeating the substance of the cell wall (crystalloid), but especially diffusing into the protoplasm (colloid), and in the same way the solution of the colloidal aniline dyes diffusing but little or not at all into the colloidal protoplasm. In consequence of this conclusion I made the experiment of dissolving the solid HOFMANN'S

* *Loc. cit.*

blue in a 50 per cent. alcoholic saturated solution of picric acid, under the supposition that the latter might mechanically carry with it into the tissue the dissolved aniline dye, and that on washing out the picric acid with water, the protoplasmic threads would be left stained. Such treatment I found to be perfectly satisfactory, the threads running through the cell-walls and, indeed, the whole of the protoplasm being stained blue, while the cell-wall either remained quite uncoloured or, if the action was forced, coloured but slightly, and to a much less extent than the protoplasmic threads which could still be easily recognised.

As regards the action of picric acid, experiment seems to show that in addition to being one of the most valuable preservative media, it also restrains the coloration of the cell-wall by solutions of such a dye as HOFMANN'S blue, which though a special stain for the protoplasm, will upon lengthy action stain the cell-wall also. Thus, if two sections be stained, one of alcohol material and the other of material which has been treated with picric acid previous to preservation in alcohol, in the former the cell-wall will be definitely stained, while in the latter little, if any, coloration will occur. Thus, the action of the picric acid is of twofold significance, not only serving as a vehicle for the passage of the HOFMANN'S blue into the minute protoplasmic filaments, but also restraining at the same time the coloration of the cell-wall.

It now only remains for me to describe in detail my method of manipulation with Chlor. Zinc Iod. and the picric acid solution of HOFMANN'S blue. Sections of fresh material are cut in water and placed in ordinary iodine solution until they are well stained. They are then taken out by means of a platinum lifter, the iodine solution is removed with blotting paper, and they are mounted in Chlor. Zinc Iod. In those cases where the blue colour is not produced until after some time, it is usually possible to see something of any threads that may be present, and thus many very conclusive observations may be made prior to staining with picric-HOFMANN'S-blue. In fact, treatment with iodine will often bring out clearly many points that HOFMANN'S blue will not, such as a satisfactory demonstration of the passage of the threads through the substance of the middle lamella, where the lamella is well developed. Further, the threads appear thicker than with HOFMANN'S blue, and in any case the treatment will give some idea of what one may expect to see after staining with the aniline dye. The time that Chlor. Zinc Iod. requires to act depends greatly upon the character of the tissue. With many dry endosperms and other cells with thickened walls it will take as long as twenty-four hours to thoroughly permeate the tissue. In my own experiments I was in the habit of mounting in Chlor. Zinc Iod. on one morning and staining on the following day. If not allowed to act for a sufficiently long time, it will be found that while some portions of the walls are swollen, others are hardly acted upon, and that the difference of refractive index of these two portions will give a very confusing appearance to the whole section, and very greatly hinder successful observation. Experiment alone can decide the time required for the complete action;

the only point of importance requiring attention is that the reagent should be allowed to act long enough.

After treatment with Chlor. Zinc Iod. the section is well washed in water until the blue or brown colour (as the case may be) has disappeared. It is then placed for about a quarter of an hour in the picric-HOFMANN'S-blue solution, and after being well washed is mounted in glycerine or glycerine jelly.

As before mentioned, the staining solution is made as follows: To 100 cub. cent. of strong alcohol (*e.g.* about 90 per cent. strength) is added an equal bulk of distilled water. The resulting solution is saturated with picric acid, and HOFMANN'S blue is added, until the liquid is of a dark blue-green colour. It is then filtered.

I used the method with Chlor. Zinc. Iod. almost entirely in my researches upon the structure of endosperm cells. I did not discover the picric-HOFMANN'S-blue modification until I had finished my work with pulvini. Thus, the results with pulvini rest mainly on the sulphuric acid modification.

On the nature of the pit membrane.

If a thin section of almost any tissue be treated with Chlor. Zinc Iod. it will be seen that the walls of almost every cell are distinctly pitted.* These pits are brought into prominence from the fact that whereas the thicker unpitted portions of the cell wall give a well-defined cellulose blue reaction, the thin pit-closing membranes stain but slightly, or in some cases apparently remain quite colourless.† Indeed, so common is this pitting, which the above-mentioned reaction demonstrates, that it would be a statement little short of the truth to say that every cell whatsoever, is pitted to a greater or less degree. Moreover, the closing membrane of the pit itself may also be pitted, as in the seed of *Lupinus hirsutus*, &c. As a rule, it is only in the case of thin walled cells that it is necessary to apply any reagent to bring this pitting into view, for the more the cell wall increases in thickness the more pronounced does the pitting become, until its appearance is at length so marked that we are accustomed to speak of it as a pitted cell *par excellence*. It is a point of special interest to note that

* Cells of *Strychnos*, *Dioscorea*, and *Tamus* are notable exceptions.

† Even in cases where an *en face* view of the pit will give the impression that no coloration has occurred, a transverse section will show that in reality it is slightly stained, and contrasts as markedly with the deep blue stain of the thick wall as the same blue staining of a young cambium cell does with that of the mature, full-grown cell which is subsequently produced. The pits in the parenchymatous cells of the petiole of *Cycas revoluta* are of special interest here. There are, as it were, two systems of pits. The larger, which are arranged in rows up the sides of the cell, face the intercellular spaces, and stain deep blue with iodine and Chlor. Zinc Iod. The smaller pits between the communicating cell walls, on the other hand, do not stain perceptibly when viewed from above. (See Plate 68, fig. 12.) DE BARY mentions pits of *Cycas* and *Encephalartos* which give a callus reaction. (See 'Verg. Anatomie,' p. 125. See also RUSSOW'S important paper, "Ueber Tüpfelbildung," &c. Sitzber. der Dorpat Natur., 1882, pp. 350-389. RUSSOW is inclined to think that no staining of the pit membrane occurs.)

in two adjoining cells * any unequal thickening that may occur always takes place symmetrically on either side of the first formed cell-wall, and in such a way that the two pits which are formed in consequence are exactly opposite one another.

Among many other examples, the thickened cells of hard endosperms and the parenchymatous tissue of all pulvini exhibit this structure to a high degree, and since it was probable that by means of these pits a communication between adjacent cells was established, the study of the nature of the pit-membrane becomes one of great importance. The result of experiments with various staining reagents may now be detailed.

As at first mentioned, Chlor. Zinc Iod. usually stains the pit membrane but little.† Instead of treating the section with this reagent alone, better and more decisive results may be obtained by first soaking the tissue in iodine, then rapidly washing to get rid of the extraneous iodine which would otherwise be precipitated over the tissue, and then mounting in Chlor. Zinc Iod., or the section may be first treated with Chlor. Zinc Iod., then washed and mounted in iodine solution. This gives good results in cases where protoplasm is left sticking to the pits as in the parenchyma cells of the pulvinus of *Amicia* or the endosperm cells of *Bomarea*.

Methyl violet gives very striking, and at the same time is apt to give very deceptive results. When a washed out section of pitted tissue that has been exposed to the action of sulphuric acid is treated with this reagent the whole of the tissue becomes rapidly stained. The protoplasm is coloured a deep purple; the cell-wall is stained violet; and the closing membrane and sides of the pit are brought into prominence since they assume a purple colour, somewhat lighter than that of the protoplasm. The middle lamella also stains deeply. Now in a much-pitted tissue, *e.g.*, that of a pulvinus, the cell wall after treatment with sulphuric acid usually becomes much swollen, causing an elongation and at the same time a narrowing of the pits, and may, moreover, in its swollen condition closely invest and surround the protoplasm. When such a section is treated with methyl violet, the deeply stained tubular pits, being placed symmetrically opposite one another on either side of the common cell wall, abut on the shrunken and similarly stained protoplasm, and give the impression that a distinct and well-defined continuity exists from cell to cell. Thus in *Amicia* the most beautiful and striking appearance is produced which is further heightened by the fact that processes from the main protoplasmic mass usually go for some distance into the pits. (See Plate 68, fig. 10.) If, however, the section be treated for some time with dilute glycerine, the colour is dissolved from the cell wall and the pits and the protoplasm alone remains stained, thus making the real state of things apparent.

The reaction with methylene blue is perhaps the most characteristic. When a section is stained with this reagent before treatment with sulphuric acid, the cell wall and the pit membrane will be deeply coloured, the protoplasm being left unstained.

* I exclude from this statement such cases as that of a cell adjoining a vessel, &c. See 'Sach's Text-book,' English edition, 1882, p. 26.

† In old cells with thick pit membranes the staining of Chlor. Zinc Iod. is, however, very apparent.

If, however, the section be first treated with the acid, then washed, and stained with methylene blue, only the closing membrane and the sides of the pits will be stained (see Plate 68, fig. 8), unless the action of the sulphuric acid be forced. Both the protoplasm and the rest of the cell-wall undergo scarcely any coloration. *Thus methylene blue, apart from its great value as a stain for cell-wall, becomes by this modification a reagent for pit membrane. Naturally HOFMANN'S blue stains neither the cell-wall nor the substance of the pit membrane. The whole results of my investigation appear to point to the conclusion that the staining of this reagent is specially confined to the protoplasm. In the case of many palm endosperms, where after the action of Chlor. Zinc Iod. and picric-HOFMANN'S-blue threads can be observed going through the closing membrane of the pits, it is the threads which are specially stained, and are in consequence defined from the substance of the pit membrane. Indeed, so characteristic is the staining of HOFMANN'S blue, that experiment seems to point to the conclusion that in those cases where a staining of the pit membrane occurs, such staining points to the presence of protoplasm.

There is, however, another special structure which is also stained by HOFMANN'S blue, and which can be distinguished from protoplasm by its solubility in strong sulphuric acid, viz.: the callus of sieve-tubes. It was RUSSOW† who first used aniline blue as a reagent for callus, and even combined it with Chlor. Zinc Iod. As I did not know what particular blue RUSSOW used, I made a number of experiments with the various blues I had at my disposal,‡ with the result that the special staining of the callus was confined to two of them, viz.: HOFMANN'S blue and water or soluble blue, one of which it is pretty certain that RUSSOW employed. Water blue is only second to HOFMANN'S blue in that it also especially stains protoplasmic structures. Now the properties of callus are somewhat peculiar.§ WILHELM showed that it was soluble in sulphuric acid, and insoluble in ammoniacal oxide of copper. In the former respect it resembles cellulose, and indeed its mode of formation—arising as symmetrical warts on either side of the cell wall—as described by JANCZEWSKI,‖ and confirmed by STRASBURGER,¶ certainly give some colour to this idea.** On the other hand, unlike cellulose it

* After keeping the section for a long time in dilute glycerine, staining of the protoplasm does take place since the glycerine dissolves the dye. This solution ultimately stains the protoplasm.

† RUSSOW, 'Sitzber. Dorpater Nat. Ges.,' 1881, April 23, and 'Bot. Ztg.,' 39, 1881, p. 723.

‡ HOFMAN'S blue stains protoplasm and callus. Soluble or water blue, ditto. Benzyl blue, protoplasm cell-wall and callus, like rest of cell wall. Insoluble aniline blue, *i.e.*, solution in spirit, as benzyl blue. Neither of the latter appear to stain the pits. Methylene blue, stains cell-wall and pit membrane Phenylene blue resembles methylene blue. Both water blue, methylene blue, and HOFMANN'S violet may be obtained at MARTINDALE'S, New Cavendish Street, Portland Place, London. The rest of the dyes that I used were obtained from the Actien Gesellshaft für Anilin Fabrication, Berlin.

§ 'Beiträge zur Kentniss des Siebröhrenapparatus Dicotyler Pflanzen.' Leipzic, 1880.

‖ 'Études comparées sur les tubes cribreux.' Cherbourg, 1881.

¶ 'Bau und Wachsthum,' p. 56, *et seq.*

** This was first noted by WILHELM. *Loc. cit.*, p. 16.

is insoluble in ammoniacal cupric oxide, and moreover it gives with Chlor. Zinc Iod. not the customary blue but an intense red-brown coloration. Lastly, the result with HOFMANN'S blue appears to point to a protoplasmic character, opposed to which conclusion is the fact that it dissolves in sulphuric acid. Thus the question appears to be, whether it is related to protoplasm or to cellulose, or whether it consists of a modified cellulose basis permeated by a protoplasmic structure.* This, however, minute study of development alone can decide, but the point I wish to bring forward is the fact that it is coloured by dyes which especially stain the protoplasm.

There is a curious parallelism in the action of callus towards HOFMANN'S blue and of pit membrane towards methylene blue, after treatment with the same reagent (viz.: sulphuric acid) which may perhaps be worth mention.

If a section of a second year stem of *e.g.*, *Vitis vinifera*, be treated with HOFMANN'S blue it will be found that both the protoplasm and the callus will be stained. If, however, sulphuric acid be allowed to act before staining, the callus will naturally be dissolved and will no longer colour, and only the protoplasm will be left stained.

If in the same way a section of pitted tissue, *e.g.*, pulvinus of *Robinia*, be treated with methylene blue, both the cell wall and the pit membrane become coloured. But if the section be first treated with sulphuric acid, the swollen or dissolved cell wall will remain unstained and only the closing membrane and the sides of the pits will alone be stained blue.

Now, if it be allowed that callus may be regarded as altered protoplasm, it might be suggested from the foregoing reactions that cell wall is to be looked upon as altered pit membrane, or rather that pit membrane is to be regarded as consisting of cell wall that has retained its original properties and has undergone comparatively little chemical change. However, I prefer at present to draw no definite conclusions from these observed phenomena but merely desire to put forward the facts.†

On the structure of pulvini.

Having thus treated of the methods employed, and made some remarks as to the nature of the pit membrane, I am now in a position to proceed with the description of my investigation of the structure of pulvini. This work was commenced in the Würzburg laboratory in the month of July, 1882, under the direction, and at the suggestion, of Professor SACHS.

I studied in detail the pulvini of *Mimosa pudica, Robinia pseudacacia, Amicia*

* See RUSSOW'S observations on Abies Picta, 'Stzb. d. Dorpat. Naturf. Gesell.,' 1881, p. 70. Also STRASBURGER, 'Bau und Wachsthum,' p. 60. RUSSOW ('Stzb. d. Dorpat. Naturf. Gesell.,' Feb. 17th, 1882), like myself, in contradistinction to JANCZEWSKI ('Mem. de la Soc. des Sc. Nat. et Math. de Cherbourg.,' vol. xxxiii., p. 209, 1882), believes that the reactions of callus point essentially to its protoplasmic nature.

† I find in reality that the above reaction of the pit membrane with methylene blue takes place in consequence of the fact that the membrane is more resistant than the rest of the cell-wall. Whether this is in consequence of the presence of protoplasm in its structure remains to be proved. This STRASBURGER also found to be the case. See 'Bau und Wachsthum,' p. 16.

zygomeris, and *Phaseolus multiflorus*. I do not intend in the present paper to enter into any discussion with regard either to the nature of irritability or the phenomena of movement of which these plants serve as illustrations, but merely to confine myself to such structural detail as is necessary for the clear comprehension and significance of my results. The principal literature of the subject has been collated by PFEFFER,* to whose researches and those of SACHS† we owe the greater part of our knowledge of what is one of the most interesting phenomena of plant life.‡

Mimosa pudica.—As a rule the main pulvini at the base of the petiole of the leaf were chiefly made use of on account of their larger size and consequent easier manipulation. The secondary and tertiary pulvini, however, gave the same result. Thin longitudinal and, as far as possible, axial sections of the fresh material were taken, since the unequal contraction and puckering-up of the tissue due to the tensions produced by the violent action was not so great as in transverse sections.

As regards its anatomical structure the pulvinus shows a thin vascular bundle surrounded by a thick layer of parenchymatous cells. The epidermis is not pronounced and the epidermal cells have undergone very little, if any cuticularisation. For the most part confined to the underside of the pulvinus are several long stiff multicellular hairs.

Immediately under the epidermis the cells are small, as are those immediately surrounding the bundle, and between these two layers occur the cells of maximum size (see Plate 68, figs. 1 and 3). From the hypodermal cells inwards the intercellular spaces, which are at first inconspicuous, become more and more apparent, until in these cells around the bundle itself a system of large communicating air spaces exist (Plate 68, figs. 3 and 4). The vascular bundle is arranged on the concentric type, the phlœm being outermost and surrounding the xylem. In the phlœm the walls of the prosenchymatous cells are greatly thickened and very highly refractive; the middle lamellæ between them are also almost inconspicuous (see Plate 68, fig. 1), the structure of which is similar to that of *Mimosa*. The cell-walls of the upper half of the pulvinus are thicker than those of the lower, which moreover is the side towards which the bending takes place, and this rule is followed in the secondary and tertiary pulvini also, viz.: that the cells of the side which becomes concave on bending, have always thinner walls than the side which becomes convex, so that whereas in the main pulvini the underside has the thinner walls, in the pulvini of the leaflets the reverse is the case. The parenchymatous cells each contain a number of chlorophyll granules and a nucleus. One or more drops of tannin are also present,§ which can be well seen,

* PFEFFER, 'Physiologishe Untersuchungen,' 1873, i. id.; 'Die periodischen Bewegungen der Blattorgane,' 1875; see also 'Pflanzen Physiologie,' 1880.

† SACHS', 'Handb. der Exp. Phys.,' 1866, p. 479, *et seq.*

‡ See also DARWIN, 'Movements of Plants,' 1880.

§ I could not detect the special pellicle mentioned by PFFEFER. See SACHS' Text-book, p. 889.

by staining the section with methyl violet, and washing with alcohol. The dye is then dissolved from everything but the tannin drops. With osmic acid they also stain a blue-black and with chromic acid a brown-yellow. The latter reagent, however, affects the protoplasm as well, and thus does not allow the individual drops to be distinguished.

On treating with Chlor. Zinc Iod. it becomes apparent that the parenchymatous cells are freely pitted, each such pit being so little stained as to appear quite transparent, thus presenting a marked contrast to the ordinary deep cellulose blue of the rest of the cell-wall. The pits, as a rule, are somewhat shallow and of small diameter except in those cells bordering on the vascular bundle, which from their peculiar configuration in consequence of the presence of large intercellular spaces exhibit on their walls pits of much larger size (Plate 68, fig. 4). The pits are greater in number on the longitudinal than on the transverse walls. The thin pit membrane between two cells is, except under very favourable circumstances, extremely difficult to observe in the unstained condition. It is perhaps brought out most clearly by staining the protoplasm with HOFMANN'S blue when the unstained wall will be seen as a thin colourless membrane separating the protoplasm of one cell from that of the other.

Even with the most favourable section there is no indication of the existence of any connexion between the protoplasm of neighbouring cells. Deep staining with iodine or with HOFMANN'S blue shows the outline of the protoplasm to be well defined and sharply limited by the cell-wall at all points.

But if the wall be swollen with sulphuric acid, and after washing stained with iodine, methyl-violet and glycerine, or HOFMANN'S blue, it will become apparent that a definite communication between the cells does exist, and that such communication is established by means of the pits (Plate 68, fig. 5). The appearance of a well-prepared section is extremely characteristic, reminding one to some extent of a gold chloride preparation of corneal connective-tissue cells. The protoplasm, as it has contracted away from the cell-wall, has adhered to the membranes of the pits, at those points in the cell-wall where pits are present; and in consequence, the whole section presents the appearance of a number of stained and interconnected irregularly-shaped stellate masses, for the narrow processes of any one mass unite at their apices with those proceeding from the neighbouring masses, thus exhibiting a well-defined reticulate arrangement. The reason that the processes proceeding from the masses of two contiguous cells are opposite one another obviously depends upon the symmetrical development of pits on either side of the cell-wall (Plate 68, fig. 3). But that the relation between two such processes is of the most intimate character is quite evident from the fact that in many instances it appears that an optical continuity exists between them, thus establishing a means of communication between cell and cell (Plate 68, fig. 5).

Successful sections are somewhat difficult to prepare, for if the sulphuric acid does not act sufficiently long, the cell-wall is either little or not at all swollen, and when in this condition cannot be permeated by the dye, and if the action has been allowed to

proceed too far, the protoplasm is attacked and the delicate connexions soon become obliterated. Moreover, at the same time the middle lamella becomes swollen and will deeply stain, which of all things is to be avoided. A regulated action of the acid gives the best results, and if the protoplasm be not sufficiently shrunk to show up the processes to the best advantage, the section need only be mounted in strong glycerine which will soon bring about the desired effect. Even in one and the same preparation, though a successful one, the acid may have acted unequally, due, it may be, to varying thickness of the section, and thus the different results produced by the acid may be observed at the same time. However, in a well-prepared section, where the action of the acid has been properly regulated, plain examples of continuity are apparent. Upon longer treatment, the further shrinking of the protoplasm causes a greater tension to be exerted upon the processes and rupture ensues. This frequently occurs, but the rupture nearly always takes place on one or both sides of the point where the thin thread-like process crosses the middle lamella, and seldom at the point itself. Thus the threads cannot be said to be merely pulled out of the pits, for rupture takes place in such a manner that a longer or shorter length still remains in the pit cavity.

Finally, when the action of the acid has been carried too far, the processes appear to have been partially destroyed, and but few can be traced as far as the swollen and now deeply-stained middle lamella. Many of the processes appear to be directly and uninterruptedly continuous from cell to cell, whilst others are swollen at the point where they cross the middle lamella. In other cases between the two ends of the strongly-stained processes there is a lighter-stained portion, which connects the two This lighter-stained area exhibits a haziness and appears to be somewhat indistinct, although well defined from the rest of the swollen cell-wall, and clear enough not to be confounded with the middle lamella (Plate 68, fig. 5). Again, when the protoplasm is but slightly contracted, and but little tension has been exerted on the threads, the point of junction of the two threads is both slightly swollen and also coloured darker than the rest. In spite, however, of the fact that in several cases direct continuity appears to exist, I am strongly of opinion, both from analogy and from such appearances as I last described, that in reality a sieve-plate arrangement occurs. It must be borne in mind that the difficulties of examination are great, both on account of the smallness of the pits and the thinness of the pit membranes, but in any case we cannot imagine that the threads go bodily through the pit, for were it so, the pits would not possess a closing membrane, and ordinary staining would soon demonstrate the existence of the protoplasm, by which the pit was perforated.

Although Pfeffer's* results appear to prove that it is the underside of the pulvinus which is especially sensitive, I have not been able to establish any difference between them as far as histological evidence goes. Nor is this to be greatly wondered at, for the method for such a discrimination is essentially rough, and one would hardly

* See Sachs' 'Text book,' p. 889.

expect such physiological differences to be made apparent by a somewhat coarse histological treatment. Certain cells occur scattered about in the tissue which are both larger and stain more deeply than their neighbours, but the latter phenomenon may be, and probaby is, caused by the presence of tannin. In the parenchyma of both the upper and lower side of the pulvinus the connexion appears to be more pronounced in the cells of the middle layer than it is in those either next the epidermis or next the vascular bundle; and since the cells are more freely pitted on the longitudinal than on the transverse walls more connexions exist through those of the one than of the other.

With regard to the middle lamella there is some difficulty, unless very careful preparation is adopted. It will in any case stain, the depth of the staining depending upon the action of the acid and of the dye, and if the treatment with the one or with the other be forced, the great coloration of the lamella will so obstruct the view that it will be impossible to see with certainty whether or not a distinct continuity of the protoplasmic processes occurs. The difficulty may, however, in a great measure be removed by long treatment with strong glycerine, which both dissolves the greater portion of the colouring matter from the lamella, and at the same time renders it sufficiently transparent for a decisive observation to be made.

The bast fibres of *Mimosa* are of peculiar interest with regard to this question. The middle lamellæ between these cells are so little developed that they are recognised with some difficulty, and which is an important fact, do not stain at all. Consequently, the additional factor of difficulty, that the presence of a well-developed middle lamella involves, is here done away with. Each bast cell is freely pitted, the pits of neighbouring cells being placed symmetrically opposite one another. When treated with sulphuric acid and stained in the usual manner the following appearance is produced. The pit membranes being somewhat thick have distinctly swollen, and in so doing have increased the distance from one another of the ends of the protoplasmic processes projecting into the pit cavity. All the processes are deeply stained, and between each symmetrically opposite pair is a small less stained portion traversing the pit membrane, which from its reactions must be protoplasm. Thus it stains with iodine, and when coloured with methyl violet is not dissolved by glycerine (Plate 68, fig. 6). It is also well brought out by HOFMANN'S blue, the staining characters of which have already been sufficiently dwelt upon. That it is not callus is clear from the fact that it does not dissolve in sulphuric acid. The structure traversing the pit membrane is somewhat difficult to observe, both on account of its very small size and its want of definition. Indeed, it rather presents the appearance of a small blue cloud between the ends of the deeply-stained and well-defined processes.

The protoplasmic processes projecting into the pits are broad at their extremities, and are at the same time more deeply coloured at that point. They gradually taper off from the bottom of the pit inwards, widening again as they join the general proto-

plasm of the cell. Each pair of processes with their above-mentioned broad ends and the cloud between them forcibly suggest a sieve-tube arrangement, and from analogy, as I shall point out later on, I believe that such is the case. I have, however, with the highest powers at my disposal been unable to resolve the stained structure traversing the pit membrane into fine lines as I had hoped to do, although the whole appearance is most strongly suggestive of a striation, the direction of which is parallel to the long axis of the pit. However, it seems certain that there is a protoplasmic communication which can be plainly seen, and is not complicated by the presence of a stained middle lamella.

Thus it appears that from the epidermal cells right up to the last living bast fibre which impinges on the first dead vessel a direct continuity from cell to cell has been established, and that such a pulvinus may be regarded as a connected whole.

Robinia pseudacacia.—As in *Mimosa*, thin axial, longitudinal sections of the main pulvini were examined. Fresh material was used in every case, and after treatment with sulphuric acid the sections were stained with either HOFMANN'S violet and glycerine, or with HOFMANN'S blue. In fundamental structure the pulvinus of this plant resembles that of *Mimosa*. Rough examinations show that it is much larger, and that its surface is quite smooth and free from hairs. The cells do not appear to be so freely pitted, nor is tannin so abundant. In many of the cells which are scattered about the tissue, and are smaller than their neighbours, are crystals of calcium oxalate, which can be well seen embedded in the protoplasm of the containing cell. The cells in certain cases possess more than one nucleus. The nuclei are large and well developed, and are brought into prominent view in the case of tissue which has been previously treated with picric acid.

After treatment with sulphuric acid it can be seen that, in a well prepared section, the cells present very much the same appearance as those of *Mimosa* (Plate 68, fig. 7). The continuity existing between the processes is not as pronounced as in the former case, and the appearance of threads going straight and uninterruptedly through the pits is not so frequent (Plate 68, fig. 7). On the contrary, there is more indication of the existence of a sieve-plate-arrangement, which is very marked in those cases which admit of successful observation. Frequently at the point of junction of two processes there is a distinct and well defined swelling which stains perceptibly lighter than the very darkly stained threads, which it connects one with the other. It can clearly be distinguished from the pit membrane and the middle lamella, and can almost certainly be resolved into a striated appearance, although the observation cannot perhaps be regarded as perfectly conclusive or perfectly satisfactory. In consequence of the presence of fewer pits on the cell walls the interconnecting protoplasmic processes are fewer in number than in *Mimosa*. The bast fibres present the same appearance as those of *Mimosa*, although the appearance, on the whole, is not so marked. The secondary pulvini display essentiallyt he same structure as the main pulvinus of the whole leaf, with the exception that the number of tannin cells is very

much greater.* When stained with chromic acid the protoplasm of the tannin cells exhibits a distinct appearance of reticulation, but from what cause I am at present ignorant.

Amicia zygomeris.—The pulvinus of this highly interesting plant was pointed out to me by Professor SACHS as well worthy of investigation. As a most striking example of both periodic and irritable movements this plant has apparently escaped general observation. It is particularly sensitive to alternations of day and night, and assumes the sleep position long before even such plants as *Robinia*. If violently shaken the leaves will, after a time, fall, and will be similarly affected some time after being cut and placed in water: the large size of the leaves rendering the least movement very conspicuous. Since it was the secondary pulvini that were especially movable, and they were, at the same time, of a comparatively large size, I used them in preference to still larger main pulvinus.

The chief characteristics of the pulvinus tissue of *Amicia* are the thinness of the walls of the parenchymatous cells, the extremely unlignified character of the vascular bundle and the remarkable development of a system of large pits, which is in this case extremely pronounced. The whole tissue is very succulent, and easily admits of thin sections being cut (Plate 68, fig. 9).

On treating with iodine and Chlor. Zinc Iod. the pits are, as usual, markedly brought into view. From the contrast of the deep blue coloration of the walls with that of the pits, it appears, at first sight, that no staining of the latter has taken place. Sections transverse to the pit, however, show that both a very slight staining of the pit-membrane has occurred, and that the membrane is extremely thin. In some of the pits small masses of protoplasm may be recognised sticking to the pit-membrane, being brought into view in consequence of their brown staining reaction. The pit-membrane is well stained by methylene blue. Scarcely any difference can be detected between the thickness of the cell-walls on the upper and under sides of the pulvinus. Except just beneath the epidermis, and next the vascular bundle, the cells are relatively large. The layer of protoplasm (primordial utricle) lining the cell-wall is thin, and the central vacuole is large. In consequence of this, very great shrinking of the protoplasm is possible, and experience shows that the successful preservation of this tissue is extremely difficult. Any reagents causing the least diffusion very soon affect the protoplasm, and the only at all successful treatment is brought about by

* The fact deserves notice that in the cases where the protoplasm displays any great activity of function, the cells of such a tissue usually contain tannin. For example, pulvini of *Mimosa Robinia*, *Desmodium*, &c., leaf of *Dionæa*, *Drosera*. Again in galls, where a stimulation of the protoplasm followed by rapid growth occurs. Notice that in *Robinia* it is the pulvini of the leaflets that move more than the main pulvinus, which have the greater quantity of tannin. The effect of tannin in producing aggregation is dealt with by A. F. W. SCHIMPER ('Bot. Zeit.,' 14, 1882) On Tannin. See also GARDINER "On the General Occurrence of Tannin in the Vegetable Cell, and a Possible View of its Physiological Significance," Proc. Camb. Phil. Soc., vol. iv., pt. vi., pp. 387–394, and Bot. Central. Bd. xvi., No. 48, p. 258.

means of saturated, watery, picric acid. Absolute alcohol is quite unsatisfactory. In the same way sulphuric acid causes very great contraction, the processes being usually ruptured, and nearly always pulled perceptibly from the pit membrane.

The cell-walls possess that peculiar semi-horny structure which is equally shared by so many of the Leguminosæ, and swell greatly with sulphuric acid. The delusive and at the same time very beautiful effects obtained by staining a section after treatment with sulphuric acid with methyl violet have already been dealt with under the head of pit membrane (Plate 68, fig. 10). As there mentioned, the bottom and sides of the pits are markedly stained by this reagent in a way somewhat similar to that of the protoplasm, and at first sight the appearance suggests that the cells are freely connected —the one with the other—by unbroken protoplasmic threads. The whole structure is remarkably like that of an enlarged representation of free cell formation. However, on treating the section with glycerine, all the deception disappears with the solution of the colouring matter, and it will then become apparent that in reality the connexion is neither so well defined nor so pronounced as in the case of *Mimosa* and *Robinia* (Plate 68, fig. 11). In no instance, so far as I can ascertain, do the processes approximate to one another in the unbroken way in which they appear to do in *Mimosa*. In the larger cells occupying the middle layer between the epidermis and vascular bundle, the protoplasm is either entirely pulled from the pit membrane, or the processes which at first connected the protoplasm of the pit with the general protoplasmic mass are ruptured so as to leave a short portion only sticking to the pit membrane. In the four last layers of cells which abut on to the vascular bundle where the cells are smaller with thicker walls and smaller vacuoles, it will be seen that although shrinking has taken place, yet the whole appearance of contraction is not so great although the protoplasm projecting into the pits has, as a rule, been pulled from the pit membrane. In the swollen pit membrane between the two symmetrically placed processes the same stained structure is apparent as occurs in the bast fibres of *Mimosa*, although the whole appearance is much more marked (Plate 68, fig. 11). The stained portion as before, and more markedly, suggests an appearance of striation, but with the strongest powers at my disposal, consistent with clear definition, I was unable to resolve the structure into fine threads. All that one can say is that its reactions point to its protoplasmic nature. In such of the other cells of the larger celled tissue as could be favourably observed, the same structure was present. It may here be mentioned that in cases, *e.g.*, *Phœnix* (Plate 69, fig. 13), where with a high power a sieve plate arrangement can be seen, and the threads clearly made out; with a low power the same appearances are produced as in the case in point, or as in bast fibres, and it seems probable that here a sieve plate arrangement does in reality occur.

The staining of that portion of the pit membrane which colours with aniline blue or which is left stained by methyl violet after prolonged action of glycerine, must not be confounded with the coloration of the bottom and sides of the pit which occurs with methyl violet alone or with methylene blue after the action of sulphuric acid. In

the latter instance the pit, as a whole, stains. In the former it is the staining of a substance other than pit membrane which runs through the latter, and which, by its different reactions, is to be separated from the pit membrane itself. Its reactions, as before-mentioned, point to a protoplasmic character.

Experiments were made with other pulvini and other organs of similar character, the results of which are detailed below. The experiments were somewhat hurried as the season was late, and although, to the best of my belief, the results are accurate, yet I do not regard them as perfectly conclusive, and I must work over the subject in detail on a future occasion.

Phaseolus multiflorus appears to be connected as *Amicia*.

Desmodium gyrans resembles *Mimosa* in structure.

Dionæa muscipula.—Sections of the tissue next the vascular bundle showed the cells to be connected as in *Mimosa*. In the epidermal and sub-epidermal layers this structure was especially evident, and some processes were seen uniting the glands with the cells.

Stamens of Cynara.—The lengthy oblong cells surrounding the central bundle appeared connected one to another principally through their end walls, in a manner almost exactly resembling that of a sieve tube. Apparently some connexion between them also took place through the side walls.

Tendrils.—In the oblong cells of the tendrils of *Bryonia*, a similar sieve-tube-like arrangement appeared to occur, especially on the end walls.

On the structure of endosperm cells.—From some points of view I could not regard the results I had obtained with pulvini as either perfectly satisfactory or perfectly conclusive. In spite of a probability little short of certainty, some doubt still remained; for it could be brought forward, that in the first place the results had been obtained by means of an extremely powerful reagent, with whose action we were by no means intimately acquainted; and, secondly, that we had no such examples of the general perforation of the pit-membrane by protoplasmic threads. And even allowing that the pit-membrane was traversed by fine threads, the great question that required answering was—Do these threads in reality cross the middle lamella, or is it only a case of the membrane itself being pitted, and the threads running up to the lamella, but no further?

In order, therefore, to put my results on as firm a basis as possible, it was necessary to experiment with my methods upon any such cases as might exist, where the passage of protoplasmic threads through the cell-wall was a confirmed fact, or to endeavour to establish, in a manner which admitted of no doubt, other instances of the existence of similar phenomena.

The first and most obvious examples of the occurrence of the perforation of the cell-wall are naturally afforded by sieve-tubes, and, in consequence, I began by investigating the results produced upon such structures by the reagents which I had employed in the case of pulvini.

In this direction I found that the method was in every way peculiarly adapted to show the intimate structure of sieve-tubes. In the course of my investigations on pulvini I had frequent opportunities for observing sieve-tubes, *e.g.*, in *Mimosa*, *Robinia*, &c. In both the above-named cases the sieve-tubes are very small, but treatment with sulphuric acid, and subsequent staining with methyl violet and glycerine, or HOFMANN'S blue, brought out these structures very successfully, and defined in an extremely clear manner the very fine threads connecting the contents of neighbouring tubes. The sieve-tubes of *Dahlia variabilis*, *Ricinus communis*, and *Phaseolus multiflorus* were also investigated. In *Ricinus* the youngest sieve-cells where perforation had not yet taken place were clearly demonstrated. In *Phaseolus* the general occurrence of a lenticular highly refractive body in the sieve-tube cavity was noticed, but I must defer a description of it until a future occasion.

But in the end the fact became apparent that although the results obtained with sieve-tubes gave very valuable proof of the success of the method I had adopted, yet that their structure could not be exactly compared to that of the parenchymatous cells of pulvini. Thus, in sieve-tubes, the cell-walls tend to assume a soft and somewhat mucilaginous character, and in them the middle lamella is but little developed, and the whole wall readily dissolves in sulphuric acid.

In the cells of the pulvinus, on the other hand, the walls greatly resist the action of the acid, and the development of the middle lamella is essentially pronounced.

There was, however, still one road left open, and that was to investigate the structure of thickened endosperm cells where all the requisite conditions were present, and what was of greater importance still, where the pit membrane was extremely thick, and would be likely to show plainly the existence of threads traversing its substance.

Some results had already been obtained in this direction, for TANGL,* in 1880, in his paper on "Open Communication between the Cells of Endosperms," had shown that in *Strychnos Nux-vomica*, *Phœnix dactylifera*, and *Areca oleracea*, a communication between the protoplasm of neighbouring cells was established by means of fine protosplasmic threads running through the cell-wall. In *Strychnos* the walls were thick and devoid of pits, and the presence of the threads was not confined to any particular portions of the cell-wall, but they occur over the whole area. In *Phœnix* and *Areca*, on the other hand, it was by means of pits that the connexion was brought about; the pit membrane being perforated in a manner very much resembling that which takes places in a sieve-tube.

TANGL'S results with *Strychnos* were fully confirmed by STRASBURGER,† but in the case of *Phœnix* and *Areca*, he states that he was unable to see the threads with the clearness conveyed by TANGL'S figure, and although he says that the pit membrane of *Phœnix* is demonstrably porous, yet the general tone of his statements

* *Loc. cit.* † *Loc. cit.*

give one the idea that he has not been fully able to satisfy himself as to the structure by direct successful observation.

I then resolved to repeat for myself TANGL'S experiments, and also to investigate in as thorough a manner as possible the endosperm tissue of other species of Palms, and of other seeds of a similar nature. This work was carried on in the Jodrell Laboratory of the Royal Gardens, Kew, during the first three months of the present year.

Of the Order Palmæ I have examined the seeds of typical representatives of a great number of the genera, and I have, in addition, investigated the structure of the endosperm of members of the following Orders, viz.: Leguminosæ, Rubiaceæ, Myrsineæ, Cornaceæ, Loganiaceæ, Hydrophyllaceæ, Iridaceæ, Amaryllidaceæ, Dioscoriaceæ, Melanthaceæ, Liliaceæ, Smilaceæ, and Phytelephasieæ.

A mere glance at the foregoing list will be sufficient to show that a very large number of seeds were required, although from the great resources of the Royal Gardens I found no difficulty in obtaining typical representatives of any of the genera, and I cannot speak too highly of the great kindness I received on every side, from the Kew authorities, both in rendering me every assistance, and enabling me to obtain whatever material I was in need of for my investigation. Especially do I owe a debt of gratitude to W. T. THISELTON DYER, Esq., the Assistant Director, not only for the help I always received from him, but also for the kindly interest he took in my work all along.

Of the methods I employed I have already spoken in the earlier part of this paper. The usual plan I adopted was to cut with a razor, wetted with water, thin sections of the seeds, which were then stained with iodine and mounted in Chlor. Zinc Iod. Usually they could be examined at this stage; the exceptions being in those cases where the pit membrane rapidly assumed the blue cellulose coloration. After the prolonged action of Chlor. Zinc Iod. they were washed in water stained with picric-HOFMANN'S-blue, and after a second washing in water were mounted permanently in glycerine (strong or dilute) or glycerine jelly.

In certain cases some slight modification of this process had to be resorted to, which was occasioned by the peculiar characteristics of the tissues in question. Thus, for example, in such endosperms as *Strychnos* or *Tamus*, where great swelling takes place upon treatment with water, the sections were cut in alcohol, stained with alcoholic iodine, and after treatment with Chlor. Zinc Iod. were washed with dilute alcohol; stained, and mounted in strong glycerine after having been well stirred in glycerine, on taking out of the staining fluid, instead of washing with water, although usually quick washing with water will succeed equally well. Again, where the pit membrane was thin, and taking up water soon became coloured blue with Chlor. Zinc. Iod., and would only for a short time retain its primary yellow coloration: such tissue was also stained with dilute alcoholic iodine.

The strength of the iodine must be altered as the nature of the material requires.

Thus *Phytelephas* or *Lodoicea* require a strong solution of iodine, while *Ruscus* or *Colchicum* will quickly assume a dark yellow with a solution of a comparatively weak strength. Treatment with sulphuric acid was also resorted to, not only as an alternative method, but also for the purpose of confirming my results with pulvini; although, from the very great thickness of the walls and the consequent enormous swelling which occurs, it was found that, as a rule, Chlor. Zinc Iod. was the preferable reagent for ordinary use.

On repeating the observations already made upon *Strychnos Nux-vomica* my results fully confirmed those of TANGL and STRASBURGER in every particular; and in thin and carefully-prepared sections it can be plainly seen that the threads do cross the middle lamella. Like TANGL, I was unable to stain the threads with reagents in the usual manner; and, in consequence, I instituted those experiments which led me to adopt that particular modification of dissolving HOFMANN'S blue in picric acid, and using it as a stain, which I have already dwelt upon in the earlier part of this paper. When by the use of alcohol the extreme swelling which takes place upon treatment with water is prevented, sections may be stained with picric-HOFMANN'S blue, and after mounting in strong glycerine may be successfully observed.

With regard to *Strychnos potatorum*, I am disposed to agree with STRASBURGER that a sieve-plate-arrangement does exist between the pits, for a striation could certainly be made out. However, the seeds I had to work upon were extremely old, and as such I look upon the results obtained with them as unsatisfactory.

As regards the structure of *Phœnix dactylifera*, when treated with iodine and Chlor. Zinc Iod. I came to the same conclusions as Professor STRASBURGER* that, although a striation could be observed, the threads were not nearly so clear as TANGL'S drawing represents, and, indeed, were made out with difficulty. After treatment with sulphuric acid, washing, and then iodine-staining, they were defined much more clearly; but the best and in every way most satisfactory results were obtained by staining the washed-out sections with HOFMANN'S violet and glycerine. In the latter case the stained protoplasm was contracted, and running through the pit membrane could be seen well-coloured threads presenting a distinct sieve-plate-arrangement (Plate 69, fig. 13).

In the same way *Areca oleracea*, usually known as *Euterpe oleracea*, at first gave a very feeble result when treated in the usual way; but, after a great number of trials and the use of strong iodine, and a prolonged action of Chlor. Zinc Iod., delicate threads could be plainly observed, which, moreover, appeared to cross the little developed middle lamella.

Having obtained the above-named results I commenced the examination of a number of palm seeds and of other seeds possessing a similar structure, in the hope of being in the end able to make some statements as to their general histology and to determine how far such a structure was of general occurrence. The following is the list of the palm seeds examined. I am indebted to Sir JOSEPH HOOKER for kindly

* *Loc. cit.*

looking over this list for me and not only making several valuable alterations, but also adding the authority for each species.

Arecineæ.
- Areca triandra. Roxb.
- Areca Catechu. L.
- Stevensonia grandifolia. Duncan.
- Rhopalostylis sapida. W. and D.
- Howea Belmoreana. Ben.
- Kentia costata. Ben.
- Archontophœnix Cunninghamii. W. and D.
- Euterpe oleracea. Mart.
- Euterpe edulis. Mart.
- Hyophorbe Verschaffeltii. Wendl.
- Synechanthus fibrosus. Wendl.
- Didymosperma distichum. H.f.
- Pinanga latisecta. Bl.
- Heterospathe elata. Scheff.
- Caryota urens. L.
- Manicaria saccifera. Gærtn.
- Cyrtostachys Renda. Bl.
- Calyptrogyne Swartzii. H.f.
- Calyptrocalyx spicatus. Bl.
- Chamædorea tinella. Wendl.
- Prestœa pubigera. H.f.
- Ceroxylon andicola. H. and B.
- Oncosperma horridum. Seem.

Lepidocaryeæ.
- Calamus calicarpus. Griff.
- Mauritia flexuosa. Linn f.
- Calamus fissus. Bl.
- Plectocomia Himalyana. Griff.
- Pirgafetta elata. Becc.
- Raphia Hookeri. M. and W.

Borasseæ.
- Latania Loddigesii. Mart.
- Lodoicea Sechellarum. Mart.
- Geonoma vaga. Griseb and Wendl.
- Bentinckia Conda-panna. Berry.

Coryphecæ.
- Thrinax, sp.
- Corypha elata. Roxb.
- Licuala Rumphii. Bl.
- Livistona Hoogendorpii. T. and B.
- Washingtonia filifeva. Wendl.
- Sabal umbraculifera. Mart.
- Rhapidophyllum Hystrix. W. and D.

Cocoineæ.
- Cocos nucifera. L.
- Cocos flexuosa. Mart.
- Bactris, sp.
- Astrocaryum rostratum. H.f.
- Syagrus botryophora. Mart.
- Martinezïa Aiphanes. Kl.
- Maximiliana caribœa. Gr. and W.
- Desmoncus, sp.
- Martinezïa caryotifolia. H. and K.
- Guilelma speciosa. Mart.
- Diplothemium, sp.

Phytelephasieæ.
- Phytelephas macrocarpa. R. and P.

Phœniceæ.
- Phœnix dactylifera. L.

In all the above seeds a direct means of communication between the cells of the endosperm was observed.

Confining myself at first to the Palmæ, I would point out that in their structure the various seeds present every possible modification both of thickness or thinness of the pit membrane, of clearness or difficulty of observation, of variations in the size of the cell, and in degree of development of the middle lamella.

In making the subsequent observations with reference to the study of a number of instances of one and the same phenomenon, I propose to deal with the subject in a somewhat general manner and to illustrate my statements by such typical examples as will best serve my purpose.

I. *Development.*—In no case have I worked out the development of any of the seeds that I have examined. On account of want of time and opportunity, it is, there-

fore, a subject which must be reserved for another occasion. I would only draw attention here, to the striking similarity which the arrangement of the protoplasmic threads joining the cells of such endosperms as *Strychnos*, *Tamus*, or *Dioscorea* presents to the same arrangement of achromatin fibres which accompanies the development of the similar structure in *Agrimonia Eupatoria*,* and the close resemblance of the barrel form, so beautifully shown by *Heterospathe*, *Bentinckia*, or *Lodoicea*, to the like form assumed by the fibrillæ between the dividing nuclei in such endosperms as *Caltha palustris*,† or, indeed, in cell division in general. As TANGL‡ remarks, it seems as if the fibrillæ persisted during the subsequent cellulose formation and deposition. The appearance, perhaps, suggests that such is the case, and that the particles of cellulose have been deposited around the threads. It may also be noticed that no instance of a reticulate arrangement of the threads has been observed. In any case it is apparent, of course, that grave alterations must be occasioned by subsequent growth and increase in size of the cells, but anything certain development alone can decide.

II. *Structure of young endosperm cells.*—A number of observations were made upon the young endosperms of *Archontophœnix Cunninghamii*, *Sabal umbraculifera*, and *Rhopalostylis sapida*. In all these cases it was found that when the cell was still living, as could be seen from the presence of a well-developed nucleus, the connexion between the cells was fully maintained, and therefore that communication had existed in any case from a very early period (Plate 69, figs. 14 and 15).

As the cells grow older profound changes take place in the protoplasm, which usually result in the death of the cell. In order to ascertain the fate of the nucleus, portions of ripening seeds of *Archontophœnix elegans* and *Rhopalostylis sapida* were treated for twenty-four hours with saturated watery picric, and well washed with alcohol, until the yellow colour of the acid had quite disappeared. Sections were cut, which were stained with hæmatoxylin, and mounted in dilute glycerine. It was then apparent that well stained nuclei were present in the cells occupying the central portion of the seed (Plate 69, fig. 15), and as one gradually traced the staining effects from within, outwards, it was seen that the nearer the periphery, the less conspicuous became the cell-nucleus, until in the outermost layers no trace of a nucleus could be detected; its substance staining less and less, and its outline becoming more and more badly defined. Thus it apparently suffers a complete disorganisation.

Along with changes of the nucleus proceed alteration of the protoplasm. In many seeds—*e.g.*, *Phytelephas*—but little protoplasmic substance appears to remain in the cell. Oil very frequently occurs as a cell content, and sometimes is present in large quantities, especially in the Cocoineæ—*e.g.*, *Cocos*, *Bactris*, &c. Small crystals may also occur, and in such examples as *Diplothemium*, *Syagrus*, and *Corypha* aleurone grains are met with. In the cases which I examined with special reference to the

* STRASBURGER, 'Zellbildung und Zellthilung,' Tafel I., fig. 15. † *Loc. cit.*, Tafel II., fig. 31.

‡ *Loc. cit.*

question the cells appeared to be quite dead, and as such they are simply preyed upon by the growing embryo. Thus, all the changes which result in their subsequent breaking down proceed from the embryo itself.

Special experiments were made with sulphuric acid, in order to observe its action in cases where a continuity was known to exist. In fresh living cells treatment with sulphuric acid, and staining with methyl violet and glycerine or HOFMANN'S blue, showed that, although the protoplasm had contracted, those portions projecting into the pits still adhered to the pit membrane, and that the threads of protoplasm running through the pit membrane were continuous on either side with the above-mentioned symmetrically opposite processes (figs. 14 and 15). The processes, in fact, appear to be held to the pit membrane by the threads in question in all cases where the continuity is pronounced. Under a low power the individual threads could not be distinguished, and the appearance then presented was that of two darkly-stained threads united by a lighter-stained area running between them—in fact, the very appearance presented by *Mimosa* and *Robinia** (*cf.* figs. 5, 7, 14, and 15). In the case of ripening seeds, the protoplasm may be made to contract slightly from the membrane, and then a similar phenomenon is induced to that which occurs in *Amicia* and the bast cells of *Mimosa* (*cf.* figs. 11 and 13), although in them it is not occasioned by loss of vitality, but rather from the fact that the threads are probably extremely fine and the continuity not so pronounced as it is in the case of the parenchyma cells. In fully-ripe seeds where the cells are dead, the protoplasm always contracts away from the cell-wall, and a similar state of things usually occurs when the cell has been killed by the action of reagents (see figs. 16 and 22). Thus, both my method and my results have received very satisfactory confirmation and elucidation.

III. *General results with ripe endosperms.*—As a rule, most of the seeds I examined were either one or, at most, two years old. I also made use of some museum specimens, but decided to reject the results I obtained with them, as I had reason to believe that in many cases those results were abnormal. As regards their favourable or unfavourable character as material for showing the perforation of the cell-wall by protoplasmic threads, seeds greatly differ one from another. In the first place, it may be stated, as a general rule, that the thicker the pit membrane the easier can the threads be distinguished. In very thin pit membranes the observation of such threads as may cross it requires great precaution and care; there is nothing, so to speak, for the eye to catch upon, and one has to detect a line within a line. It is this very fact that causes endosperm tissue to be so favourable for such an investigation as the present one; for here, not only, as a rule, are both the cells and the pits unusually large, but, what is much more important, the pit membranes are thick. In many cases, however, this is not the case, and an examination of such examples as *Manicaria*, *Mauritia*, or *Caryota* is quite sufficient to prove that the

* The results with *Bomarea* also confirm this.

successful observation of the threads crossing a thin pit membrane is a matter of extreme difficulty; and it also serves to show that in other cases where the pits are very small and the membranes very thin this difficulty is so increased as to become almost an impossibility.

But apart from any consideration of the pit membrane, the ease or difficulty of observation also appears to depend greatly upon the peculiar characteristics of the seed itself. For instance, as I stated at the outset, *Phœnix dactylifera* and *Euterpe oleracea* are inclined to be unfavourable material. In *Euterpe edulis*, on the other hand, the connecting threads can be easily demonstrated. Of numerous other instances, *Geonoma*, *Plectocomia*, *Areca triandra*, *Areca catechu*, and *Cocos nucifera*, afford examples of cases where difficulties of observation occur.

Among the most favourable material for examination are the endosperms of *Bentinckia*, *Stevensonia*, *Thrinax*, *Heterospathe*, *Syagrus*, *Corypha*, *Howea*, and *Lodoicea* (see figs. 16, 17, 18, 22, and 25). The degree of development of the middle lamella varies greatly. As a rule, in thickened endosperms it attains but little development, or, even if this be not the case, it stains but little, and its refractive index varies only slightly from that of the general cell-wall. In *Calamus*, *Sabal*, *Raphia*, and *Ptychosperma*, the lamella is decidedly pronounced. In such cases as *Stevensonia* and *Calamus* (Plate 69, fig. 24) both the middle lamella and the threads are well developed; and though in them there is some difficulty in determining whether the middle lamella is actually perforated by the protoplasmic threads, yet, as a rule, careful examination and preparation will decide that in the vast majority of cases it can be seen that such perforation does occur, and such examples as *Heterospathe*, *Kentia*, *Mauritia*, or *Bentinckia* do away with all possible doubt (figs. 16, 19, 22, 23). As to the manner in which the communication between the endosperm cells is established, experiment shows that there are two possible ways which essentially depend upon the configuration of the cell.

In such exceptional cases as *Strychnos*, *Tamus* (Plate 70, fig. 33), and *Dioscorea*, where the walls are extremely thick, and, at the same time, devoid of pits, the communicating protoplasmic threads run through the cell-wall. A section of such an endosperm exhibits the threads, which are seen freely perforating the wall, except at the corners of the cell, at the point where the junction of several cells occurs.

The usual mode of union, however, is by means of pits. As I have mentioned elsewhere, the presence of pits in the cell-wall, due to unequal thickening, is of almost universal occurrence, and it is through the closing membranes of such pits that the protoplasmic threads run. This, in fact, appears to be by far the most common and typical way in which the continuity of the protoplasm of adjacent cells is brought about.

In other, and perhaps less frequent cases, examples of both modes of connexion occur; the communication taking place not only through the pits, but through the

walls as well. I have observed that this happens in *Kentia Belmoriana, Kentia costata, Lodoicea, Bentinckia,* and *Asperula* (see figs. 16, 17, 19, 31); but I am led to believe that such union is of much more general occurrence. In all the foregoing examples, the threads running through the walls are more especially obvious in the cells just below the surface, and gradually become less and less visible, as one approaches the central tissue of the seed. This appears to me to be simply an arrangement for insuring that every facility should be given for the passage of nutritive material from without inwards, and also that it should have opened to it as many channels as possible. It is obvious, for instance, that the amount of plastic formative substance required for building up such a tremendous endosperm as that of *Lodoicea* must be very considerable, and even supposing its growth to be slow, the drain on the nutritive material must be large, and the rate of its flow must be very great. Consequently the increased facilities for easy transmission must be of great advantage. And not only in the development of the endosperm, but also upon germination, is this structure of great use to the plant, for at that period the outer cell-layers will have become very dry, and consequently the difficulty of their being broken down by the absorbent foot of the cotyledon will be increased. But at the same time, owing to the greater development of a system of channels in them, they are more easily permeated and wetted by the cell sap holding in solution the ferment which will bring about their final disorganisation.

The form presented by the aggregate of threads traversing the pit membrane is usually that of the well-known basket or barrel-shape which is met with in connexion with nuclear division. In many instances, and especially in *Bentinckia,* the shapely sweep of the curving threads, and the graceful arrangement of the whole thread-complex is extremely striking and beautiful (Plate 69, fig. 16). In other cases the bending of the curve is not so marked, and in very thin pit membranes—*e.g., Synechanthus, Livistona,* &c.—the threads appear to be altogether straight.

In the instances where the threads go through the cell-wall their direction is seldom straight, but usually bent, and resembling in arrangement the appearance presented by the achromatic fibres during free-cell-formation.

Every variation occurs both as regards the size of the cells, the distribution of the pits, and the number and thickness of the threads. Thus, whereas the cells of *Caryota urens* and *Lodoicea* (Plate 69, fig. 19) are large, those of *Thrinax* and *Geonoma* are small. In such endosperms as *Manicarea* and *Chamædorea* the pits are very numerous, while in *Washingtona* but few are present. In *Calyptronoma* the threads are few and somewhat stout, while in *Oncosperma* they are very numerous and fine. In *Bentinckia* and *Heterospathe* they are also many in number. The threads are made very conspicuous by staining with iodine and treatment with Chlor. Zinc Iod., for the latter reagent appears to cause a decided precipitation of iodine upon them as well as upon the general protoplasm which is accompanied by an increase in

apparent diameter. That this is actually the case may be demonstrated either by reversing the operation and staining with iodine after treatment with Chlor. Zinc Iod. and subsequent washing, or by staining with picric-HOFMANN'S-blue. (Compare Plate 69, figs. 22 and 23.)

As a rule nothing can be seen of the threads when a section of endosperm tissue is mounted and stained in the usual manner. But to this statement *Bentinckia* affords an exception, for here an appearance of striation can be detected, and in *Stevensonia* staining with HOFMANN'S violet alone makes the threads apparent. Treatment with iodine, picric acid, or with a mixture of iodine and glycerine will also often bring them into view, *e.g.*—*Lodoicea* (Plate 69, fig. 20), *Latania*, and *Bentinckia*.

Experiments with the object of injecting the threads with colouring solutions met with no success. Pieces of the endosperms of *Latania* and *Calamus* were fitted into a bored india-rubber cork, which was then tightly fastened into one end of a manometer tube, the shorter arm of which contained the solution of the colouring matter, and the longer held the mercury by means of which the injection-pressure was induced. First, a solution of water-blue in water was employed, and as this caused swelling of the wall a solution of insoluble blue in alcohol was used in preference. However, when exposed to the pressure of a column of mercury of sixty inches no injection occurred.

Besides the particular methods I have chosen for the elucidation of this subject, many others were tried with little or no success. Sections of *Bentinckia*, as being favourable material, were treated in the usual way with solutions of gold chloride and silver nitrate, but with no result. In every case it was found that it was necessary to swell the cell-wall before staining. After swelling with Chlor. Zinc Iod. and washing, silver nitrate was again tried, and this time with some small amount of success. I adopted a modification of treating the section with sulphuretted hydrogen-water, after exposure in a 2 per cent. solution of silver nitrate for half-an-hour, and subsequent washing, instead of reducing the silver by the action of light, as in the usual process. The result was perhaps better, but still far from satisfactory, and would be quite inapplicable in any case where the threads were not particularly well developed. Some sections, after swelling, were treated with an alcoholic solution of tannin, and when washed were shaken up with a solution of ferric chloride. The wall then coloured the usual blue-black, and the colourless threads could be seen fairly well. Other sections, again, were soaked in a solution of ferric chloride, and after washing were treated with a solution of potassium ferrocyanide. In this case the threads were less clearly defined than with tannin and iron. Lastly, sections were treated for some time with a solution of corrosive sublimate, in the hope that an insoluble compound might be formed with the remains of the cell protoplasm. After washing the section was shaken up in sulphuretted hydrogen-water, but with no good result. Thus all these experiments pointed to the fact that my methods, if not perfectly satisfactory, were at least fairly successful.

In concluding the subject of Palm endosperms I might make a few remarks upon some particular examples which appear to be of equal interest.

In all the seeds nearly related to *Calamus* the structure is very typical (Plate 69, fig. 24). I have already noticed the great development of the middle lamella in these endosperms. Another interesting fact is, that in *Calamus* and *Metroxylon* a well-marked cubical crystal is present, imbedded in the wall of each cell. It seems as if there had been a period in the life of the cell when the protoplasm had required to get rid of some of the calcium oxalate resulting from the metabolic activity of the protoplasm. This was consequently thrown down in the form of a crystal which adhered to the cell wall, and in the subsequent thickening which occurred, was gradually covered in until it was at length surrounded on all sides by the cellulose.

Lodoicea sechellarum is of interest, not only as affording one of the clearest examples of the perforation both of the wall and pit membrane, but also because of its very unique distribution (Plate 69, fig. 19).

In *Oncosperma* the threads are excessively fine, and certainly suggest the extreme probability of the existence of threads which are so delicate as to be invisible (Plate 69, fig. 21). In fact, I am inclined to believe that this really is so in such endosperms as *Cocos nucifera*. To this seed *Martinezia caryctifolia* presents a useful transition. With iodine it can be seen that very fine threads do go through the almost smooth walls, but upon treatment with picric-HOFMANN'S-blue the individual threads cannot be distinguished, and only a blue coloration occurs. In *Cocos*, which has essentially the same structure, I was unable to observe threads, though I cannot doubt that such threads do exist. In all the *Cocoineæ* the walls are thin and must be carefully examined. They are, however, of extreme value, both from the point of view of analogy and comparison. In *Syagrus* (Plate 69, fig. 25) and *Desmoncus* the threads are well seen. *Heterospathe elata* is a particularly favourable endosperm for demonstrating the perforation of the middle lamella, which here is but little developed (Plate 69, figs. 22 and 23). The threads appear very clearly with iodine. In *Phytelephas*, although the walls are extremely thick the pits are small, the pit membrane somewhat thin, and the threads are demonstrated with difficulty. The cells contain but little remains of the protoplasm, and several results have induced me to think that the amount of solid matter in the perforating thread channels is so small that the channels are practically empty (Plate 70, fig. 26).

These results seem to show that in all the Palmæ the structure of the endosperm cells is similar.

Endosperms other than those of Palms.—As I have remarked elsewhere, the endosperm of Palm seeds is particularly favourable material for an investigation of the perforation of the cell wall by protoplasmic filaments. And, speaking generally, in the examination of most endosperms other than those of the Palmæ, additional difficulties are presented which greatly interfere with successful observation. Especially does it become apparent that the thickness of the pit membrane is not

nearly so great, and this fact both increases the difficulties of making out the threads, and in consequence of the rapid blue coloration of such a thin membrane causes the observations with iodine and Chlor. Zinc Iod. frequently to be almost valueless and often an impossibility. It is in such cases that my staining method comes to be so important.

Often it would seem that the threads are so excessively fine that they cannot be resolved as separate filaments, and the appearance presented by the whole aggregate of threads crossing the pit membrane is simply that of a blue coloration. In this direction the results with *Bomarea* are of special interest, as they tend to give weight to the view that my experiments have led me to adopt, viz.: that a well defined blue coloration, after the action of Chlor. Zinc Iod. and picric-HOFMANN'S-blue, points to the presence of protoplasmic threads in the cell-wall.

Professor STRASBURGER * states, in the case of the endosperm cells of *Ornithogalum* and in the pith cells of *Taxodium*, that the pit membranes are demonstrably porous, and that a striation can be observed crossing the membrane upon action with iodine and Chlor. Zinc Iod. He also represents this striation in figs. 17, 18, 19, and 23, Tafel I.; and again in fig. 23, Tafel II., he shows that a similar striation may be seen in the closing membrane of the pits in the thickened cells of the seed-coat of *Viscum*.

As far as regards *Ornithogalum* I can fully confirm his results, and the fact of the existence of a similar structure in *Taxodium* and *Viscum* is one of great value.

Staining with picric-HOFMANN'S-blue, subsequent to the action of iodine and Chlor. Zinc Iod., will demonstrate in the case of *Ornithogalum* that the pit membrane is distinctly blue, while the rest of the cell-wall is practically colourless, and will also bring out more clearly the striation of the pit membrane, due to the presence of threads. This, however, is by no means a favourable case.

Sections of the endosperm cells of *Bomarea oligantha*, after swelling and staining, gave me good results. If examined in a somewhat cursory manner it is at once observed that the pit membrane is well coloured and distinctly delineated from the rest of the cell-wall (Plate 70, fig. 27). In some instances it can be observed that instead of the whole pit membrane being uniformly coloured, it may be traversed by one or two coloured bands which run through the otherwise colourless substance of the membrane (refer to figure). In favourable instances well defined striation can be seen. In an *en face* view of the pits it becomes evident that the pit membrane exhibits essentially the same appearance as that presented by a sieve-plate (Plate 70, fig. 28), and the appearance of the two coloured bands is explained from the fact that in some instances the whole membrane is not necessarily perforated, but that the perforation and hence the sieve-plate structure may be confined to particular areas of the membrane of the pit. This particular distribution of perforating areas also explained the appearance of bifurcation, which is sometimes presented by the apex of much contracted protoplasmic

* 'Bau und Wachsthum,' p. 16, *et seq.*

processes when pulled out of the pit cavity in consequence of the action of strong sulphuric acid, or other dehydrating agents.

The staining results with *Bomarea* appear to me to give great support to the idea that a pronounced coloration of the pit membrane by picric-HOFMANN'S-blue after the action of iodine and Chlor. Zinc Iod. gives evidence of the presence of protoplasmic threads in the cell wall and therefore of perforation.

In *Ruscus*, although the cells are large, the pit membranes are very thin and quickly coloured with iodine and Chlor. Zinc Iod. After staining in the usual manner fairly well defined threads can be seen (Plate 70, fig. 29). The same is the case with *Iris* and *Xiphium*.

Colchicum is a particularly plain example of perforation of the pit membranes, which are here somewhat thick. Both with iodine and with HOFMANN'S blue the individual threads are easily distinguished (Plate 70, fig. 30). Of Dicotyledons exhibiting a similar structure, *Ardisia polycephala* is an example of some interest on account of its peculiar reaction with iodine. With a dilute solution of this reagent the substance of the cell-walls give a blue reaction, exactly resembling that of starch. Stronger solutions rapidly cause a dark brown coloration. The seeds of *Ardisia crenulata* behave in the same way. The same has been observed in the seed of *Pæonia*, and has been long known in the case of the phlœm of *Lycopodium;* the so-called fungus cellulose ; and (when the iodine solution is of a certain strength) in mucilage cells. In *Nemophila*, although the cells are small, an appearance of striation is plainly evident (Plate 70, fig. 32). The structure of the horny seeds of certain of the *Rubiaceæ, e.g., Coffea, Galium*, and *Asperula* (Plate 70, fig. 31) is of some interest. The cell-walls present a somewhat crumpled appearance, and there is no definite arrangement in their shape. After treatment with strong iodine and a lengthy action of Chlor. Zinc Iod., a system of fine threads is clearly brought into view. Where the wall is pitted, the threads go through the pits, or, if not, through the thick wall, as the case may be. This was observed in *Asperula* only. The rest were not examined in detail.

The structure of the seeds of *Tamus* and *Dioscorea* are very important as affording additional confirmation of TANGL'S results with *Strychnos*. The thick walls of these seeds present no pits, and are of the same transparent horny nature as those of *Strychnos Ignatia*. After treatment with iodine and Chlor. Zinc Iod. the very numerous threads which freely perforate the entire thickness of the cell-wall gradually come into view, and resemble in both arrangement and properties those of *Strychnos*. The fact that the threads cross the middle lamella is even better demonstrated in *Tamus* than in the former instance, for here the development of the lamella is not so great. The cell-walls soon swell very strongly, and in so doing the threads are broken up into a number of points, as TANGL has observed, and in the swollen portion of the wall at last become invisible (Plate 70, fig. 33). In *Dioscorea* the threads are much finer than in either *Strychnos* or *Tamus*. In both instances threads can be observed uniting all

the cells, including of course those directly below the surface. In this respect they differ from *Strychnos* as far as their demonstrable character goes.

In such of the *Cæsalpiniæ* as possess endosperms a similar pitted structure of the cells occurs. The existence of threads was observed only in *Bauhinia*, but I cannot doubt that other leguminous seeds of the same structure will show the same occurrence of threads, *e.g.*, *Sophora Japonica* and *Gleditchia* mentioned by Von MOHL.*

Subjoined is a list of the seeds examined. In those whose names are printed in italics it was actually observed that there was a protoplasmic continuity from cell to cell. The rest were not examined in detail.

Leguminoseæ.
- *Bauhinia variegata.*

Rubiacea.
- *Asperula odorata.*
- Galium aparine.
- Galium spurium.
- Coffea Arabica.
- Sherardia arvensis.

Myrsineæ.
- Ardisia crenulata.
- *Ardisia polycephala.*

Cornaceæ.
- *Aucuba Japonica.*

Loganiaceæ.
- *Strychnos Nux-vomica.*
- *Strychnos Ignatia.*
- *Strychnos potatorum* (?)

Hydrophyllaceæ.
- Nemophila discoidalis.
- *Nemophila parviflora.*
- Phacelia pimpernelloides.

Melanthaceæ.
- *Colchicum speciosum.*

Liliaceæ.
- Asparagus officinalis.
- Asparagus sp.
- *Ornithogalum tenuifolium.*
- *Ornithogalum narbonense.*
- Yucca, sp.

Smilaceæ.
- Polygonatum Japonicum.
- *Ruscus aculeatus.*

Iridaceæ.
- *Iris pseudacorus.*
- *Xiphium vulgare.*
- Iris ochroleuca.

Dioscoreaceæ.
- *Dioscorea dæmonorum.*
- *Tamus communis.*

Amaryllidaceæ.
- *Bomarea oligantha.*

The above results have established not only that protoplasmic threads do perforate the cell-wall, and thus bring adjacent cells into communication with one another, but that such perforation is of very frequent occurrence. My results with endosperm ells have fully confirmed those which I obtained with pulvini, and have both elucidated the structure that occurs in those organs, and given every support to the methods that I employed in their investigation. It would thus appear that not only in the endosperms of Palms, but in those of other plants in general, the cells are placed in connexion one with the other. It may be objected that I have used thick walled endosperms in every instance. I gave my reasons for so doing, and although I have not as yet examined the structure of thin walled endosperm cells, I have but little doubt that the same means of communication takes place in them also, for every range of difference of thickness of the cell-walls occurs, not only in the same order but

* Von MOHL, 'Vegetable Cell,' English translation, p. 33.

in families of that order, that differ but little one from another. Russow's results are also of especial value here.

Results with Plasmolysis.—At an early stage in this investigation certain phenomena in connexion with experiments upon the preservation of tissues forced themselves upon my notice. What was especially striking was the different result which was obtained when different tissues were treated with the same reagent, and under the same conditions.

Thus, upon examination of sections of the pulvini of *Mimosa*, *Robinia*, and *Amicia*, which had been all carefully preserved in absolute alcohol, it will be seen that the degree with which the protoplasm is contracted from the cell-wall varies greatly in the three cases. In the cells of *Mimosa* the protoplasm will have undergone but little contraction, and the whole tissue will show signs of successful preservation. In *Robinia*, on the other hand, an appreciable contraction has evidently taken place, and in *Amicia* this state of things has attained a maximum, for almost every cell exhibits the much shrunken protoplasm lying freely in the cell cavity, and separated on all sides from the cell-wall. Since in every instance the cells are full grown, and are under equal conditions, it would seem probable that the protoplasm is held closer to the cell-wall in some cases than in others.

After having obtained my results with *Mimosa*, *Robinia*, and *Amicia*, it seemed the more probable that the above appearances were in reality a consequence of the intimate union between the cell-wall and protoplasm which my investigations had shown to exist, and the pronouncedness of which appeared to vary.

In consequence of these and other considerations, I was led to study, in a detailed manner, the effect of plasmolysing such cells, since it seemed to be almost certain that the phenomena accompanying such a condition would afford additional confirmation of the results I had already obtained with somewhat powerful reagents.* According to De Vries† when the plasmolytic condition is induced in a cell by means of dilute dehydrating agents, the protoplasm (primordial utricle) separates entirely from the cell-wall, and appears as a much contracted vesicle lying freely in the cell cavity.

On the other hand, both Pringsheim‡ and Nägeli§ had noticed that in certain cases the protoplasm appears to separate with some difficulty from the cell-wall, and that it was frequently connected to it by means of one or more threads in those cases where great contraction had taken place.

It had also been long known that in filamentous *Algæ*,‖ the protoplasm upon contraction is often connected to the cell-wall by threads. These, however, may be rather described as isolated cases, for no generalisations were made, nor was any

* Proc. Roy. Soc., Nov. 11, 1882.

† 'Untersuchungen über die Mechanischen Ursachen der Zellstrehung.' Leipzic, 1877.

‡ 'Bau und Bildung der Pflanzenzelle.' 1854.

§ 'Pflanzenphysiologishe Untersuch.' 1855.

‖ Hofmeister, 'Die Pflanzenzelle.' 1867.

particular attention drawn to the fact; on the contrary, it has been generally accepted that on plasmolysis the protoplasm is quite free from the cell-wall.

However, in repeating these experiments I find that in all the cases I have examined the contracted protoplasm is always connected to the cell-wall by means of very numerous protoplasmic threads.

The above phenomena were also discovered subsequently and independently by BOWER,* whose excellent paper on the subject appeared shortly after my own. My experiments were first made upon pulvini, but were afterwards extended to tissues in general (figs. 34, 35, 36, 37).

The most detailed observations were made upon transverse sections of the pulvini of *Amicia zygomeris* and *Robinia pseudacacia*, after treatment with 2·5 per cent., 5 per cent., and 10 per cent. solution of sodium chloride, but since the results obtained in other cases differ so little, one may describe the phenomena which accompany plasmolysis in general terms.

If a dilute solution of salt be employed, *e.g.*, 2·5 per cent., the protoplasm will gradually contract away from the cell-wall, and will at length frequently appear to lie quite freely in the cavity. In other cases the protoplasm will adhere to the cell-wall at certain points. But if the section be examined for some time, it will be seen that delicate strings of protoplasm will gradually come into view, and increase in number, until at length the contracted protoplasmic mass will present the appearance of a sphere suspended in the cell cavity by innumerable fine protoplasmic strings (Plate 70, fig. 37).

If contraction be rapidly brought about by means of a stronger solution, *e.g.*, 10 per cent., it will be observed that the protoplasm experiences some difficulty in separating from the cell-wall, and may even become divided up during the process into two or more portions (Plate 70, fig. 35), each of which rapidly assumes a spheroidal shape; also several somewhat thick threads may be seen connecting the protoplasm to the cell-wall or the protoplasmic masses to one another (figs. 35 and 36). Subsequently the finer threads come into view. I am inclined to believe that it is these thicker threads which have been hitherto seen, and that the finer threads have, up till now, escaped observation; and although, as BOWER† remarks, the difference between the thicker and the finer threads is only one of degree, yet the importance of the observation is in no way diminished thereby.

The thicker threads frequently present nodal swellings of a perfectly spherical form. These spherical nodes may either abut on to the cell-wall or may occupy any other position upon the thread. When, by chance, rupture of the threads occurs, part contracts to the central protoplasmic mass, and part forms a small sphere on the side of the cell-wall.

The first indication of the existence of the fine threads is afforded by an appearance

* Quart. Jour. Micr. Sci. Jan. 1883.

† *Loc. cit.*

of striation, which gradually becomes more and more defined until distinct threads can be observed. At first the diameter of the threads gradually diminishes from the protoplasm to the cell-wall, so that it is impossible to trace the thread over the whole of its course (Plate 70, fig. 40); but after some time it comes more clearly into view, until at length it is apparent that it extends right up to the wall in question.

The thickness of the threads varies greatly. Up to a certain point, more and more threads come into view the longer the cell is observed, until at length the appearance presented will be that of a central contracted sphere of protoplasm from which radiate out to the cell-wall numerous fine threads, some of which are of an appreciable size, other smaller though still well defined, and others so difficult to see that their presence is only indicated by a faint striation traversing the space between the protoplasm and the cell-wall.

The phenomenon of the gradual definition of the threads appears to suggest that a thickening of their substance has taken place, and as BOWER * has observed, this in reality does occur.

He has seen also that the nodal swellings appear to travel from the protoplasm to the cell-wall, and is of opinion that the thickening of the threads is due in a great measure to a drawing out of fresh substance from the main protoplasmic body. He also suggests that lateral coalescence of the strings may occur. My view of the case, however, differs from his. It is certain that at first the protoplasm quickly contracts, owing to the rapid diffusion which occurs. The water diffuses from the cell vacuole into the salt-solution, much more quickly than the salt-solution diffuses into the water, so that the contraction of the protoplasm reaches its maximum when it has lost the greatest amount of water. After a time osmosis ceases, but not until the strength of the fluid, both inside and outside of the protoplasm, is the same. And in the subsequent equilibrium which occurs, the protoplasm, which had before suffered an abnormal contraction, owing to the rapid loss of the water it had contained; now takes up in exchange a small quantity of the salt-solution, and the ultra shrinking (so to speak) is relieved,† and a definite swelling of the protoplasm takes place. Thus the tension on the threads is no longer so great, and, owing to their elastic character, they thicken up and are thus brought into view. Subsequently they cease to thicken, and by the time the shrunken protoplasm has regained its equilibrium they become quite lax. Both BOWER and myself have observed that, after some length of time has elapsed, the threads execute lateral vibrations which are possibly caused by currents due either to diffusion or to temperature.

It seems probable that the action of the salt-solution, unless very dilute, causes grave changes to take place in the protoplasm. Ordinary cells do not give much evidence of this, since on washing out with water they regain their usual appearance.

* *Loc. cit.*

† It loses, in fact (if I may be allowed to use the expression), some of its water of constitution, and takes up in its stead the salt-solution.

If, however, *Spirogyra* cells be plasmolysed, it will be seen that the whole structure has been much affected, for the chlorophyll bands will no more resume either their accustomed appearance or arrangement, and a general swelling of the cell takes place.

The strings of protoplasm which normally traverse the cell vacuole in ordinary living cells frequently exhibit the same appearances as those which are presented by plasmolysed threads, and nodal swellings may also occur. I have observed this particularly well in the hypodermal cells of potato tubers.*

I have also frequently noticed that as a result of plasmolysis many chlorophyll grains will tend to aggregate around the nucleus as if some connexion with the latter existed, such as PRINGSHEIM observed in *Spirogyra*.†

The point of special interest to me was to ascertain whether these threads bore any relation to the pits. As I stated in my paper before the Royal Society, I have observed several well defined instances in which threads do go to pits, and in Plate 70, fig. 34, which is a made-up figure embodying in one representation the results of numerous individual cases, I have attempted to illustrate such appearances. In one instance, where plasmolysis had been quickly induced by means of a strong salt solution, two spheres of protoplasm occupied the two opposite pit depressions, from each of which a thread ran to the main protoplasmic mass. However, numerous experiments have convinced me that no reliance can be placed upon the results obtained by plasmolysis, as giving any certain clue to the existence of protoplasmic continuity. With this opinion BOWER also agrees.‡ In fact, the greater proportion of threads bear no relation to pits, and in such an experiment as plasmolysing a hair of *Primula sinensis*, it is seen that as many threads go to the longitudinal as to the transverse walls, and are thus present on the free walls, as well as those separating contiguous cells (see also Plate 70, fig. 39).

As I mentioned in the earlier part of my paper, my efforts to fix and stain these plasmolytic figures did not meet with perfect success, although picric acid gave very satisfactory results. I am, however, inclined to think that additional shrinking was produced by the use of glycerine, and the method deserves another trial. As a result of the staining, both the threads and the protoplasm were well brought into view, but a very great proportion of the threads were ruptured, and appeared as little spheres attached to the cell-wall. I was unable to trace the protoplasm into the cell-wall, but at that time I had not adopted my plan of staining with picric-HOFMANN'S-blue. It is possible that with this reagent some results may be obtained. In my paper before the Royal Society,§ I stated that I had succeeded in showing the passage of the protoplasm through the cell-wall when the wall was left intact, and not swollen by reagents; the method consisting in treating thin sections of fresh material with saturated picric

* See figure of cell of hair of *Cucurbita*. SACHS' 'Vorlesungen,' p. 752.

† PRINGSHEIM, 'Uber Lichtwirkung und Chlorophyllfunction.' Leipzic, 1881. Tafel XIV., fig. 4.

‡ *Loc. cit.*

§ *Loc. cit.*

acid. There are two mistakes in that statement. First I should have said "treating plasmolysed sections:" and what is of more importance, I am inclined to believe that my observations were not perfectly trustworthy. I had two particularly plain instances of an apparent passage of protoplasm through the cell-wall, one of which I have represented in Plate 70, fig. 38. Although it still seems perfectly clear and plain, I am almost convinced that some abnormal appearance has been produced, either by distortion of the section, or owing to the fact that, intersecting the two coloured protoplasmic threads, are thin pit membranes which I cannot resolve.

As regards plasmolysis, numerous tissues were examined, and in all the same occurrence of strings was observed. Both BOWER and myself believe that the phenomenon is universal. As definite instances where actual observations were made I may mention the pulvini of *Mimosa, Phaseolus, Rhynasia, Oxalis, Biophytum, Apios, Desmodium, Maranta* and *Marattia;* various roots, *e.g., Beta;* petioles, *e.g., Primula* and *Ficus;* leaves, *e.g., Primula;* young endosperm cells, *e.g., Rhopalostylis, Sabal,* and *Ancuba.* Stems and other structures examined from time to time gave the same results. These results, taken in conjunction with those of BOWER, make it extremely probable that the same phenomenon is displayed by every living cell whatsoever.

In attempting to explain these appearances which accompany plasmolysis one has only hypothesis to offer. BOWER* suggests two views—(1) that the main mass of protoplasm on retreating may leave the cell-wall still completely lined with a thin film of protoplasm; (2) that the peripheral part of the protoplasm being entangled as a network among the deposited microsomata may, on the contraction of the main mass, be drawn out at the points of entanglement into fine strings like those observed; while the surface of the wall is left free, and not covered by a film of protoplasm.

But it seems to me that all the above phenomena may be explained from the mere fact that the cell-wall is so perfectly wetted (to use a physical phrase) by the protoplasm; for as STRASBURGER'S† results show, the connexion between the cell-wall and the protoplasm is one of the most intimate description, even if any direct perforation of the cell-wall by protoplasmic filaments be left out of the question. The very same effects may be obtained with stringy mucus adhering to a glass tumbler. My results have certainly shown that the connexion between protoplasm and cell-wall is much closer than was imagined to be the case; but I am inclined to doubt whether the existence of protoplasmic threads in the cell-wall at all influences the phenomenon of plasmolysis, for they are equally well displayed over the whole surface of the wall, and bear no relation even to such pits as those occurring in the young endosperm cells of *Archontophœnix* and *Rhopalostylis,* where well pronounced continuity is known to occur. But I am bound to admit that it is a question of hypothesis against hypothesis, and I look forward with interest to the results of

* *Loc. cit.*

† 'Bau und Wachstum,' p. 246.

plasmolysing such a cell as *Tamus communis* (Plate 70, fig. 33). In concluding the subject I should like to state my views as to the reason why plasmolysis does not give any reliable assistance to the subject of the perforation of the cell-wall by protoplasmic threads.

When the protoplasm separates from the cell-wall in consequence of the action of dehydrating agents, it always tends to assume a spheroidal form, in consequence of the action of the two forces of pressure and tension, which endeavour to bring about a state of equilibrium. Now the pulling force that the living protoplasm must exhibit in contracting from the cell wall and assuming its spheroidal condition must be very considerable. As we have seen from the appearance presented by such sections as Plate 70, fig. 40, there is a tendency on separation for the protoplasm to adhere rather to the main protoplasmic mass than to the cell-wall, and in consequence of this the protoplasm of the fine filaments going through the cell-wall will tend to be pulled out of its canal, and thus the thread proceeding from it will be no thicker than one which arises from the general cell-wall, and will therefore not be especially apparent. In instances where plasmolysis is very rapidly induced, the protoplasm quickly contracts, and even becomes divided up into several masses. Then it may possibly happen that, owing to the particular combination of forces, a minute sphere of protoplasm may be retained, sticking to the pit membrane (as in Plate 70, fig. 34), although it may equally well adhere to the cell-wall (as in Plate 70, fig. 35).

But with such strong reagents as sulphuric acid the case is different. Owing to the rapid death of the protoplasm, the assumption of that spheroidal form attended with the exhibition of the usual rending force between the protoplasm and the cell-wall is prevented. The factor of life no longer asserts itself, and the contraction produced is now merely a mechanical shrinking, in consequence of dehydration, and the separation tends to take place rather between protoplasm and cell-wall than between protoplasm and protoplasm. Thus any intimate union which may exist between the protoplasm of the cell and the protoplasm running through the cell-wall tends to be maintained, and if sufficiently pronounced is made evident.*

I am now in a position to bring my paper to a conclusion.

I have succeeded in demonstrating that in living tissues a means of communication between adjacent cells exists. My results have been confirmed by Russow, whose valuable contribution I have already mentioned. The wide field that this discovery opens is so great, and the whole bearing of the subject is so enormous, that it would be useless for me either to attempt to sketch its significance or indicate the important inferences which arise therefrom, in the present paper. We are now in a position if not to understand, at least to get a clearer insight into, such phenomena as the downward movement of a sensitive leaf upon stimulation, of the wonderful action of a germinating embryo on the endosperm cells, even those which are far

* In connexion with this subject, see De Bary's figure of the sieve-tube of *Vitis* after the action of iodine and potassic iodide (*loc. cit.*, fig. 75, p. 186).

removed from it, and finally of the whole cell mechanism. The passage of protoplasm from cell to cell, which numerous observations have showed must occur, can now be explained, and the mere fact of the possibility of this taking place increases very materially our knowledge as to general mechanics of the vegetable cell.

Although I am aware of the danger of rushing to conclusions, I cannot but remark that when these results—which were foreshadowed by SACHS and HANSTEIN when they discovered the perforation of the sieve-plate—are taken in connexion with those of RUSSOW, it appears extremely probable that not only in the parenchymatous cells of pulvini, in phlœm parenchyma, in endosperm cells, and in the prosenchymatous bast-fibres, is continuity established from cell to cell, but that the phenomena is of much wider, if not of universal occurrence.

Finally, I have to acknowledge the many kindnesses I have received during this investigation. Of Professor SACHS' kindness to me it is impossible for me to speak sufficiently highly. The mere fact that it was at his suggestion that this work was undertaken will show how much I owe him. To my friend and former teacher, Dr. S. H. VINES, I am indebted for much valuable advice. Especially must I also express my most sincere gratitude to my friend, Dr. D. H. SCOTT, not only for his valuable criticisms and suggestions, but for the many assistances that he has given me in every possible way during the whole of this difficult work.

NOTE.

(Added January 12th, 1884.)

Since the communication of the above I have written two more papers on the same subject, viz.:

1. "On the Continuity of the Protoplasm through the Walls of Vegetable Cells," Proc. Roy. Soc., December 20, 1884, which deals with the confirmation of my methods and the further establishing of my results. Since in the endosperm cells of *Bentinckia Conda-panna* the threads can be seen by merely mounting a section in dilute glycerine, such a preparation is taken as normal, and can be then compared with similar sections, in the preparation of which reagents have been employed. Such comparison is in every way satisfactory. I have further confirmed the existence of a continuity in *Dionæa*, and have established that in the parenchymatous cells of the leaf bases of *Aucuba Japonica* and *Prunus lauro-cerasus* distinct threads can be made out, crossing the pit-closing membrane. I then make some remarks as to the function of the threads.

2. "On the Continuity of the Protoplasm through the Walls of Vegetable Cells," Arbeiten des Botanischen Instituts in Würzburg. Bd. III., Heft I.

This is a fairly complete paper, embodying all the results I have obtained up to the present time.

Description of Plates.

PLATE 68.

Fig. 1. Transverse section of the pulvinus of *Trifolium repens* which in its principal details resembles that of *Mimosa pudica*. (× 55.)

Fig. 2. Longitudinal section of a portion of the pulvinus of *Trifolium repens*, showing the cells immediately beneath the epidermis. (× 105.)

Fig. 3. Longitudinal section of a portion of the pulvinus of *Mimosa pudica*, showing the cells immediately beneath the epidermis. The intercellular spaces are small and badly developed. (× 235.)

Fig. 4. Cells of the pulvinus of *Mimosa pudica* which are situated immediately around the vascular bundle. The intercellular spaces are large and conspicuous. (× 235.)

Fig. 5. Cells of the pulvinus of *Mimosa pudica*, situated midway between the epidermis and the vascular bundle, after treatment with sulphuric acid, staining with methyl violet, and washing with dilute glycerine. The protoplasmic contents are shrunken and deeply coloured. The remains of the middle lamellæ can be seen. Certain of the processes appear to join uninterruptedly from cell to cell. In others between the two darkly stained ends is a lighter stained area uniting the two. The latter is believed to be the typical and only true means of continuity. (× 550.)

Fig. 6. Portions of two bast-cells from the pulvinus of *Mimosa pudica* after treatment with sulphuric acid, and staining with methyl violet and glycerine. (× 1020.)

Fig. 7. Cells of the pulvinus of *Robinia pseudacacia*, situated as in *Mimosa* (fig. 5), after treatment with sulphuric acid and staining with methyl violet and glycerine. The typical mode of connexion between adjacent cells is better seen than in *Mimosa*. The appearances of an uninterrupted continuity are not so frequent. (× 550.)

Fig. 8. Cells of the pulvinus of *Robinia pseudacacia* after treatment with sulphuric acid and staining with methylene blue. The bottom and sides of the pits are stained. (× 105.)

Fig. 9. Longitudinal section of a portion of the pulvinus of *Amicia zygomeris*. In certain of the cells conspicuous pits are apparent. (× 235.)

Fig. 10. Cells of the pulvinus of *Amicia zygomeris*, situated midway between the epidermis and the vascular bundle, after treatment with sulphuric acid, staining with methyl violet, and mounting in dilute glycerine. Between the adjoining masses of the much shrunken protoplasm are numerous fine stained processes uniting the two. These in reality represent the stained

bottoms and sides of the much swollen and resistent pits. The swollen cell-wall abuts directly on to the protoplasm. By long treatment with dilute glycerine all the colour becomes dissolved from the pits, and the protoplasmic masses are then left fairly isolated one from another, or by prolonged treatment with sulphuric acid the resistent pits become swollen, and then stain like the rest of the wall. (× 1020.)

Fig. 11. Cells of the pulvinus of *Amicia zygomeris* situated immediately around the vascular bundle, where the cell-walls are thick and the pits deep and well developed, after treatment with sulphuric acid and staining with methyl violet and glycerine. The protoplasmic processes tend to adhere to the pit-membrane, and between any two contiguous processes is a lighter stained area. (× 1020.)

Fig. 12. A cell from the rachis of the leaf of *Cycas revoluta*, treated with iodine and Chlor. Zinc Iod. The pits opposite the intercellular spaces stain deep blue, whereas those separating the contents of adjacent cells are but feebly coloured. (× 235.)

PLATE 69.

Fig. 13. A cell from the ripe endosperm of *Phœnix dactylifera* after treatment with sulphuric acid and staining with methyl violet and glycerine. Some portions of the wall remain but little acted upon. The protoplasmic processes of the main shrunken mass have separated with difficulty from the pit-closing membrance, and the protoplasmic threads which traverse that structure, and normally abutted on to the ends of the protoplasmic processes of the pits, are well stained and brought into view. Compare figs. 6 and 11. (× 550.)

Fig. 14. Young endosperm cell of the seed of *Archontophœnix Cunninghamii* (*Seaforthia elegans*) after treatment with sulphuric acid and staining with methyl violet and glycerine. The pit processes of adjacent cells are united by fine protoplasmic threads, after the manner of a sieve-tube. This compares with figs. 5 and 7. (× 550.)

Fig. 15. Young endosperm cell of *Rhopalostylis sapida* (*Areca sapida*) after treatment with sulphuric acid, methyl violet, and glycerine. The processes from the shrunken protoplasm which enter the pits adhere to the pit-closing membrane, and the opposite processes of adjacent cells are united to one another and held in position by delicate protoplasmic threads perforating the pit-closing membrane. (× 550.)

Fig. 16. Cells of the ripe endosperm of *Bentinckia Conda-panna* after treatment with Chlor. Zinc Iod. and staining with picric-HOFMANN'S-blue. The proto-

plasmic cell contents have undergone degeneration, and many oil drops are present. Traversing both the thick pit-closing membranes, and also the general cell-walls, are complexes of fine protoplasmic threads. (× 550.)

Fig. 17. Portions of cells of ripe endosperm of *Howea Belmoreana* (*Kentia Belmoreana*), cell contents not shown, treated with Chlor. Zinc Iod. and picric-HOFMANN'S-blue. The threads traverse the pit-closing membranes and the general cell-walls. (× 550.)

Fig. 18. Cells of ripe endosperm of *Howea Belmoreana*, with protoplasmic cell contents. Treated as before. (× 550.)

Fig. 19. Portions of cells of the ripe endosperm of *Lodoicea Sechellarum* (the double cocoa-nut) treated with iodine and Chlor. Zinc Iod. Protoplasmic threads traverse the pit-membranes and general cell-wall. Cell contents not shown. (× 550.)

Fig. 20. Piece of cell-wall of same mounted in a mixture of iodine and glycerine. Threads fainter. Those traversing the unpitted portion of the wall do not appear to perforate as far as the free surface. The delicate channels containing protoplasm are widest in the region of the middle lamella, and colour with little or no swelling. In consequence of incomplete swelling the remaining portions of the threads are not visible. (× 550.)

Fig. 21. Portions of cells of the ripe endosperm of *Oncosperma horridum* treated with iodine and Chlor. Zinc Iod. The threads are very fine; much finer than it is possible to represent them in a drawing. (× 550.)

Fig. 22. Cells of the ripe endosperm of *Heterospathe elata* treated with Chlor. Zinc Iod. and picric-HOFMANN'S-blue. Middle lamella but little developed. Threads fairly thin. (× 550.)

Fig. 23. Portion of same treated with iodine and Chlor. Zinc Iod., showing that by such treatment the threads appear much thicker, owing to the precipitation of iodine upon them. (× 550.)

Fig. 24. Ripe endosperm cell of *Calamus callicarpus* treated with Chlor. Zinc Iod. and picric-HOFMANN'S-blue. Cell contents in this and many following omitted. Middle lamella well developed. Embedded in the cell-wall are crystals of calcium oxalate. (× 550.)

Fig. 25. Portions of ripe endosperm cells of *Syagrus botryophora* treated as before. Pits shallow and little developed. (× 550.)

PLATE 70.

Fig. 26. Cell of ripe endosperm of *Phytelephas macrocarpa* (vegetable ivory) treated with iodine and Chlor. Zinc Iod. (× 550.)

Fig. 27. Portion of cell-wall of almost ripe endosperm of *Bomarea oligantha* treated with Chlor. Zinc Iod. and picric-HOFMANN'S-blue. In the case of one of the pit-closing membranes the badly developed threads are not present over the whole of its surface. (× 550.)

Fig. 28. *En face* view of same. In the upper of the two pits the threads run as in the last described example. In the lower they are distributed equally over the surface. The figure is badly drawn, for in the upper pit the sections of the threads should have been more plainly apparent, and in the lower the unstained portions should have been represented stained, and *vice versâ*. (× 1020.)

Fig. 29. Portion of cell-walls of ripe endosperm of *Ruscus aculeatus* treated with Chlor. Zinc Iod. and picric-HOFMANN'S-blue. Pit-membranes thin. Threads badly developed and seen with difficulty. (× 550.)

Fig. 30. Portion of cells of ripe endosperm of *Colchicum speciosum* treated as before. Threads much better defined. (× 550.)

Fig. 31. Portion of cell-walls of ripe endosperm of *Asperula odorata* treated with iodine and Chlor. Zinc Iod. Threads traverse pit-membranes and walls. (× 550.)

Fig. 32. Portion of cell-walls of ripe endosperm of *Nemophila parviflora* treated with Chlor. Zinc Iod. and picric-HOFFMANN'S-blue. Cells small. Pit-membranes thin. Threads difficult to see. (× 550.)

Fig. 33. Portion of cell-walls of ripe endosperm of *Tamus communis* treated with iodine and Chlor. Zinc Iod. Part of the wall much swollen and coloured blue, in consequence of the usual cellular reaction. In this swollen area the threads can no longer be detected. In the lower half of the figure the apparently unswollen walls are commencing to swell, and the protoplasmic threads are breaking up into small points, instead of presenting the appearance of lines, as in the walls of the upper half of the section, which are still fairly intact. (× 550.)

Fig. 34. Cell of pulvinus of *Robinia pseudacacia* after treatment with a 10 per cent. solution of common salt. Appearance presented some two hours after plasmolysis. Certain of the threads can be seen going to pits. (× 550.)

Fig. 35. Cell of same tissue examined about ten minutes after mounting in 10 per cent. salt solution. (× 550.)

Fig. 36. Similar cell treated in the same way. Examined half an hour after treatment. (× 550.)

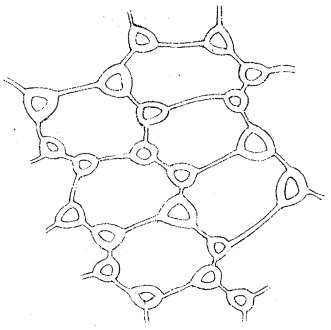
Fig. 37. Cells of pulvinus of *Apios tuberosa* treated with 5 per cent. salt solution. Examined three hours after plasmolysis. (× 440.)

Fig. 38. Cells of pulvinus of *Apios tuberosa* after treatment with 10 per cent. salt solution, saturated watery picric acid, and HOFMANN'S blue. Some of the threads are fairly preserved. Two thick threads appear to perforate

two pits, and unite the protoplasmic masses of the neighbouring cells as stated in text (refer to paper). (× 550.)

Fig. 39. Cell of the lamina of *Trichomanes pyxidiferum* as seen ten minutes after plasmolysis with a 10 per cent. salt solution (after BOWER). The threads appear to have no fixed relation to the pits. (× 550.)

Fig. 40. Similar cell as seen two hours after plasmolysis (after BOWER). (× 550.)



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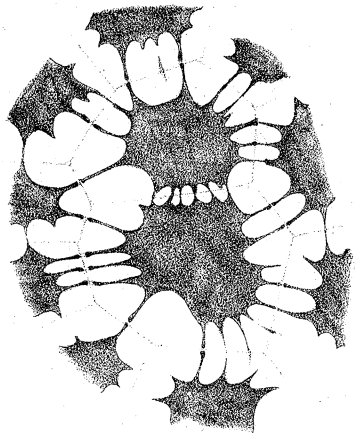

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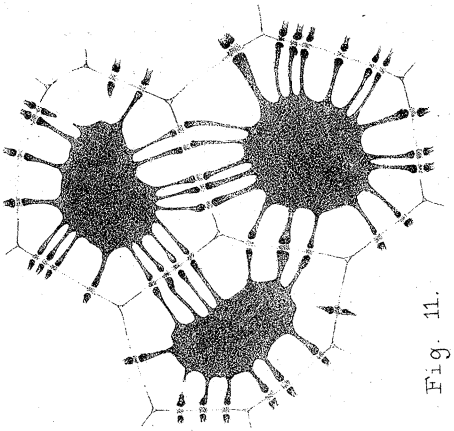

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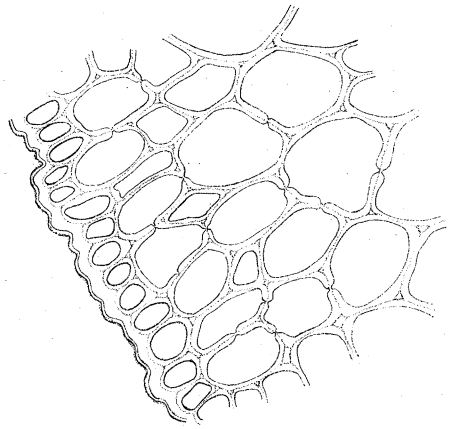

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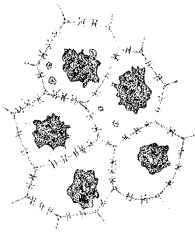

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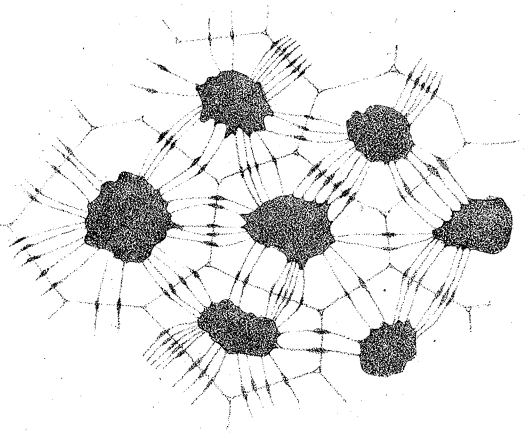

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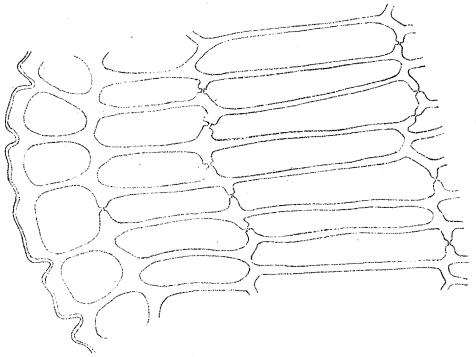

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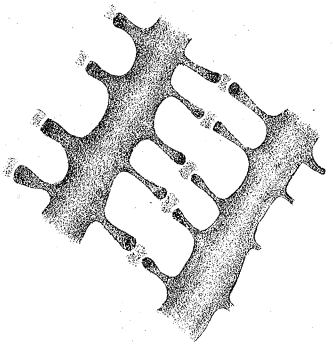

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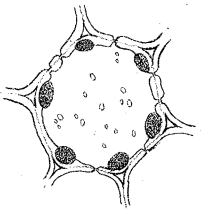

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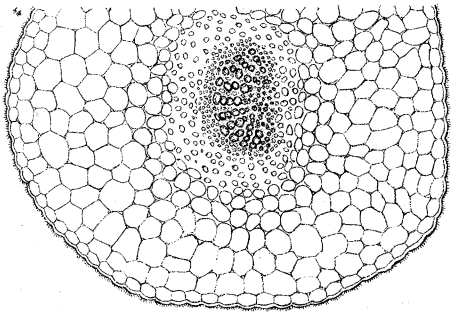

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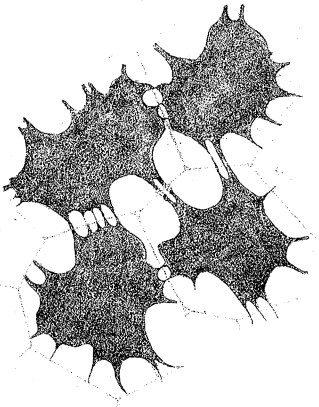

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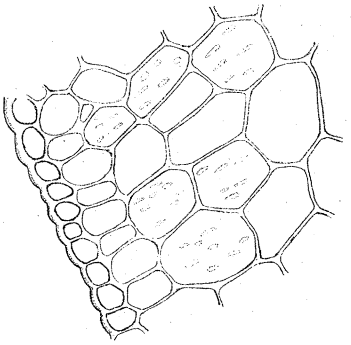

Fig. 9.

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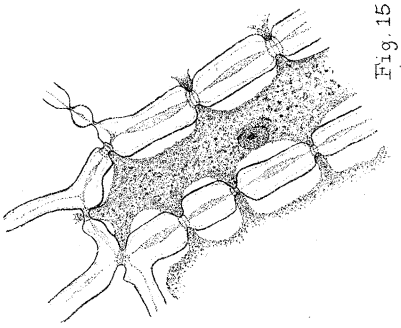

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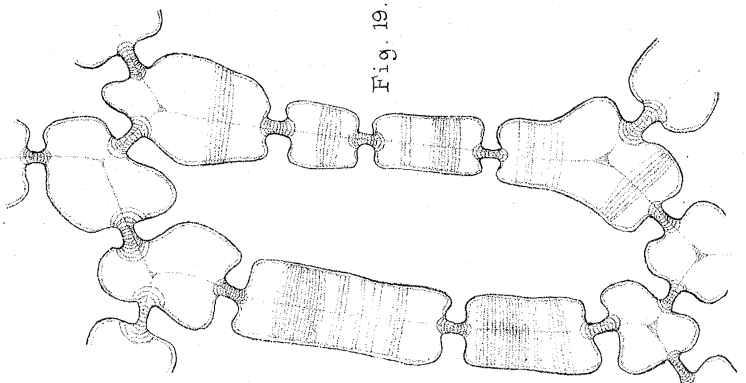

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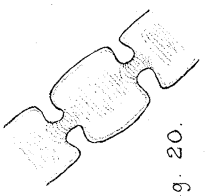

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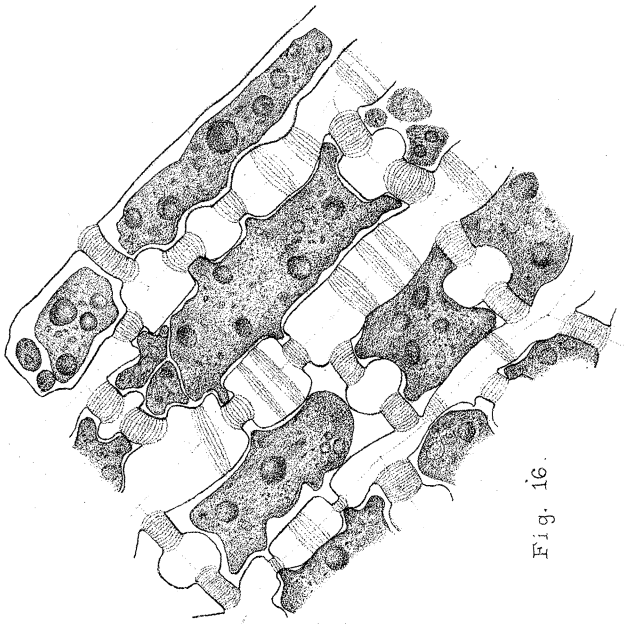

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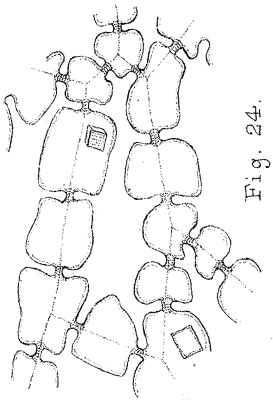

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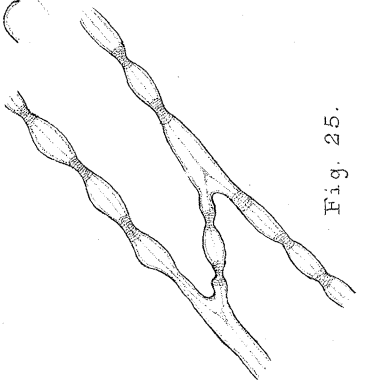

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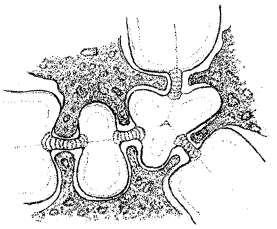

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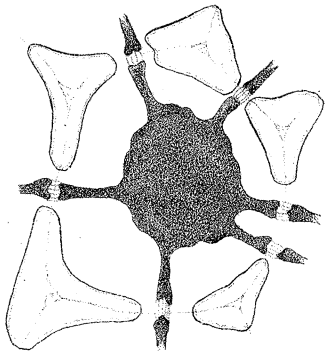

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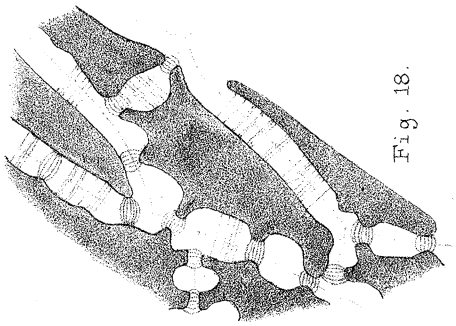

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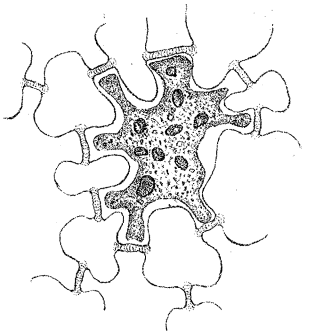

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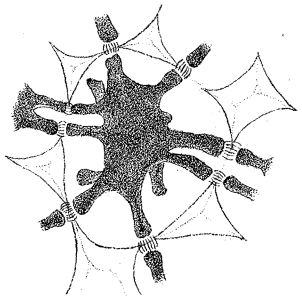

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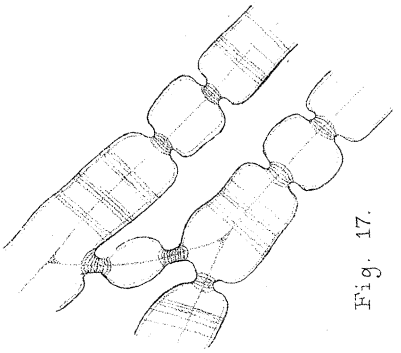

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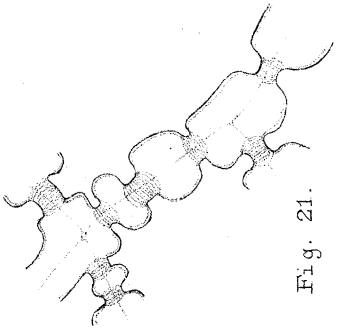

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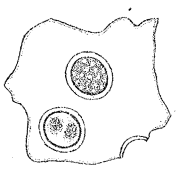

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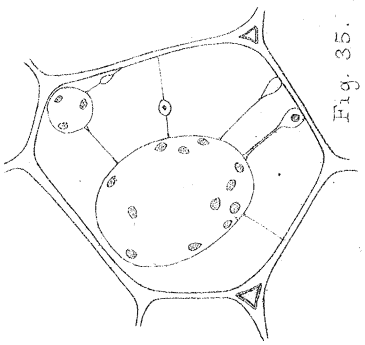

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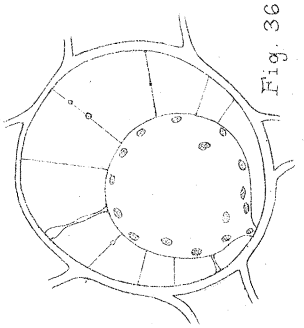

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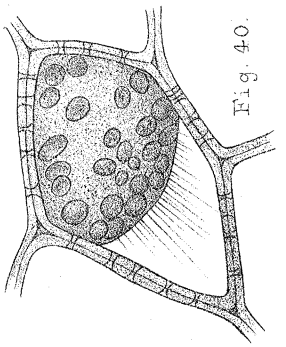

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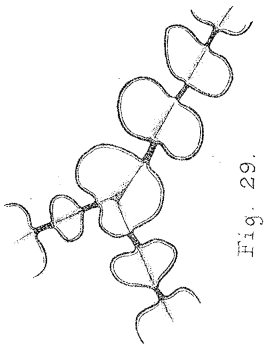

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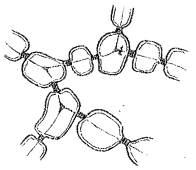

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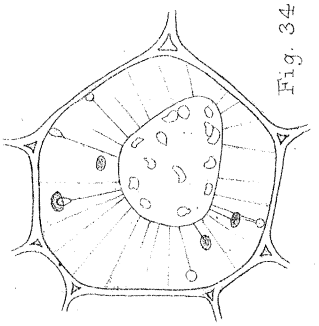

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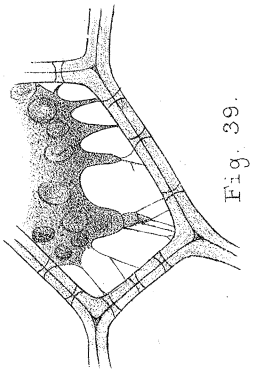

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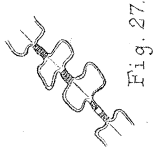

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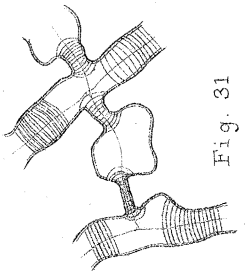

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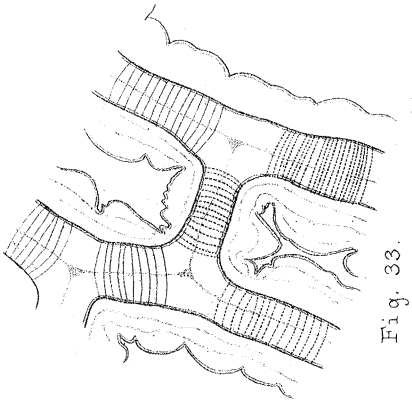

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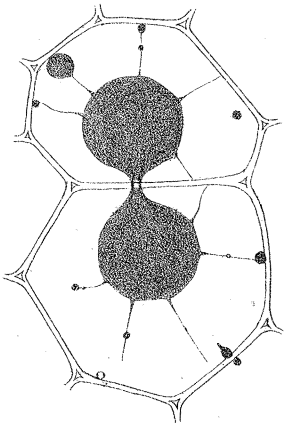

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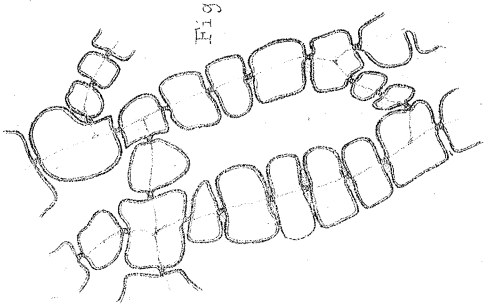

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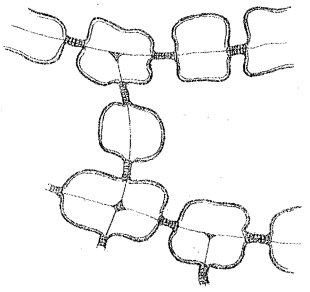

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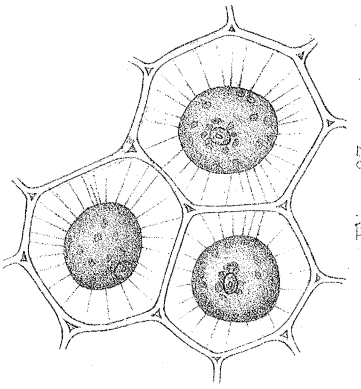

Fig. 37.