IV.

On the continuity of the protoplasm through the walls of vegetable cells.

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A most important addition to our knowledge of the histology of tissues was made in 1863 by Sachs [1]) and in the following year by Hanstein [2]) when they demonstrated that in the sieve-tubes first described by Hartig [3]), there are perforations in the transverse partition walls, through which pass filaments of protoplasm, and that thus there is continuity between the protoplasmic contents of adjacent cells.

For a long time this discovery, although admirably confirmed and supported by the researches of Wilhelm [4]), Janczewski [5]) and Russow [6]), remained as an isolated fact until in 1880 Tangl [7]) published certain results which afforded some practical proof of the correctness of the suggestions as to the existence of a closer connection between cell and cell which had been first made by Hofmeister [8]) and subsequently more thoroughly and emphatically enunciated by Sachs [9]) and Strasburger [10]), for Tangl succeeded in showing that in the ripe endosperm cells of *Strychnos*, *Phoenix* and *Areca*, either the general cell-wall, or the closing membranes of the pits were traversed by fine threads of protoplasm.

1) Sachs. 'Flora 1863'. p. 68.
2) Hanstein. 'Die Milchsaftgefäße'. Berlin 1864. p. 23 et. seq.
3) Hartig. 'Bot. Zeit. 1854'. p. 54—54.
4) Wilhelm. Beiträge z. Kenntniß &c. Leipzig 1880.
5) Janczewski. Etudes comparées sur les tubes cribreux. Cherbourg 1882.
6) Russow. Sitzber. d. Dorpat. Nat. Gesell. 1882. p 350—389.
7) Tangl. Jahr. f. wiss. Bot. XII p. 170—190.
8) Hofmeister. See Sachs' Vorlesungen. p. 102.
9) Sachs. 'Vorlesungen über Pflanzen-Physiologie'. p. 102.
10) Strasburger. 'Bau und Wachsthum'. p. 246.

In the summer of 1882 while working at the Würzburg laboratory under the direction of Professor Sachs, I succeeded in demonstrating that in various pulvini, a direct communication between the cells is established by means of delicate protoplasmic filaments which traverse the pit-closing-membranes [1]). In subsequent papers [2]) I have detailed my further experiments and results, which are moreover confirmed by the researches of Russow [3]) who has proved that in the bast parenchyma cells and in the phloem-ray-cells of numerous plants e.g. *Populus*, *Salix* &c, the closing membranes of the pits are perforated by fine protoplasmic threads. Thus at length evidence appeared that in cells such as those of pulvini which unlike sieve-tubes cannot be regarded as modified for the purpose of serving as mere channels for conduction, and unlike inert endosperm cells are in active life — in such cells there is also an actual communication between one cell and another.

In the present paper I propose to give an account of my work as far as it has as yet progressed in which I claim to have proved that in various pulvini, in the cells of the leaf of *Dionaea*, in the parenchyma-cells of the stamens of *Berberis*, in a great number of endosperm cells, and in various ordinary vegetable tissue, there exists a demonstrable connection between the protoplasmic contents of adjacent cells.

I. Methods.

As it was important in a research of this kind that the material employed should be preserved with the minimum amount of change, a number of experiments were instituted in order to determine which of the many preservative reagents generally in use was the most serviceable. When — as I shall point out later on — the plasmolytic condition is induced in a cell by treating it for some time with a 10 per cent solution of common salt, the protoplasm is caused to contract and separate away from the cell-wall, to which however it is still connected by delicate protoplasmic strings. Such plasmolysed tissue affords very favourable material for testing the relative fixing and preserving power of reagents. In addition I also investigated the effects produced on sections of fully grown tissue possessing large vacuoles, and upon the filaments of *Spirogyra* and other filamentous Algae. Alcohol, 1 per cent Osmic acid, 5 per cent Chromic acid, alcoholic and watery solutions of alum, and corrosive sublimate, saturated watery and alcoholic Picric acid were used. The strengths of the solutions were also varied, as occasion required since it seemed to me that owing to the diluting effect of the cell

1) Gardiner. Quart Journ. Micr. Soc. Oct. 1882.

2) Gardiner. Roy. Soc. Proc. Nov. 11. 1882 d. Roy. Soc. Proc. April 16. 1883 & Roy. Soc. Proc. Dec. 20. 1883.

3) Russow. loc. cit.

sap, some modifications might be required for vegetable tissues [1]). Gold chloride and Silver nitrate were also employed. The experiments pointed to the fact that treatment of small pieces of fresh tissue with a saturated watery solution of Picric acid gave the best results although these results were far from being perfectly satisfactory. When small pieces of the tissue were treated for some time ($^1/_4$ hr. to 2 hrs.) with this reagent, and subsequently with dilute (50 p. c.) and progressively stronger alcohol, the close relation existing between the protoplasm and cell-wall was fairly maintained, the latter having undergone but little shrinking. Still the amount of shrinking was sufficient to modify considerably the normal relations between protoplasm and cell-wall, and moreover the protoplasm was found to have become rigid and brittle. In consequence of these objections I regarded it as most satisfactory to give up the attempt to use preserved material; hence all the observations described below were made upon fresh material alone. [2])

I then proceeded to make a number of very careful observations upon the staining properties of as many dyes as I could obtain. Most of them were not remarkable for any well-defined selective staining power, but in the end I succeeded in finding two very excellent reagents for botanical

1) From my more recent Experiments I find that I had been working with too strong solutions of Osmic and Chromic acid. In the case of young tissue where the cells are full of protoplasm, exactly the same treatment may be resorted to, as in animal structures, but with fully grown and much vacuolated cells, much more care is required all rapid diffusion must be avoided, and the strength of the solution with which such tissue is treated, must be only slowly and progressively increased. The staining solution must also be approximately of the same specific gravity as that of the fluid in which the tissue is, at the time. I found that as regards Osmic and Chromic acid a 1% solution gave good results. The tissue is placed in such a solution for 12 or 24 hours. It may then be removed to 30% alcohol for a day, and into 50%, 70%, 90% and absolute as required. If it is to be mounted in glycerine it must in the same way be gradually brought into water: into very dilute glycerine (1 Glycerine to 10 water), and so on. In all cases small pieces of tissue must be used. The black staining produced by Osmic acid may be removed by treating the sections with Chlorine-water or by suspending them in alcohol through which Chlorine is allowed to bubble. The alcohol must be kept cool by means of a current of cold water. It is best not to act on large pieces of tissue, both because the bleacking then takes a long time, and because it is not well in a delicate investigation to expose a tissue to the prolonged action of chlorine. The method is a slight modification of that first proposed by MAYER (MITT. Arch. 1874. p. 321). As regards Picric acid, the saturated solution may have to be diluted with 3 times its bulk of water. For sea-weeds, Florideae &c the following method gives good results. Make a saturated solution of Picric acid in sea-water, add to it 3 or 4 times its volume of sea-water. The material is treated for $^1/_4$ hour to 4 hr and then placed in 30% alcohol &c. Finally for any ordinary investigation there is no doubt that absolute alcohol is the most useful reagent.

2) Alcohol material may be used but it must be recollected that it will always cause the protoplasm to shrink from the cell-wall, and the relation between the pit-membranes and the protoplasm will no longer be maintained.

research viz. Methylene blue which especially stains the cell-wall, and Hoffmann's blue [1]) which especially colours the protoplasm. The only precautions required are that a fairly dilute solution of the dye should be made in Alcohol of a strength not greater than 50 per cent, and that the stained section should be washed in water [2]).

All experiments made with the view of attempting to detect the presence of protoplasmic filaments in the cell-wall when the wall was normal and intact met with but little success [3]), so that in investigating the subject of protoplasmic continuity the method of swelling the cell-wall, and subsequently staining with a dye which was found to especially stain the protoplasm, was adopted. Either Sulphuric acid or Chlor-Zinc-Iod. was used as the swelling agent, although every preference was given to the latter. Naturally Potash might have been used, but on account of the difficulties which attend the thorough washing out of that reagent and in consequence of its often deleterious action on the dyes, it was rejected when it was found that Chlor-Zinc-Iod. answered every purpose. Since for the two reagents employed the methods are somewhat different it seems best to treat of them separately.

1. *Method with Sulphuric acid.*

During the earlier part of my work I was accustomed to use Sulphuric acid, in combination with Hoffmann's Violet. This latter reagent, at the time of staining, colours equally both protoplasm and cell-wall. If however the section be treated for some time with dilute glycerine, the staining of the cell-wall is removed, and the protoplasm alone remains clearly stained [4]).

In working with Sulphuric acid, the fresh material is first cut in water. A section having been taken up with a platinum spatula and the excess of water removed with blotting paper, a drop of strong Sulphuric acid is placed upon it, and allowed to act for a short time — usually a few seconds. The section is then plunged into water and rapidly washed. After several wash-

1) Since the various aniline dyes even though possessing the same name differ materially in their staining reactions, I may mention that this particular blue is known as Hoffmann's blue (Aniline blue) and may be obtained from Morelli in Würzburg.

2) Like Mayer ('Mittheilungen aus der Zoologischen Station zu Neapel'. Vol. II 1880) I found that dyes containing a high percentage of alcohol stain more diffusely than those of weaker grades.

3) In certain instances where the threads were well developed, e. g. Bertinckia, Tamus etc. the threads can be distinguished when the sections are merely mounted, in water, or dilute glycerine & if a little Jodine be added they are very clearly brought into view. This is a very important point, and proves that the phenomena detailed in the present paper are not artificially induced by reagents.

4) A very useful reagent for the demonstration of sieve-tubes may be made by dissolving the Hoffmann's violet in strong Sulphuric acid. After treatment with this solution the sieve-tubes are well brought into view, and moreover all lignified tissue assumes the usual gold yellow tint, as after treatment with Aniline chloride and hydrochloric acid.

ings it may be stained and mounted. As a staining reagent either HOFFMANN's violet or preferably HOFFMANN's blue may be used. In the case of HOFFMANN's violet the section is quickly stained, washed in water and then placed for 24 hours or more in dilute glycerine which dissolves out a great portion of the dye from the stained cell-wall and at the same time removes the peculiar staining of the pits which, if allowed to remain, is apt to lead to very delusive results. The section is finally mounted in glycerine.

When HOFFMANN's blue is used a moderate quantity of the dye is dissolved in a 50% solution of alcohol to which has been added a few drops of acetic acid. After staining, the sections are washed in water and mounted in glycerine. Or a sufficient quantity of the dye may be dissolved in a 50% solution of Alcohol which has been saturated with Picric acid, until the solution assumes a dark greenish blue tint. This solution — the properties of which will be dealt with under the head of Chlor-Zinc-Jod. — I shall speak of as Picric HOFFMANN's blue. After staining, the sections are washed in water and mounted in glycerine as before, or after treatment with alcohol they may be cleared with clove oil, and mounted in Canada balsam.

2. *Chlor-Zinc-Jod.*

In TANGL's method, which was the same as HANSTEIN had employed for demonstrating the perforation of the sieve-plate, sections of endosperm were stained with Jodine and mounted in Chlor-Zinc-Jod. In such dry tissue as that of ripe endosperm cells, the cell-walls do not turn blue but merely remain stained with the ordinary yellow brown due to Jodine. The protoplasm on the other hand assumes a very dark brown colouration and after some time there comes into view a series of striae traversing the thickened cell-wall which from their colouration, and from the fact that their depth of staining varies pari passu with that of the protoplasm, are taken to be essentially protoplasmic in character.

Although in such cases where it can be applied the method is of great value, it will be seen that it is attended also with some disadvantages; for, firstly, in tissues containing a higher percentage of water the walls assume the ordinary cellulose blue, which at once prevents the threads from being seen, and secondly, on account of the extensive and very varied [1]) staining properties of the Jodine the results obtained by it alone cannot be taken as entirely conclusive. Nevertheless, where practicable, TANGL's method is of great use to give at least an idea of the existence of the protoplasmic filaments, and moreover the staining of the threads with Jodine is much more distinct than with any other reagent I have yet been able to employ.

1) Thus besides its well known reactions with protoplasm, cell-wall and starch, Jodine gives a blue colour with mucilage, with the cell-walls of certain fungi, with the phloem of Lycopodium, with the cell-walls of the endosperm cells of *Paeonia officinalis* (VINES) and of *Ardisia crenulata* and *Ardisia polytoca* (GARDINER).

To obviate the difficulties I have mentioned above I attempted to use the same modification as I had done in the case of Sulphuric acid viz. to treat first with Chlor-Zinc-Iod. and, having well washed out the section, to stain with Hoffmann's blue. In this however, like Tangl, I was at first unsuccessful, for although by the Jodine and Chlor-Zinc-Iod. treatment, well defined threads were plainly seen, yet on staining, no colouration whatever was produced. However from a number of experiments that I instituted in order to ascertain why this occurred, I observed that when such sections were treated with solutions of well coloured crystalline bodies, such as picric acid, gold chloride, chromic acid, something of the threads could be seen. This led me to believe that I had to deal merely with a phenomenon of diffusion, for my aniline dyes were essentially colloidal in character, and it seemed not improbable the solution of such colloidal substances would not diffuse into the delicate strands of colloidal protoplasm. Consequently I adopted the modification of dissolving the Hoffmann's blue in a 50% solution of alcohol saturated with picric acid and on washing out I found the threads well stained — the picric acid bodily carrying, as it were the solution of the dye into the fine protoplasmic strands. Picric acid has also another valuable property in that it tends to prevent the staining of cell-wall by dyes which, although possessing an especial affinity for the protoplasm, will stain the cell-wall also unless some such restraining reagent be used.

I am now in a position to give my method with Chlor-Zinc-Iod. in full. Sections are stained with Jodine and mounted in Chlor-Zinc-Iod. Then if the material is favourable one may see something of the threads, or at any rate obtain some information as to their probable presence or absence. After being exposed to the action of Chlor-Zinc-Iod. for about 12 hrs. the sections are well washed; stained with Picric Hoffmann's blue, washed again in water, and finally mounted in glycerine, or what is often better still, placed in alcohol, first dilute, and at length absolute; cleared with clove oil, and mounted in Canada balsam. In those cases where the tissue rapidly swells under the action of the reagent, as in the endosperm of *Strychnos nux-vomica*, *Bauhinia* and *Tamus*, the action need not be so prolonged, and the excessive swelling must be prevented by the use of alcoholic Jodine at the outset, and in a similar manner it may be washed with alcohol instead of with water, otherwise the threads will be so displaced and altered as to be almost or entirely invisible.

These then are the two principal methods. As regards the management of the reagents, and the length of time they must be allowed to act in order to obtain a satisfactory result, it is clear that the manipulation must be varied to a certain extent to suit the requirements of the various kinds of tissue, as it is thin walled or thick, easily swollen or swollen with difficulty. The use of Sulphuric acid is attended with by far the greater amount

of difficulty, for if it be allowed to act for too short a time the cell-wall will not be sufficiently swollen, while if the treatment be somewhat prolonged, the middle lamellae of the cells are liable to swell, and at the same time stain, and, when in such a condition, will hinder all successful observation of the threads which may traverse their substance. Upon still further action the protoplasm itself commences to be attacked. With Chlor-Zinc-Iod. on the other hand, where the action is much more regulated and gradual, but little precaution as to length of time need be observed.

Besides the difficulty of regulating its action, there are still other and grave objections to the use of Sulphuric acid. One of these is that no matter how carefully the acid is added to the tissue, and no matter how quickly the washing in water is accomplished, there will be a very considerable evolution of heat attending the hydration of the acid, which is liable to accelerate its action and to cause very grave changes in such delicate structures as line protoplasmic filaments traversing the cell-wall. Secondly the folding up and general displacement of the tissue consequent upon the action of such a violent reagent, greatly increases the already existing complications which attend all observations connected with minute histology.

For these reasons, while I still regard Sulphuric acid as a very valuable reagent, both for swelling up resistent tissues upon which Chlor-Zinc-Iod. has but little action, and for demonstrating in an unusually clear way the remarkable manner in which the apices of the protoplasmic processes, entering the pits, cling to the pit-closing-membrane, yet I am convinced that it must be looked upon as the less satisfactory of the two, and that the phenomena produced in consequence of its action can only be rightly interpreted in the light of the more certain results obtained by the use of Chlor-Zinc-Iod. Finally I am of opinion that for all tissues which will swell sufficiently under its action, my Chlor-Zinc-Iod. method may be regarded as perfectly satisfactory, and that after treatment with Picric-Hoffmann's-blue and subsequent washing in water, nothing but protoplasmic structures will be stained. In clear instances where a thick closing-membrane is plainly traversed by threads, it can be demonstrated with ease that while the individual threads are well stained, the substance of the pit-membrane itself experiences no colouration, even when the section has been exposed to the action of the dye for a long time. When the pits are smaller and the threads less clearly defined it is more difficult to observe that the substance of the pit-membrane is still free from colouration, and when owing to the thinness of the closing-membrane, all appearances even of striation cease to be recognizable, we are only able to observe an apparent staining of the entire membrane. But I am convinced from my own tentative experiment, and I think anyone who follows this paper to the end, will be convinced also that such staining points not to the colouration of the substance of the pit-membrane, but to the staining of protoplasmic filaments traversing its structure.

With regard to other manipulative details I should mention that besides a platinum lifter, I also used platinum needles and that I was careful to thoroughly brush all the sections with a camel-hair-brush, both, after the action of the acid, or of Chlor-Zinc-Iod. and after staining. This I regard as a detail of some importance.

In order to prove that the threads traversing the cell-wall were in reality protoplasm, I employed with success a solution of Molybdic acid in strong Sulphuric acid, which has the advantage of swelling the cell-wall and at the same time colouring the protoplasm. If Molybdic acid be dissolved in strong Sulphuric acid a colourless solution is obtained which, with Alcohol or many other substances of an organic nature, gives a beautiful blue colour. So delicate is this reaction that the blue colour is developed even when the liquid is kept in a stoppered bottle in consequence of the previous introduction of some foreign matter of an organic nature. I found that such a solution while not affecting the cell-wall for some time gave a fine blue colouration at once with the protoplasm. If then a section of some living endosperm such as *Tamus* be treated with this reagent, it will swell up the cell-wall and will commence to dissolve the protoplasm; the fine threads perforating the walls will remain for some time unacted upon, and while the main protoplasmic mass will assume an intense blue, the threads in addition will be perceptibly coloured.

At an early stage in this research I was struck with the peculiar properties of the pit-membrane as compared with those of the rest of the cell-wall.

For instance, after staining with Jodine and Chlor-Zinc-Iod., whereas the general cell-wall assumes the usual blue tint, the pit-membrane is but slightly coloured, and indeed when the membrane is somewhat thin may not appear to colour at all, although the examination of a fine transverse section of the pit will prove that a definite staining has taken place. But the depth of the staining certainly appears less than one would expect in proportion to the thickness of the membrane.

Methylene blue stains both the wall and the pit-membranes a fine light blue, and after the action of Sulphuric acid the swollen wall assumes a much lighter tint, owing to the fact that the quantity of the dye taken up by the cell-wall is now distributed over a relatively larger space. If a section be cautiously treated with Sulphuric acid, washed and stained, it will be seen that whereas the general swollen wall is coloured a light blue, the bottoms and the sides of the pits will still assume the darker blue colour of the unswollen cell-wall, and will thus be clearly marked out. If however another section be treated for a longer time with acid, or if the same section be a second time exposed to its action, it will be seen on staining that no special colouration of the bottoms and sides of the pits can be detected, but that the whole swollen wall is of an uniform light tint.

This phenomenon evidently points to the fact that the substance of the pit-closing-membrane and of the layers immediately surrounding the pit-cavity are more resistent than the rest of the cell-wall. Exactly the same thing was noticed by STRASBURGER [1]) who proved that by cautious treatment of a section with dilute Sulphuric acid or with weak Ammoniacal oxide of Copper the middle lamella and the pit-closing-membrane remained while the rest of the cell-wall suffered solution. With stronger solutions of the above reagents he likewise found that the pit-membrane also disappeared. Evidently the fact of not using some staining reagent prevented him from demonstrating that the sides of the pits, as well as the pit-membrane itself, are more resistent than the general cell-wall.

Exactly the same phenomena are observed when a section after cautious treatment with Sulphuric acid is stained with Methyl violet. In the case of Methylene blue the protoplasm is not coloured, but when Methyl violet is used a deep staining of that structure occurs, the tint of which is the same as that of the bottoms and sides of the pits: for whereas the general cell-wall assumes a violet colour, the protoplasm, the pit-membranes and the pit sides appear of a deep purple. Now since protoplasmic processes from the main protoplasmic mass may project for some distance into the swollen pits, when such a stained section of pitted tissue is examined it appears as if there were in any two contiguous cells, filaments of protoplasm of a purple colour traversing the thickness of the violet cell-walls by means of the pits, and thus establishing a direct continuity of the protoplasm from cell to cell. However after prolonged treatment with dilute glycerine this purple colour dissolves from the pits, and the protoplasmic processes are left clearly seen and may or may not be the means of establishing a continuity between the cells. As in the case of Methylene blue so also here a more lengthy treatment of the tissue with acid will swell up the pit-membranes, and when in that condition the pits will assume the same colour as the rest of the cell-wall [2]).

II. Observations.

Having thus described my methods at some length, I can now proceed to give an account of the results I obtained with pulvini and other organs in which a continuity of the protoplasm was shown to exist, and as the principal object of the paper is to study the relation which exists between protoplasm and cell-wall. I shall only quote such anatomical and physiological details as are necessary for the proper understanding of the organ in question.

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1) STRASBURGER. 'Bau und Wachsthum'. Pages 16 & 22.

2) Whether this resistent character of the pit-membrane is due to the fact that it contains protoplasm in its structure must for the present be left an open question.

In structure the typical pulvinus consists of an axial vascular bundle which is surrounded by some eight layers of parenchyma cells. The whole organ is invested by a feebly developed epidermis and the parenchyma cells are very conspicuously pitted especially on their longitudinal walls. I studied in detail the pulvini of *Mimosa pudica*, *Robinia pseud-acacia*, *Amicia zygomeris* and *Phaseolus multiflorus*, of which Mimosa gave the best results. Thin longitudinal sections of a fresh pulvinus of Mimosa are cut in water: treated with Sulphuric acid, and stained as already described. Then if the operation has been successfully accomplished, the cell-walls will have undergone considerable swelling so as to be rendered almost invisible, and the protoplasmic bodies will present the appearance of a number of deeply stained irregularly shaped masses lying in the swollen substance of the cell-wall. From these main protoplasmic masses radiate numerous processes towards the pits, and in any two neighbouring cells the processes from the one central mass are exactly opposite those proceeding from the other, thus presenting a most characteristic appearance and resembling somewhat a preparation of corneal connective tissue which has been stained with gold chloride.

The appearances I have described are the natural outcome of the action of Sulphuric acid upon fresh sections of vegetable tissue and the whole process may be watched under the microscope. It will then be apparent that the following effects are produced. The protoplasm is almost immediately killed, and in this process, although some slight contraction may take place, it remains for the most part perfectly passive. The cell-wall rapidly commences to swell and in so doing drives before it the passive protoplasm, which in virtue of its previous vacuolation easily admits of being squeezed into a much smaller space. The effect of the swelling wall, as far as the pits are concerned, is to cause a narrowing of the diameter of the pit-cavity, while the closing membrane itself swells but little. The processes from the general cell-protoplasm which enter the pits are left in their normal position, although they are necessarily somewhat constricted, and, what is a remarkable fact, that portion of each process which immediately abuts on the pit-closing-membrane, usually sticks to the latter structure, and is held in position even though owing to the swelling of the cell-wall, a very appreciable tension may be set up and the processes may be drawn out into strands of great tenuity. In other instances, and especially when the action of the acid has been prolonged, the processes contract away from the closing membranes altogether. The narrowing of the diameter of the pit-cavity naturally assists to maintain the processes in position in a merely mechanical manner, but apart from this one can recognize with ease that the apices of the protoplasmic processes adhere with considerable tenacity to the closing membranes of the pits. For the same thing occurs in thin walled tissue where the narrowing of the pit diameter is inappreciable, and where moreo-

ver treatment with Jodine and Chlor-Zinc-Jod. demonstrates that the swollen sides of the pits are quite free from, and do not embrace the delicately drawn out protoplasmic processes That part of the process abutting on to the pit-closing-membrane has either a broad apex, or it may be drawn to a line point. In two opposite pits the protoplasmic processes may either both have broad ends, or both pointed ends, or finally a broad-ended apex may have opposite it one whose termination is drawn out to a delicate point, all these various appearances depending upon the degree of action of the acid. The middle lamellae remain but little acted upon, and appear as a delicate network marking out the limits of the cells. By proper treatment with Glycerine all the staining may be dissolved from them, and after being mounted for some time in strong Glycerine they may be rendered so inconspicuous as not to interfere with successful observation.

Having thus stated in detail the various phenomena presented by the cell-wall and the general cell-protoplasm, I now come to the most important part of the subject which deals with the evidence as to the occurrence of a continuity of the protoplasm between one cell and another. The only suggestion that we have hitherto had of such protoplasmic continuity was the remarkable fact of the hanging on of the protoplasm to the pit-membrane, but this fact although it may be taken to afford some indication of the probable existence of a communication between adjacent cells, of itself actually proves but little; for it is the existence of protoplasm in the cell-wall which has to be demonstrated and protoplasm may equally well be present traversing the pit-closing-membrane whether the protoplasmic processes adhere to the pit-wall or whether they contracted away. This is an important point.

Thus it is the pit-membrane which must be carefully examined. In a well prepared section of the pulvinus of *Mimosa* there appear after treatment with acid at first to be several ways in which the continuity of the protoplasm between adjacent cells is established. In some instances it appears as if fairly thick protoplasmic processes traverse the pits bodily, so that the protoplasmic mass of one cell is directly continuous with that of its neighbour as if in fact the pits were open, and possessed no closing membrane. In other instances it appears as if each process had become drawn out in the pit into a very delicate strand and that the two opposite attenuated strands effect a junction by means of a small perforation in the pit-closing-membrane. Lastly it appears as if a sieve plate arrangement occurs. I propose to consider these cases in some detail, and before so doing I think it is necessary that I should state some of the difficulties which attend the observation of such structures, and also some of the precautions which must be taken in order to avoid a false interpretation of the appearances which I have described above. Firstly the tenuity of the processes, and the thinness of the pit-membranes necessitate the use of high powers, which must necessarily be manipulated with very great care. Again, owing to the action of

such a strong reagent as Sulphuric acid great displacement of the tissue is liable to occur. Lastly and most importantly the direction of the long axes of the pits, and consequently of the protoplasmic processes lie in all planes, and it is obvious that many processes which lie in a plane inclined even at a very slight angle to the plane of the coverslip will appear to touch one another and be continuous, although as a matter of fact they may be separated by a considerable space. Consequently the greatest precautions must be taken. All extraneous light should be kept from the eyes and it is best to work with the microscope under a wooden screen made for the purpose which admits light to the mirror only by means of a hole cut in the front. By careful observation one must learn to recognize exactly the effects which the acid produces, and the whole phenomena attending the swelling of tissues. It must be remembered that the middle lamella completely surrounds each cell, and that in a section the network of lamellae occur in all planes, and that the various constituent lamellae appear to intersect one another at all angles. In the examination of the processes and of the intercepting closing membranes the greatest possible care must be taken to ascertain that the long axis of the particular pit in view lies as nearly as possible at right angles to the line of vision, or what is the same thing, lies in a plane parallel to the plane of the coverslip.

I will now deal with the various resemblances which as I have stated simulate an actual occurrence of a protoplasmic continuity. As to the appearance of a somewhat thick thread bodily traversing the junction between two opposite pit-cavities and necessitating the idea of open pits, it can be easily shown that it is fallacious, for thin sections either of fresh or alcoholic material, stained with Jodine and mounted in Chlor-Zinc-Iod. demonstrate that in every case a closing membrane is present, and that the ends of the two opposite processes are sharply defined from it. Such an appearance is due either to the fact that the plane of the long axis of the pits is inclined at an angle to that of the coverslip, or that the pit-membrane has not been swollen, and since it is very thin, slightly stained, and at the same time in the thicker sections difficult to define, it appears at first sight as if no pit-membrane were present, but a careful examination will generally enable one to see that an intercepting closing membrane is in reality present in all cases.

With regard to the fine processes appearing to perforate the closing membrane it is obvious that this necessitates the existence of a small pore in that structure. But by the most careful examination of pit-closing-membranes I have failed to detect the existence of any such perforation, and as in many instances the threads are of an appreciable size they such a perforation of the pit-membrane ought certainly to be recognized. On the contrary in the whole of my work on endosperms and other tissues there was no single instance of such a simple perforation, but a sieve arrangement was present

in every case. I have been able to explain such appearances in the following ways. They may be produced: First, from the apparent overlapping of unconnected threads which lie in a different plane to that of the coverslip: Secondly, when two extremely attenuated threads lie very close to one another, they may appear to be continuous, and may give the eye the impression that they really join, although great care and accurate focussing will prove that such is not the case: Thirdly, that such an attenuated thread coming from a main protoplasmic mass may be intercepted by a middle lamella lying above it which is free from it, but which appears to be pierced by it. Frequently it will be seen that the attenuated process has not yet reached its own pit, and further that the process on the other side of the pit may be wide at its extremity, and that it is between these two that the true pit-membrane intervenes.

Having thus disposed of two of the appearances of protoplasmic continuity which may be produced when such a tissue as that of the pulvinus of *Mimosa* is treated with strong Sulphuric acid and stained, it only remains for me to deal with the last of those mentioned above which is moreover undoubtedly real namely, the appearance of a sieve-structure in the pit-closing-membrane. In this instance one observes the following appearance. Fixing on a favourable case in which two well defined broad protoplasmic processes are opposite one another, one can observe that between these two darkly stained ends, and traversing the pit-membrane, there is a lighter stained area which appears to bridge over the swollen closing-membrane and to unite as it were the two opposite and deeply coloured processes. The form presented by this stained portion is usually that of a flattened sphere, the diameter of each flattened end being the same as that of the pit-closing-membrane, while in the direction of its greatest breadth it exceeds this diameter in consequence of its spheroidal shape. In favourable instances, and with a high power, an appearance of striation may be detected in this stained area, the striae running in a direction parallel to the long axis of the pits, but making a curve in their course across the pit-membrane instead of traversing it in a straight line. The appearance of striation is however exceptional, and as a rule, nothing more than the colouration, and the form that such colouration assumes can be made out. Between two opposite processes with pointed or attenuate ends the same structure naturally occurs, and when the processes have contracted away from the closing membrane, it follows that a distinct interval between the deeply stained processes and the lighter stained area will occur though in successfully prepared sections one cannot help being greatly struck with the wonderful tenacity with which the protoplasm clings to the closing membranes of the pits. In the parenchyma cells where the middle lamella is well developed and resistent, this structure, even after the prolonged action of glycerine, offers some slight impediment to observation, but in the prosenchymatous

cells surrounding the vascular bundle even this difficulty is removed, for there the middle lamellae become quite invisible. They too exhibit the same structure, but no striation can be observed, and moreover on account of their great swelling the protoplasmic processes projecting into the pits are separated by an appreciable distance from the pit-closing-membranes.

From the complete reliance which may be placed upon the staining of Picric Hoffmann's blue, as a result of very numerous tentative experiments I am in a position to state that this peculiar colouration of the pit-membrane is due to the presence of protoplasm in its substance. From a careful examination of what takes place in other instances, and from a thorough comparison of this structure with numerous other structures of a like kind, one may further state that this staining is in reality caused by and is the expression of, a number of delicate protoplasmic filaments traversing the pit-membrane after the manner of the threads in sieve-tubes; that these filaments join on to the ends of the protoplasmic processes entering the pits, and that the whole closing-membrane is in fact a sieve-plate.

In formulating this statement it is important that we should clearly understand what are the exact points to be taken into consideration, in making a reliable comparison between this and other tissues. There are two principal facts to be dealt with, viz the thickness or thinness of the pit-closing-membrane and the size of the threads which traverse it. With a thick wall and a fairly thick thread we have the best possible conditions, and the whole structure will be easily seen. This actually occurs in many endosperms. Again there is the combination of a well defined thread, and a thin membrane. This state of things, which also occurs in certain endosperms is obviously much less favourable. With a thick wall and a thin thread the difficulties increase; and lastly the simultaneous occurrence of a very thin membrane and a very thin thread gives us the very worst combination, in which it is next to impossible, if not impossible altogether to see anything of any structure whatsoever and owing to the extreme thinness of the closing membrane so little colouration takes place that we cannot even define any staining, which would suggest the presence of protoplasmic filaments in that structure.

In *Mimosa* we have to deal with a case of some difficulty, for we have thin threads, and a somewhat thin closing membrane, but although the combination may be unfavourable it is certainly not at its worst, for there is a distinct and well defined colouration, and in addition an appearance of striation. In such tissues as the base of the leaf-stalk of *Prunus laurocerasus*, the pit-membranes are thicker, and in addition, the middle lamellae of many of the cells are quite invisible after swelling. Such a tissue treated by the Chlor. Zinc. Jod. method stained with Picric Hoffmann's blue, and mounted in Canada-balsam demonstrates that in the clearly stained pit-membranes a distinct striation, and even distinct threads can be seen. From

this stage to that of complete clearness of structure where the threads may even be counted, the tissues of various endosperms give many examples and offer every gradation. Moreover when those which with a high power exhibit well defined threads, are examined under a low power of the microscope, they present exactly the same appearance as *Mimosa*, the stained area has the same figure of a compressed sphere in the closing membrane, and the threads which can no longer be distinctly recognized, give an appearance of striation or only a mere colouration. Every peculiarity in the structure of the endosperm threads, such as that frequently displayed in the peculiar sweep of the threads, giving rise to a much flattened spheroid form, are all faithfully reproduced in the tissues of pulvini and the like. These considerations and results, which will become even more evident after I have detailed my work with endosperms, will I think prove that a sieve-structure does prevail, and that it is moreover the only true means whereby a protoplasmic continuity is established in *Mimosa*. The fact of protoplasmic continuity is also very greatly supported by the wonderful adhesion of the protoplasm to the base of the pit-membranes. With Chlor. Zinc. Iod., at least as far as regards Alcoholic material, the tissue of the pulvinus of *Mimosa* swells but little, and after such treatment the evidence as to the presence of protoplasmic filaments traversing the closing membrane is limited merely to a definite staining of that structure. [1])

After swelling with Sulphuric acid the pulvinus of *Robinia* displays essentially the same structure as that of *Mimosa*; but there are much clearer examples of a sieve-arrangement, for the stained area connecting to neighbouring protoplasmic processes, shows a much more evident striation. In *Amicia* on the other hand I was unable to observe any appearance of striation, but only a uniform and apparently structureless stained area. In *Phaseolus* also the evidence as to continuity is limited to a mere staining of the pit-closing-membrane.

It is also of extreme interest to note that the degree of tenacity with which the apices of the processes cling to the pit-closing-membranes in the various examples I have named, bears some very definite relation to the degree of development of the threads crossing the pit-closing-membrane.

Thus in *Mimosa* the processes projecting into the pits continue to stick to the pit-membrane even though the tension upon them, as exhibited by their extreme tenuity, is very great. In many instances indeed they stick so closely to the membrane as to produce every impression of the existence of a direct continuity between the cells; and when, owing to the protoplasm having undergone considerable shrinking, such a tension has been brought to bear upon them that rupture finally ensues, such rupture, in a very great

1) I was unable to detect any difference between the appearance presented by the upper and lower sides of the pulvinus of Mimosa.

number of cases seldom occurs at the closing membrane itself, but nearly always, on one or on both sides of this point.

In *Robinia* in the same way the processes from the main protoplasmic mass are very attenuated, although they do not appear as tense as those of *Mimosa*, neither is the appearance of direct continuity so often visible. At the same time however the apices of the processes do not contract away from the pit-closing-membrane, but abut on to, and are placed in connection with each other, by means of, the striated, stained, and flattened sphere-like structure, of which I have so often spoken.

In *Amicia* on the other hand, the processes usually contract away very appreciably from the closing membrane, so that between this stained structure, and the more deeply coloured processes, a very considerable space may intervene, while in *Phaseolus* by the most careful preparation it is almost impossible to obtain sections which demonstrate that the processes entering the pits, bear any intimate relation to the pit-closing-membrane. I may remark that these phenomena bear no direct relation to the degree of development of the pits, for the pitting which occurs in *Amicia*, for instance is much more pronounced than that of either of the other three. [1])

In the prosenchyma cells surrounding the vascular bundle, the processes entering the pits appear always to contract away from the pit-membrane, but between the two processes the same stained area occurs as in the parenchyma cells.

In the organ of movement of *Desmodium gyrans*, I have but little doubt that the same structure prevails as in *Mimosa*, but on account of the extremely small size of the cells, and of the tissue in general I have been unable to make any definite observations.

A detailed examination of the leaf of *Dionaea muscipula* showed that in the parenchymatous cells there was an actual continuity, and the pit processes entering the pits clung to the closing membranes as in *Mimosa*. In the epidermal cells I could not observe that such was the case.

The walls of the cells of the secreting glands which abut on to the general tissue of the leaf are freely pitted, and it seemed to me that in some instances I could detect the existence of a continuity between them and the cells of the leaf parenchyma, but here again I can as yet make no positive statement. In the parenchymatous tissue of the stamens of *Berberis*, I could detect a definite colouring of the pit-closing-membrane. In the stamens of *Centaurea* and *Cynara* the cells are full of protoplasm, and exhibit little if any vacuolation. After treatment with Sulphuric acid, great contraction of the protoplasm occurs, and in any two adjacent cells, the protoplasm in contracting from the transverse walls, and also from certain areas on the

1) It is of great interest to note that the degree of tenacity with which the protoplasmic processes cling to the pits is in direct relation to their degree of sensitiveness.

longitudinal walls, does so with difficulty, and presents at those places the appearance of a somewhat drawn out mass, having a broadened apex which narrows as it joins the main protoplasmic body, reminding one very strongly of the appearance presented by the contents of such sieve-tubes as those of *Vitis* and *Cucurbita* after the action of Alcohol or strong Sulphuric acid. But I could not detect any connecting filaments, nor could I satisfactorily determine that the intervening wall was stained. The broad processes when viewed "en face" presented a spotted appearance suggesting the presence of short projecting filaments, but I can at present make no satisfactory statement concerning them. My investigations as to tendrils are also still incomplete.

The tissue of the base of the petioles of various leaves, is remarkable both for the great development of the pits in the cells, and for the thickness of the closing membranes.[1] In many instances the protoplasmic processes cling very markedly to the closing membranes even when treated with Chlor. Zinc. Iod., which does not kill the protoplasm as quickly as Sulphuric acid, and there may be detected in the pit-closing-membrane either a stained area showing no striation; a striated area, or in some instances definite threads which unite the apices of neighbouring processes and thus establish a communication between adjacent cells. Thus in *Aucuba Japonica* and *Prunus lauro-cerasus* distinct threads crossing the pit-membrane may be demonstrated. In *Acer pseudo-platanus* there is a doubtful striation while in *Ilex aquifolium* and *Aesculus hippocastanum* there is only a stained area in which no structure can be made out. All these results with leaf petioles were obtained with Chlor. Zinc. Iod. and Picric-HOFFMANN's blue.

Although the results which were obtained from a study of the tissues I have mentioned above, appeared to prove with the greatest certainty, the existence of a continuity of the protoplasm between adjacent cells yet one could but recognize, that if there could be brought forward instances in which the sieve-structure could be clearly seen and the individual threads easily demonstrated, the foregoing results would not only be more thoroughly established, but would be set upon the firmest possible basis, and proved beyond all possibility of doubt. There were at the time no other generally received instances of such continuity of the protoplasm except in the case of sieve-tubes — which after all could hardly be regarded as normal living cells — and in the dead endosperm cells of *Strychnos* where the structure was somewhat different. TANGL had indeed stated, and as I shall show later on, rightly stated, that in *Phoenix* and *Areca* a sieve-structure was present, but his results in this direction were not wholly confirmed by STRASBURGER[2],

1) This was noticed by VON MOHL. See. Über die Verbindung der Pflanzen-Zellen unter einander. Tübingen 1835.

2) STRASBURGER. 'Bau und Wachsthum'. Pages 23 and 25.

who like myself was unable to see the threads, with anything like the same distinctness, that TANGL had represented in his figures, when sections of the endosperms were treated with Jodine and Chlor. Zinc. Jod.

In consequence of these considerations I determined to turn my attention to the study of thick-walled cells, and I naturally commenced with those of thickened endosperms, which on accounts of their large size and the thickness of their closing membranes, seemed to offer the most favourable conditions for an investigation of this kind and rendered it extremely probable that such threads as might exist would be easily seen. Of the structure of these cells I made a fairly detailed examination, although the conclusions which were arrived at, will admit of being shortly summed up. By means of the methods which I mentioned at the beginning of this paper I examined in detail the seeds of some fifty species of Palms, besides those of representatives of the following orders *Leguminoseae*, *Rubiaceae*, *Myrsineae*, *Loganiaceae*, *Hydrophyllaceae*, *Iridaceae*, *Amaryllidaceae*, *Dioscoriaceae*, *Melanthaceae*, *Liliaceae*, *Smilaceae*, and *Phytelephasieae* in all of which I found that the cells were placed in communication with one another by means of delicate threads traversing the walls of the cells. [1])

Palmeae.

I. *Arecineae*

Areca triandra. Roxb.
Areca Catechu. L.
Rhopalostylis Sapida. W. & D.
Stevensonia grandifolia. Duncan
Howea Belmoriana. Ben.
Kentia costata. Ben.
Caryota urens. L.
Manicaria saccifera. Gaert.
Didymosperma distichum. H. f.
Pinauga latiseeta. Bl.
Heterospathe elata. Scheff.
Cyrtostachys Renda. Bl.
Archontophoenix Cunninghamii. W. & D.
Euterpe oleracea. Mart.
Euterpe edulis. Mart.
Hyophorbe Verschaffeltii. Wendl
Synechanthus fibrosus. Wendl
Calyptrogyne Schwartzii. H. f.
Calyptrocalyx spicatus. Bl.
Chamaedorea tiuella. Wendl
Prestoea pubigera. H. f.
Ceroxylon andicola. H. & B.
Oncosperma horridum. Scem.

II. *Lepidocaryeae*

Calamus caliearpus. Griff.
Calamus fissus. Bl.
Mauritia flexuosa. Linn. f.
Pleetocomia Himalyana. Griff.
Raphia Hookeri. M. & W.
Pigafetta elata. Bece.

1) I append below a complete list of the endosperms examined. To Sir Joseph Hooker I am indebted for kindly looking over for me and arranging the list of Palms.

III. *Borasseae*

Latania Loddigesii. Gacrt.
Lodoicea Sechellarum. La Bill.
Geonoma vaga. Grisel & Wendl
Bentinekia Conda-panna. Berry.

IV. *Corypheae*

Thrinax sp.
Corypha elata. Roxb.
Licuala-Rumphii. Bl.
Livistona Hoogendorpii. T.& B.
Washingtonia filifera. Wendl
Sabal umbraculifera. Mart.
Rhapidophyllum Hystrix. W. & D.

V. *Phoeniceae*

Phoenix dactylifera. L.

VI. *Cocoineae*

Coeos nucifera. L.
Cocos flexuosa. Mart.
Baetris sp.
Astrocaryum rostratum. H. f.
Syagrus botryophora. Mart.
Martinezia Aiphanes. Kl.
Maximiliana cariboea. Gr. & W.
Desmoncus sp.
Martinezia caryotifolia. H. & K.
Guilelma speciosa. Mart.
Diplothemium sp.

Leguminoseae.

Bauhinia variegata.

Rubiaceae

Galium aparine and Asperula odorata.

Myrsineae

Ardisia crenulata and Ardisia polytoca.

Loganiaceae

Strychnos nux-vomica and Strychnos Ignatia.

Hydrophyllaceae

Nemophila parviflora.

Iridaceae

Iris pseudacorus and Xiphium.

Amaryllidaceae

Bomarea oligantha.

Dioscoriaceae

Tamus communis and Dioscorea daemonorum.

Melanthaceae

Colchicum speciosum.

Liliaceae

Ornithogalum umbellatum and Asparagus officinialis.

Smilaceae

Ruscus aculeatus.

Phytelephasieae

Phytelephas macrocarpa.

The endosperm cells of seeds which are distinguished by their horny or cartilaginous character, are usually remarkable for the great thickness of their walls and often for their large size.

In by far the greater number of instances such cells are freely pitted although some rare exceptions to this typical structure are met with, as in the cells of *Tamus communis*, and *Strychnos nux-vomica*, the walls of which are uniformly thickened, and display no pitting whatsoever. Endosperm cells display every possible modification both of their size, of the thickness or thinness of the pit-closing-membranes and degree of development of their middle lamella. Thus while they are large in such endosperms, as *Lodoicea*, *Caryota* and *Manicaria*, they are equally small in *Geonoma*, *Chamaedorea*, and *Nemophila*. The pit-membranes though extremely thick as in *Howea*, *Latania* and *Heterospathe*, are usually thinner as in *Manicaria*, *Synechanthus* or *Syagrus*, while in *Bomarea*, *Ruscus* and *Aucuba*, their degree of development differs but little from that which occurs in ordinary vegetable tissue. The middle-lamella is often very inconspicuous as in *Tamus*, *Strychnos*, *Howea*, *Bentinckia* or *Latania*, but in other cases, e.g. *Stevensonia*, *Calamus* and *Ptychosperma* it is unusually pronounced.

With regard to their cell-contents one notices that as the seed ripens, great changes take place which finally end in the death of the cell. The nucleus usually becomes diffuse in outline and at length refuses to stain with Haematoxylin, and the protoplasm begins to show great alteration, and to diminish in quantity. [1])

Aleurone-grains may be present as in *Tamus* and *Corypha*, and crystalloids as in *Martinezia* and *Diplothemium*, but more usually the protoplasm becomes scanty, and in its stead large granules make their appearance and very generally drops of oil, as in *Cocos*, *Strychnos*, *Kentia*, and *Brahea*. [2])

Finally the embryo represents the only living part of the seed, and upon germination, it simply preys upon, and gradually absorbs the dead endosperm cells, and whatever nutritive matter they may contain.

When sections of such endosperms, after having been swollen with Chlor. Zinc. Jod., and stained with Jodine — in those cases where owing to the small percentage of water present, the usual cellulose blue is not produced — or with Pieric Hoffmann's blue, are carefully examined, it can be

1) In some seeds however it may be doubted as to whether the cells are dead e.g. *Tamus*. The fact was only noted in a few typical instances.

2) In the case of oily seeds the oil must be extracted with ether before treatment with Sulphuric acid or Chlor-Zink-Jod., otherwise it will smear over the sections.

demonstrated, that a continuity of the protoplasm between adjacent cells is established, in pitted cells by means of protoplasmic filaments traversing the pit-closing-membrane and in unpitted cells by means of filaments traversing the thickness of the walls. In certain instances the filaments may traverse both the walls, and the pit-closing-membranes.

These delicate threads or filaments, like the cells themselves, may present all possible modifications, as to size, as to destribution and as to structure. Seen with the greatest ease in such endosperms as *Thrinax*, *Bentinckia*, *Stevensonia*, *Latania*, *Howea*, *Heterospathe* and *Lodoicea*, they are less clearly demonstrated in *Sabal*, *Manicaria*, *Mauritia*, *Phoenix*, *Euterpe* (Areca) and in most of the *Cocoineae*, while in *Bomarea* and *Iris* little more than a striation can be made out, and in very many of the thin-walled endosperms merely a staining of the pit-membrane. The threads may be thick as in *Heterospathe*, *Lodoicea* and *Bentinckia*, or thin as in *Manicaria Dypsis*, *Oncosperma* and *Kentia*, and similarly they may be many or few in number.

The filaments obviously traverse the pit-closing-membrane, or the general cell-wall by means of delicate perforations in these structures, and thus in the case of pitted cells the closing membranes are exactly comparable to a sieve-plate, while in unpitted cells the whole area of the cell-wall must be regarded as a gigantic sieve-structure. Although in active living cells these fine channels contain living protoplasm, yet as the cells die the same changes take place in the contents of these channels as in the contents of the cells themselves. Thus in some instances, e.g. ripe seeds of *Phytelephas*, on account of the general scantiness of the protoplasm, the channels are almost empty, and contain only a few granules which stain but slightly with Jodine, and dissolve readily in Sulphuric acid. In *Heterospathe* in the same way the protoplasm has become so altered that although it will stain quite well with Jodine, it colours with difficulty — if at all with Picric HOFFMANN's blue, while in other instances where the modification is not so great e.g. *Bentinckia*, *Kentia*, *Lodoicea* the same well-defined blue staining of the threads occurs, which takes place equally in all normal protoplasm.

Having thus dealt in a somewhat general manner, with some of the characteristics common to endosperm cells, and the threads which traverse their walls, I will proceed to describe a few special examples in order to give some idea of the structure that a treatment with Chlor. Zinc. Jod., and Jodine or Picric HOFFMANN's blue, brings into view. *Latania Loddigesii* may be taken as a typical example of an endosperm in which communication between adjacent cells apparently takes place through the pits only. The protoplasmic threads which can be demonstrated either with Jodine, or with Picric HOFFMANN's blue are plainly seen to traverse the pit-closing-membrane.

The whole figure of the thread-complex as seen in longitudinal section

is that of a flattened sphere, the individual threads traversing the pit-membrane in a direction parallel to the long axis of the pit. The most external threads on either side of the thread complex in passing across the pit-membrane, bend out in a graceful curve, reminding one of the meridian lines represented on a globe, except of course that they do not converge so as to meet at a point as the meridian lines do at the north and south poles, but end bluntly at the intercepting free surfaces of the pit-closing-membrane. As one gradually approaches an imaginary line joining the centres of the two free surfaces of the pit-membrane, the curve of the threads becomes less and less, until immediately around this line, their direction becomes practically straight. In an en face view of the pit one sees the ends of the threads as small stained points, dotted over the closing membrane, and furthermore the well-defined circle of the pit itself appears to be surrounded by a less well defined and concentric circular area, which is the expression of the bending outwards of the threads; the outline of this area marks the limits of the curve of the most external of the threads; and in it, the separate threads of the thread-complex can be observed curving upwards towards the free surface of the pit-closing-membrane.

In unpitted cells in the same way e.g. *Tamus*, *Dioscorea* and *Strychnos* the threads do not run in a perfectly straight direction across the thickness of the cell-wall, but in each face of the wall, along which it is in contact with neighbouring cells, they become more curved the further they are from the central point of the face, in the very same way, as in the achromatin fibres observed by Strasburger, in the nuclear division attending free cell formation. Finally in certain instances, e.g. *Bentinckia*, *Howea*, *Lodoicea*, *Kentia* and *Asperula* both these means of communication are exemplified, for the pit-membranes and the general cell-walls too are traversed by protoplasmic threads. In *Hyophorbe*, *Livistona* and *Wallichia* the threads do not appear to curve, but traverse the closing membrane, in almost straight lines.

In *Euterpe* (*Areca*) *oleracea* and *Phoenix dactylifera*, I was unable to find that the threads were well defined after treatment with Chlor. Zinc. Iod., and Iodine, but after staining with Picric Hoffmann's blue, or after treatment with Sulphuric acid and Methyl violet they came quite as clearly into view, as Tangl represented in his figures of them.

In *Ornithogalum* also I was able to confirm Strasburger's results, and I have no doubt that the same structure would be equally well demonstrated in the cells of *Taxodium distichum*, and *Viscum album*. [1])

In *Bomaria oligantha* the closing membranes of the endosperm cells are somewhat thin, and the separate threads are hard to observe in longitudinal section but in an en face view of the pit the sieve-structure is clearly

1 See. 'Bau und Wachsthum'. Taf. I. Fig. 17 and Taf. II. Fig. 29.

visible. In *Aucuba Japonica*, and many other thin walled endosperms there can only be demonstrated a definite staining of the pit-membrane.

In Strychnos neither TANGL nor myself have been able to detect the presence of threads traversing the cell-wall in the layers of cells immediately beneath the free surface of the seed, but in *Tamus*, they can be seen in all the cells equally. Again in *Kentia*, *Howea*, and *Lodoicea* the threads traversing the general cell-walls could not be made out in the cells situated towards the centre of the endosperm tissue, but only in the more exterior layers, while in *Bentinckia* they can be demonstrated in a section of any part of the seed. In many instances it is a matter of some difficulty to observe that the threads actually cross the middle lamella, but in those cells in which that structure is but little developed, such as *Heterospathe*, *Latania*, *Lodoicea*, *Bentinckia*, *Tamus* &c the undoubted perforation of the middle lamella is plainly and conclusively evident. In ripe endosperm cells, the protoplasm has become so altered, or is so small in quantity, that it frequently shrinks from the cell-wall and it does not appear as if the threads traversing the closing membrane, were really continuous with the protoplasmic processes entering the pits, or in unpitted cells with the general protoplasmic body; but if living cells be taken, and after having been swollen with Sulphuric acid (which at once kills and fixes the protoplasm) are stained with Picric HOFFMANN's blue, it will be apparent that the threads do unite with the general cell-protoplasm: that they are continuous with the pit processes, and that moreover these processes are in consequence actually held on to the pit membrane for example, in *Archontophoenix* or *Rhopalostylis*.[1])

The threads as demonstrated by Jodine are more distinctly brought into view than with Picric HOFFMANN's blue and they also appear decidedly larger in size. The latter phenomena seems to be produced in consequence of the fact that the Chlor. Zinc. Jod. besides marking out and intensifying the staining action of the Jodine upon the actual threads themselves, gradually precipitates in virtue of its dehydrating properties the Jodine filling the capillary tubes. Thus Jodine appears to demonstrate the actual size of the channels, in addition to staining the threads which in ripe dead seeds have undergone a definite amount of shrinking, whereas Picric HOFFMANN's blue in any case demonstrates the actual size of the threads alone. Moreover it appears as if there was even a further aggregation of precipitated Jodine around that already known down, for the channels appear abnormally large, and consequently the threads abnormally thick and the fact that in sections which have been first treated with Chlor. Zinc. Jod., and subsequently stained with Jodine, the threads have appreciably diminished in thickness, and appear less strongly defined certainly affords some evidence that such

[1]) This completely confirms my results with Mimosa, Robinia, &c.

a precipitation of Jodine actually occurs. Under this treatment threads present much the same appearance as with Picric Hoffmann's blue. Frequently the threads are not of an uniform size, but are thicker near the middle lamella, than near the free surface, but I am disposed to think that this is due to the greater swelling of that portion of the wall which adjoins the general cell-cavity. The pores or channels traversing the pit-membrane are extremely fine, and my attempts to inject a coloured liquid into small pieces of the endosperm which, by means of an india rubber cork, were connected with a mercury manometer, and exposed to a very considerable pressure, met with no success. Although I employed an alcoholic solution of Aniline Blue in order to avoid swelling up the wall and thus closing the channels I could with the endosperms of *Latania* and *Calamus*, obtain no injection whatever of any of the delicate pores.

As far as I was able to observe, there appeared to be no connection between the tissue of the embryo, and that of the general endosperm. In germinating seeds e. g. those of *Kentia*, and *Phytelephas*, the gradual breaking down of the tissue under the action of the ferment, which is probably derived from the cells of the encroaching foot or feeder can be followed with ease. While the uninjured cells give with Jodine and Chlor. Zinc. Jod. the usual yellow colour of the dry seeds, the cells whose walls are undergoing degeneration, assume the ordinary cellulose blue, most probably in consequence of increased hydration. Moreover the changes are propagated from cell to cell through the medium of the delicate channels which become widened out and finally break down altogether. This occurs with regard to the channels traversing the general walls, and to those of the pit-closing-membrane also.

In spite of almost conclusive appearances pointing to the fact that the threads consist of protoplasm, I felt that some other definite proof was wanted to show that such was really the case. The staining with Jodine cannot be taken as a proof, since besides its multifarious staining properties, it demonstrated that all the threads, no matter how altered from their original protoplasmic character, were equally coloured. The staining effects of Picric Hoffmann's blue may I think be taken as quite conclusive for as I pointed out, this reagent fails to colour even protoplasm when much altered. In consequence of these considerations I had recourse to the solution of Molybdic acid in Sulphuric acid: the staining of which I think further demonstrates beyond doubt that in living cells these threads consist of protoplasm.

Observations on Plasmolysis.

In order to see whether a study of living cells would afford any evidence, confirmatory or otherwise, of that close relation existing between the protoplasm and cell-wall, which my results had demonstrated, I com-

menced to investigate in as complete a manner as possible the phenomena attending Plasmolysis. It had been long known that when living cells are exposed to the action of some strong dehydrating agent such as dilute acids, dilute Chlor. Zinc. Jod., strong sugar or salt solutions, the protoplasm often appeared to separate with difficulty from the cell-wall, or was held on to the wall at certain points by fairly thick protoplasmic processes. Thus VON MOHL[1]) had remarked that upon treatment with acids his primordial utricle adhered to the cell-wall,[2]) PRINGSHEIM in Fern prothalli, in *Riccia*, *Vallisneria* and *Cladophora* also remarked that the protoplasm after treatment with dilute Chlor. Zinc. Jod. or strong sugar solution, separated with difficulty from the cell-wall and was often drawn out into strands which still clung to that structure. NÄGELI,[3]) and also HOFMEISTER[4]) in the case of *Spirogyra*, and various filamentous algae established the same fact. But after such treatment the protoplasm is gravely affected, often appearing partially coagulated as it were, and subsequently dies. It was HUGO DE VRIES[5]) however who most fully investigated the action of dehydrating agents on the living cell, and by his important results materially increased our knowledge of general cell mechanics. He employed only dilute solutions of such a strength that while they brought about the condition of Plasmolysis, they exercised but little hurtful influence on the protoplasm itself. This observer found that when living cells are treated with progressively stronger solutions of some neutral salt e.g. 4, 6, and 10 per cent of nitre, the protoplasm shrinks from the cell-wall until at length it appears as a much contracted spherical mass lying freely in the cell cavity. But in repeating DE VRIES' experiments I found,[6]) in every instance I examined, that the contracted primordial utricle does not lie free but is always connected to the cell-wall by innumerable fine protoplasmic strings. This discovery was also subsequently and independently confirmed by BOWER[7]) who was experimenting on plasmolysis for a very different object, namely that of finding whether the inducing of a plasmolytic contraction of the protoplasmic body would be a good method for preparing the apical region of the prothallus, so as to show the form and arrangement of the individual cells.

I employed as dehydrating agents solutions of common salt of the following strengths viz. 2.5 p. c., 5 p. c. and 10 p. c. and I was able to demonstrate not only that by the action of strong solutions the protoplasm suffers apparent partial coagulation, separates with difficulty from the cell-

1) VON MOHL. Vegetable cell. English translation. p. 37.
2) PRINGSHEIM. Bau und Bildung der Pflanzenzelle. 1854.
3) NÄGELI. Pflanzenphysiologische Untersuchungen, 1883. Heft I.
4) HOFMEISTER. Die Pflanzenzelle, 1867.
5) H. DE VRIES. Unters. ü. die mechanischen Ursachen der Zellstreckung. Leipzig 1877.
6) GARDINER. Royal Society Proceedings. Nov. 14. 1882.
7) BOWER. Quart. Journ. Micr. Sci. Jan. 1883.

wall, and is then frequently connected to the cell-wall by somewhat thick strands in addition to the finer ones which may also be present, but also that when the plasmolytic condition is more gradually induced by the use of dilute solutions the contracted protoplasmic body remains connected to the cell-wall by excessively fine strands which may at first be invisible, but subsequently come into view. The former plasmolysis is that which was described and observed by von Mohl, Nägeli, Pringsheim and Hofmeister. In such instances the protoplasm suffers very grave injury, as evidenced from the fact that if by washing with water, one attempts to bring back the protoplasm into its normal relation with the cell-wall, great displacement of the general protoplasm and of the chlorophyll-grains occurs and the protoplasm further becomes swollen and disorganised. In the case of the plasmolysis induced by dilute solutions but little recognizable change is produced and on washing out with water the cell assumes its ordinary normal appearance.

Naturally the phenomena produced in consequence of the action of strong Sulphuric acid are due to plasmolysis in its coarsest form, but the same kind of plasmolysis may be partially induced even by less powerful reagents. Thus on treating a section of most tissues, e.g. a transverse section of the pulvinus of *Phaseolus multiflorus* with a 10 p.c. solution of common salt, the protoplasm will be observed to contract away from the cell-wall until finally it appears as a spherical mass which is connected to the cell-wall by several fairly thick strings of protoplasm.

In other cases instead of contracting as one main mass, it may stick to the cell-wall at certain points, and in the subsequent contraction which ensues, it may become divided into two or even three masses of varying size. All these masses rapidly assume a spherical outline and it is usually easy to see, that they are connected to the cell-wall and to each other by obvious protoplasmic strings. The threads may either be perfectly uniform or may exhibit here and there a nodular thickening of a spherical form. Subsequently many more fine threads will come into view. If the salt be washed out with water the protoplasm may again be brought to fill out the cell, but at the same time pronounced disorganisation of the protoplasm is observed to have taken place and obvious abnormal swelling also occurs. An examination of the cells of *Spirogyra* when thus treated will at once convince one that this is actually the case, since here the distortion and displacement of the chlorophylle bands is very obvious, and marked.

If however the plasmolysis be brought about with a 5 p.c. salt solution the contraction of the protoplasm is much more gradual. It contracts with great regularity into a single rounded mass and usually appears at first to be perfectly free from the cell-wall. But after a time there gradually appears as Bower well observes "a faint striation in the space between the protoplasmic body and the cell-wall running in a radiating manner between

them" which finally gives way to an appearance of numerous and extremely delicate threads which in the course of some 15 or 20 minutes after the addition of the salt solution come plainly into view. They are highly refractive, frequently nodulose and usually simple threads, although in some instances they may be bifurcated near their apex. At first very tense, they gradually become more and more slack, and are finally so loose, that they execute lateral vibratory movements, probably caused by water currents due to differences of temperature. While in the tense condition they may rupture, and then each free end contracts, the one to the main mass and the other to form a minute sphere lying on the side of the cell-wall. After death their refractive index appears to alter, and they become ropy instead of brilliant and sharply defined. It is the existence of these finer threads which were discovered by BOWER and myself.

The attempts made to fix these plasmolytic figures did not meet with much success, although if a section which has been treated with a 10 p. c. salt solution, be rapidly washed in water, treated for some time with saturated watery Picric acid, and gradually transferred into weak and gradually stronger alcohol, it may be finally stained with aniline blue with the thicker threads fairly fixed.

Naturally my great object was to endeavour to find whether in pitted tissue these threads bore any relation to the pits, but after many observations I came to the conclusion that this was not the case. Very frequently when the plasmolytic condition is induced by the action of strong salt solution (10 p. c.) it can be seen that many of the thicker threads go to the pits, and also that in two adjoining cells many threads on different sides of the common pit-membranes are exactly opposite one another. Again in stained sections the same fact can be demonstrated. However I find like BOWER that in as many if not in more instances the strings bear no relation whatever to the pits, and since the above phenomena attending plasmolysis take place as far as I am aware in all cells alike, it follows that in such cells as those of the epidermis or of filamentous Algae as many strings run to the free walls as to those which separate adjacent cells.

In the coarser plasmolysis as caused by very strong reagents such as Sulphuric acid, Chlor. Zinc. Jod., or Alcohol, I believe that the sticking of the threads to the pit-membranes, as observed by HOFMEISTER [1]) in *Spirogyra*, by DE BARY [2]) in the sieve-tubes of Vitis, by BOWER [3]) in the spicular cells of *Welwitchia* and myself in the numerous instances I have cited in this paper, does afford some evidence in favour of the existence of a continuity of the protoplasm between of adjacent cells.

1) HOFMEISTER. l. c.
2 DE BARY. Vergl. Anat. p. 186.
3) BOWER. Quart. Journ. Micr. Sci. Jan. 1883.

It now remains for me to put forward some explanation of the phenomena of Plasmolysis. There are two questions to be answered. The first is, why is it that the main protoplasmic mass when contracted from the cell-wall remains connected with it on all sides by delicate protoplasmic strands? The second is, what is the explanation of the fact that these strands are at first invisible, and then gradually come into view? It will be observed that I am dealing here only with the Plasmolysis caused by dilute solutions of a neutral salt.

To explain the first question Bower suggests two views — "1) that the main mass of protoplasm on retreating may leave the cell-wall still completely lined with a thin layer of protoplasm; 2) that the peripheral part of the protoplasm being entangled as a network among the deposited microsomata, may on contraction of the main mass be drawn out at the points of entanglement into fine strands like those observed, while the surface of the wall is for the most part left free and not covered by a film of protoplasm."

Unfortunately neither of these views admit of being practically tested. Although a careful examination of most delicate sections of material which has been plasmolysed, fixed with Picric acid, and stained with Hoffmann's blue does not demonstrate the existence of such a thin layer, but only shows the little spherical masses which are either connected with a strand going to the main mass, or are formed in consequence of the rupture of strands, and demonstrates moreover that these masses are sharply defined from the cell-wall, yet it is perfectly possible that such a delicate layer might be present and yet be invisible. Again if the cell-wall be actually formed by the apposition and coalescence of microsomata as described by Strasburger, it is not impossible as Bower points out that some portion of the peripheral protoplasm may be entangled as a network among the deposited microsomata. But whether this be so or not I am of opinion that a still simpler explanation may be given. To borrow a simile from physics, the cell-wall is so perfectly wetted, so to speak by the protoplasm, and at the same time this latter body is so extremely plastic, that it appears not improbable that when Plasmolysis is induced, the protoplasm while separating at certain points, from the cell-wall, adheres strongly to it at others, and is thus drawn out into a number of delicate strands, in the same way as a piece of stringy mucus adhering to the side of a glass tumbler may be drawn out into strands of great tenuity. That particular combination of forces which exists at the time, determines which part shall adhere and which shall come away.

As to the second question Bower again puts forward two explanations. The increase in thickness of the strands may be produced "1) by the drawing out of a fresh supply of substance from the main protoplasmic body or 2) by the lateral coalescence of originally separate strands." I am disposed to think that in certain instances the latter phenomenon may occur, but it

seems to me difficult to imagine by what means a drawing-out of fresh substance from the main protoplasmic mass is occasioned. My view is as follows. When the cell is acted upon by the salt solution, the contraction of the primordial utricle which takes place, is caused by the fact that there is a rapid diffusion of the less concentrated cell-sap of the vacuole into the more concentrated salt solution. Now the water passes from the vacuole into the salt solution much more quickly than does the salt solution into the vacuole. Finally the protoplasm becomes for the time abnormally shrunken in consequence of the rapid progress of the dehydration and while in this state the strands connecting it to the cell-wall, will be at their maximum degree of tenseness, and will be drawn out to such a degree of tenuity as to be invisible. But after a time when the diffusion is beginning to cease, and the entire solution tends to assume a uniform specific gravity, a certain quantity of the salt solution will pass back into the shrunken protoplasm to supply the place of the water which it had so violently abstracted: the whole body will swell, the strands will become less tense and at the same time will stricken, and in so doing will gradually come into view. Finally owing to the further expansion on the part of the protoplasmic body they will become so slack as to admit even of lateral vibratory movement.

It might be supposed that these strands of attachment, as we may term them, which are thus brought into view in plasmolysed cells are held in position, in consequence of their being connected, in the mode described above, with similar stands in neighbouring cells. This is doubtless true in some cases, but by no means in all: for the strands of attachment in the case of any one pit are more numerous than the filaments actually perforating the closing membrane, and in the case of an unpitted cell-wall like that of *Tamus* they far exceed in number the filaments which actually traverse the wall.

It is obvious that in Plasmolysis the perforating filaments in the cell-wall, or pit, will tend to be pulled out of their channels and there is no reason why the strand coming from such a filament should appear different from those coming from the general and apparently imperforate cell-wall. At any rate a careful examination of the plasmolysed cells of many endosperms, and other like tissues, gave no clue as to there being any discernable difference between the threads.

In the coarser Plasmolysis induced by the action of powerful reagents the protoplasm is soon coagulated, and killed, and hence the assumption by the contracting protoplasm, of the rounded form, and the formation of strands of attachment between protoplasm and cell-wall is prevented. The protoplasm now remains in a passive condition and is mechanically held to the wall at those points where the most intimate relation between the two exists.. Such shrinking as occurs is the expression of the rapid dehydration of the vacuole, but only those portions of the protoplasm contract which are

the least intimately connected to the cell-wall, and the shrinking does not extend to the whole protoplasmic body.

III. Criticisms and Conclusions.

As may be readily imagined there are other observers besides myself who have investigated the subject of the continuity of the protoplasm between the walls of adjacent cells. On TANGL's results and RUSSOW's most excellent paper I have no observation to make, since it is hardly necessary for me to say that I regard them as in every way satisfactory and conclusive. It only remains for me to criticize the papers of three other investigators viz. FROMMANN[1]), ELSBERG[2]) and HILLHOUSE[3]). As I have already dealt at some length with the investigations of FROMMANN and ELSBERG in my paper "on some recent researches on the continuity of the protoplasm through the walls of vegetable cells"[4]), I need here only allude to the principal points which these observers attempt to establish, and at the same time give my own results and opinions upon them. Briefly stated the principal facts involved in FROMMANN's statements are: That open passages of very appreciable size are of very frequent occurrence in the common cell-wall: That Chlorophylle corpuscles and protoplasmic reticula occur imbedded in its substance: That the intercellular spaces may contain protoplasmic granules and networks: That these networks of protoplasm may be traced into the cell-wall, and are particularly clearly defined in the case of the epidermal cells, running from the cell lumen out into the cuticle. Although I was aware that every one of these statements would be received with some surprise by almost any botanist who is at all acquainted with the histology of tissues, I investigated in as careful a manner as possible those particular tissues in which Professor FROMMANN had obtained his most favourable results namely in the leaves of *Rhododendron ponticum* and *Dracaena draco*. Having shown that, as far as I was able to observe on treatment with Jodine and Chlor. Zinc. Jod., a pit-closing-membrane was present, I pointed out the extreme improbability both on morphological and physiological grounds that chlorophyll-grains should be imbedded in the substance of the cell-wall, and mentioned that it was hardly necessary to state that after the most careful examination no such case was observed. Numerous preparations treated and stained in various ways, showed no signs of their being either granules or nets or finally any protoplasmic structure whatsoever in the intercellular spaces. I then dealt with the possibility of following the protoplasmic structure into the substance of the cell-wall. Since Professor FROMMANN's obser-

1) FROMMANN. Beob. über Structur und Bew. d. Protoplasma der Pflanzenzellen Jena 1880.

2) ELSBERG. Quart. Journ. Micr. Sci. Jan. 1883.

3) HILLHOUSE. Bot. Central XIV. 1883. In Nr. 89—94. 124. 4.

4) GARDINER. Quart. Jour. Micr. Sci. March 1883.

vations in this direction were as far as one could see from the text, made upon sections of material which were simply mounted in expressed cell-sap, sugar solution, or dilute glycerine, one obviously comes to the conclusion that his results were obtained with little previous preparation of the tissue. Alluding to the results of TANGL and myself who had both of us failed to detect the presence of protoplasmic threads in cell-walls without at least previous swelling, I stated that I was quite unable to see any network or reticulation of any kind in the epidermal cells of *Rhododendron* and *Dracaena*, but at the same time I described appearances in those cells, which had probably misled Professor FROMMANN, namely that in the upper and side walls of the cells of *Dracaena*, what appears to be a reticulate structure can be observed, but that such structure was caused by the presence of a number of waxy granules imbedded in the cuticle which were appreciably acted on by ether or boiling Alcohol and dissolved in a 5 per cent solution of Potash.

In *Rhododendron* in the same way there is a distinct striation of the cuticularised layers, which cuticle however does not abut immediately on to the cell cavity, but is separated from it by a thin layer of unaltered cellulose.

ELSBERG had also noted open pits in the cells of the petal of *Nierembergia gracilis* and in the cells of the petiole of *Ficus elastica* he had found that "what has been sometimes described by authors, especially in growing tissues, as intercellular spaces and middle lamellae in the cellulose, were revealed to be in a number of instances, accumulations of living matter wedged in between the plant cells." These results were obtained by the use of Gold Chloride and Silver nitrate. The latter reagent gave him the best results and he observed that when a section of the petiole of *Ficus* had been thus treated, and exposed to light, the cell-walls were seen to exhibit a number of exceedingly small dark stained areas (cellulose) in which was a reticulum of non-staining protoplasm. I showed first of all that this staining was only confined to the cut surfaces and was not present in the entire thickness of the wall, and that further the whole appearance could be entirely removed by brushing the sections with a camel-hair brush. Finally I demonstrated that the patches were granules of reduced silver, which had been thus reduced by certain of the cell contents, which I showed to be tannin. I also showed that as far as my results had been carried I was forced to conclude that Silver nitrate and Gold Chloride as usually employed, were unsatisfactory for botanical research, and had given me no assistance in the study of the continuity of the protoplasm.

It only remains for me now to deal with HILLHOUSE's paper. The method employed by this observer was briefly as follows. Sections of fresh material or of material which had lain for some days in absolute alcohol, were cut with a razor wetted with alcohol. They were then placed on a slide and treated for some minutes with dilute Sulphuric acid which was afterwards

removed by means of a pipette, and in its place was added a small quantity of concentrated acid in which the section was allowed to remain for a period of from 20 to 48 hours. With the section still lying on the slide, the acid was removed; the section was well washed in distilled water and stained either with Jodine or Ammonia Carmine. These preparations were made from the cortical tissue of the stem or of the base of the leaf of various plants, but the tissue of *Prunus*, *Ilex*, *Acer* and *Aesculus* were those which were principally studied in detail.

Stated generally this observer found that after such treatment although in some cases the protoplasmic processes projecting into the pits could not be traced further than to the base of the pit-membrane, yet that in many instances it could be demonstrated that the processes of adjacent cells were directly continuous through the pit-closing-membrane. In one instance (*Prunus*) however, he noticed that in a case where the acid had only been allowed to act for a short time, the processes appeared not to be directly continuous, but to be united by means of a sieve-structure which perforated the closing membrane.

Before criticizing Mr. Hillhouse's conclusions I should like to say a few words about his methods. To an external observer, whether chemist or botanist, the exposure of such a delicate structure as that of a thin section of vegetable tissue, to the action of such a powerful reagent as strong Sulphuric acid, for periods varying from 22 to 48 hours, would I think appear to be somewhat severe treatment, and its lengthy use implies no little confidence on the part of the author in the solidity of the connecting filaments of the protoplasm by means of which such a continuity might be expected to be maintained. My own experience certainly leads me to believe that after treatment with concentrated acid for a much shorter time than that mentioned above, the protoplasmic processes entering the pits would certainly be attacked, and much more, any protoplasmic filaments of a still more delicate nature. In fact if one watches the action of strong Sulphuric acid containing Molybdic acid in solution, in order that the protoplasm may be stained, and brought more clearly into view, on sections of the living endosperm cells of *Tamus communis* it is seen that the threads begin to be acted upon in less than 10 minutes, and certainly in a half an hour, hardly any trace of them can be detected, and the same thing occurs in sections which have been treated with Sulphuric acid alone, and subsequently stained. Another objection is that although such lengthy treatment may dissolve the cell-walls, in so doing it causes the approximation of layers of cells which were before an appreciable distance apart (being kept apart by their cell-walls) and thus causes the protoplasmic contents of such cells to be in a like manner approximated; to lie in the same plane; and even to overlap one another, either bodily, or by means of their protoplasmic processes. The whole section is so soft and non-resistent that a very slight movement of

the acid, much less washing with water is liable to cause very sensible displacement of the tissue. In the case of resistent middle lamellae these structures swell and stain. Again my results as to the action of Carmine, whether Ammonia or alum Carmine, point to the fact that it has but little selective powers, staining at the same time both the protoplasm and the cell-wall, and I therefore am forced to regard it as unsatisfactory for such an investigation as the present. Finally where one relies on the fact of the clinging of the protoplasmic processes entering the pits, to the pit-membrane, it is a mistake to use either alcohol material or a razor wetted with alcohol since by so doing the protoplasm is rendered rigid and brittle, and it can neither be drawn out into strands, nor will it adhere to the membrane when any appreciable tension is set up.

I therefore worked over Mr. HILLHOUSE's results with the greatest possible care. I may state at the outset that the material he employed is in many respects extremely favourable, both on account of the conspicuous development of the pits, the comparative thickness of the pit-membrane and the non resistent character of the middle lamellae. In this respect both *Prunus* and *Acer* deserve especial notice.

Naturally Mr. HILLHOUSE's view of a direct continuity necessitates the existence of a small perforation in the pit-membrane. What strikes one at first on looking over his figures of Prunus is that if figure 4 which represents two somewhat swollen processes on opposite sides of the pit united by a sieve-structure traversing the pit-membrane, be true, and if he obtains such a structure by the cautious and regulated action of Sulphuric acid, how is it that when the acid has been allowed to act for a much longer time, he obtains the appearance of direct continuity, unless indeed we are to suppose that both means of communication are present in one and the same tissue. But on the whole, one is led to infer that the direct continuity is the typical structure.

In order to examine the matter for myself I thoroughly investigated the structure of the cortical tissue of the base of the leaf, and of the stem, in *Prunus*, *Ilex*, *Acer*, *Aesculus* and *Aucuba Japonica* which latter is perhaps the best material of all. I found that after the lengthy action of the acid, the difficulties attending manipulation and observation were very great. After a treatment of 24 hours with Sulphuric acid, I found that very thin sections were usually so disintegrated and displaced as to prevent any satisfactory examination of them. The thicker sections are more resistent, and on the whole the protoplasm withstands the action of the acid, much better than one might expect. In many cases the processes are almost entirely dissolved, and the protoplasmic bodies present a spherical form, having projecting from them at certain points a few extremely short protrusions. In other instances the processes remain fairly intact, their apices ending bluntly in a somewhat swollen rounded extremity as Mr. HILLHOUSE has

described. Here and there stretching across the tissue, or between the contents of neighbouring cells were a number of what one might fairly call slime strings, produced apparently by the violent action of the acid upon the protoplasm, and although in many cells the middle lamellae were almost invisible, in other parts of the tissue they appeared swollen up, and became stained with Iodine, or Carmine, although less so with Picric Hoffmann's blue. In nearly every instance, and certainly in the great majority of instances, no trace of any direct continuity could be detected. In others the close approximation of two attenuated processes at first sight seemed to suggest such an appearance as Mr. Hillhouse has described, but careful focussing usually determined, either that no actual union occurred, or that the processes came from cells which overlapped and were not in the same plane. In fact in no one clear and undoubted instance was I able to satisfy myself of the existence of a direct continuity, but it appeared to me that the processes ended at the pit-membrane.

I then proceeded to treat sections according to my method, mounting them either in Canada balsam or in glycerine as occasion seemed to require. Such treatment as far as I could observe, proved conclusively, that in all the tissues examined the processes entering the pits could only be followed as far as to the pit-closing-membrane and that they were clearly and distinctly bounded, by that structure, although in every case the processes of adjacent cells were connected through the pit-membrane by a lighter stained area. In *Prunus* and *Aucuba*, this area presented the well known form of a flattened sphere, and in it distinct threads could be seen. Hence here a sieve arrangement is present. In *Acer* merely a doubtful striation could be detected, and in *Ilex* and *Aesculus* merely a staining in pit-membrane.

Consequently as it seems to me, the one point which is satisfactorily proved in Mr. Hillhouse's paper, is that in *Prunus lauro-cerasus* a continuity between the contents of adjacent cells is established by means of delicate protoplasmic filaments which in the manner of a sieve-structure perforate the pit-closing-membrane.

As regards the occurrence of a direct continuity, both in *Prunus* and in the other tissues, I can neither confirm his results nor do I believe them capable of confirmation: and although by his own researches he has not demonstrated the existence of a continuity of the protoplasm in *Ilex*, *Acer* and *Aesculus*, I have shown that such a continuity does exist and that it is made possible by means of delicate filaments which in the manner of a sieve-structure traverse the pit-closing-membrane. There is a minor point that the threads do not appear to me to go as Mr. Hillhouse draws them, straight through the closing membrane so as to cause the whole thread complex to assume the form of a cylinder, but they bend in the way I have so often described so as to assume the form of a flattened sphere.

Final conclusions.

I will now briefly sum up my principal results. At the outset I may point out that they have all been obtained with fresh material, which appears to me to considerably increase their value; although as I have shown alcohol material may be employed. I am aware that most of the observations have been made upon thick-walled tissue, in consequence of the fact that the thinner the pit-membrane the more difficult does observation become.

But in allied genera and species, some of which show the existence of a continuity, and some do not, there seems to be but little doubt that the same structure is in reality common to them all.

As regards the modes in which the continuity of the protoplasm of adjacent cells is maintained, I have established in the material upon which I have worked.

1. That in pitted cells the pit-membrane is traversed by numerous delicate protoplasmic filaments, connecting the protoplasmic processes which occupy the pit-cavity, on each side of the common closing membrane:
2. That in unpitted cells e.g. *Tamus*, *Strychnos*, *Dioscorea*, very delicate protoplasmic filaments traverse the cell-wall throughout its whole extent. Such cells are however of rare occurrence;
3. That in some cases, e.g. *Lodoicea*, *Bentinckia*, *Howea*, and *Kentia* in which the cells are pitted, these delicate protoplasmic filaments traverse not only the pit-membrane, but also the general cell-wall;
4. That the significance of pits is to afford a means of communication between adjacent cells through the agency of the porous pit-membrane.

From the observations mentioned under section 3 I am inclined to believe that the passage of protoplasmic filaments through the unpitted portions of cell-walls is by no means uncommon, but the demonstration of this is difficult owing to the extreme fineness of the filaments. As regards the significance of the various structures described, I am of opinion that in all cells whatsoever the walls are perforated, and that the perforations are traversed by protoplasmic filaments. Thus sieve-tubes must be regarded as merely special examples of a general structure.

Taking all the cases, in which the passage of protoplasmic filaments from cell to cell has been demonstrated we find that they present great variation as regards the size of the channels. The largest and coarsest form is afforded by such structures as sieve-tubes; next come the perforations in endosperm cells in which the whole structure is more delicate, and finally the perforations in pulvini and the like, which are excessively fine. But the general principle or type of structure is the same throughout, though in some cases the perforations are confined to limited areas of the cell-wall

(sieve-plates, pits) and in others the whole cell-wall is perforated, constituting one large sieve-structure.

The physiological significance of such communication between adjacent cells appears to me not to be the same, in all cases. I am led to the conclusion that the sieve-structures of endosperm cells like those of sieve-tubes serve as channels for the passage of food material, and are probably of equal importance during germination in connection with the transference of unorganised ferments, while as regards the filaments in such tissues as pulvini, their chief significance is that by their means the protoplasm of isolated cells becomes connected and that thus the communication of impulses from one part of the plant to another is insured. For instance there can be little doubt that the conduction of a stimulus, which can be readily observed in the leaves of *Mimosa pudica* is effected by this means.

The presence of these very minute perforations in the cell-wall need not lead to any modification of the generally accepted ideas as to the mechanics of the cell, more particularly with regard to the maintainance of its turgid condition. It must be borne in mind that the turgidity of a cell depends upon its protoplasm, and so long as this forms a perfectly closed sac, the cell can be turgid. In the case before us, the protoplasm does constitute a closed sac, for the filaments by which the protoplasmic bodies of neighbouring cells are connected, are solid.

In conclusion it only remains for me to perform the pleasant duty of thanking those who have given me their help during this investigation, and first I would acknowledge the debt of gratitude that I owe to Professor Sachs for his uniform kindness and consideration to me during the time I was working in his laboratory. The fact that it was at his suggestion that I commenced this work will alone be sufficient to show how great that debt is. From my friend and teacher Dr. S. H. Vines I have received constant assistance and advice all through this most difficult investigation. To my friend Dr. D. H. Scott I am also indebted for much valuable aid and criticism.