"The Histology of the Cell Wall, with Special Reference to the Mode of Connexion of Cells. Preliminary Communication." By WALTER GARDINER, M.A., F.R.S., Fellow and Bursar of Clare College, Cambridge. Received August 11, 1897.

Since 1883* I have repeatedly endeavoured to discover some refined and generally applicable method by means of which the fine fibrillæ, or "connecting threads," traversing the cell membrane might be identified with certainty, and the fact of their existence settled beyond dispute. I was also anxious to be in a position to investigate the development of the threads in endosperm tissue. My researches met with little encouragement until 1894, when I succeeded in finding a new method, by means of which I obtained excellent results with the young developing endosperm tissue of *Tamus communis*. This I have further elaborated, so that either the original method, or modifications of it, can be applied to tissues generally. In the present communication I propose to give a brief account of my researches, leaving a more detailed description to a future occasion.

The methods used by earlier observers for the investigation of connecting threads are essentially based upon those of Sachs and of Hanstein, by means of which they demonstrated the characteristic structure of sieve-tubes. Tangl's important results of 1880, which were confirmed and extended by myself in 1883, were in fact obtained by Hanstein's method as such, and are the outcome of quite special conditions, and a happy combination of circumstances, depending upon the fact that in dry ripe seeds the tissue is so poor in water that with the iodine and Schulze's solution (Chlor. Zinc. Iod.), the cellulose fails to give the usual blue colour, and thus allows the darkly stained threads to come into view. The method ceases to work with unripe seeds, or even with ripe seeds which contain a certain percentage of water, and with ordinary tissue is quite useless.

Certain modifications of the method of Sachs, and the method of Hanstein, which may be described in general terms as involving both a more regulated application of the swelling agents and the use of aniline dyes in place of iodine, were first and independently introduced by Russow and myself in the years 1882 and 1883. As regards my own researches, my first results in 1882 were obtained by a modification of Sachs' method, and consisted in swelling sections of fresh tissue with sulphuric acid, and then staining with Hofmann's violet (methyl violet) washed out with glycerine, or with Hofmann's blue dissolved in picric acid (picric Hofmann's blue). This was succeeded

* Gardiner, 'Roy. Soc. Proc.,' No. 229, 1883.

in 1883 by a second method, which was a modification of that of Hanstein, and consisted in treating sections of fresh material with iodine solution, swelling with Chlor. Zinc. Iod. and staining with picric Hofmann's blue or methyl violet. Certain results obtained by this latter treatment were so promising that in my final paper in 1883, with the customary rashness of youth, I described the method as being "perfectly satisfactory;" but no long period elapsed before I found in practice that it was of but limited application.

Speaking generally, and excepting Poirault's* researches, I think one is justified in saying that since 1883 little or no advance has been made in the improvement of methods, and that later observations rest mainly on small modifications of the methods of Russow and myself.

The careful and detailed work of Kienitz-Gerloff† unfortunately serves in great measure to demonstrate the unsatisfactory nature of the results obtained by the sulphuric acid method, and to prove that unless the threads are of exceptional size, as in *Viscum album*, or as in sieve-tubes, the method is unreliable. The above remarks equally apply to such of my own results as depend upon the sulphuric acid modification.

An advance was, however, made by Poirault. In place of experimenting on sections of fresh tissue, he killed and hardened *pieces of tissue* in dilute iodine solution, and from the preserved material he then cut sections, which were swollen with Chlor. Zinc. Iod., or sulphuric acid, and stained either with eosin, Poirrier's acid brown, methyl violet, crocein, or aniline green. Poirault's researches are limited to the ferns and other vascular cryptogams, and lie buried, so to speak, in his paper "Anatomical Researches on the Vascular Cryptogams." While certain of his figures are, perhaps, not entirely convincing, the results of his research are most important, and of great interest. I am ashamed to say that I was unaware of the existence of this paper until the autumn of 1895, which was a year after I had elaborated the main lines of my own method, and applied it with success to the study of young endosperms. The great merit of Poirault's modification is that here for the first time provisions are made for preserving and hardening the tissue before taking sections. New dyes are also used. With certain kinds of tissue this method appears to have given excellent results.

I may now introduce my own researches. In the course of observations on this particular branch of cytology, certain salient facts come to the fore. In the first place one learns that material preserved in alcohol does not appear to be suitable for the investigation, and consequently fresh tissue has been used. Secondly, that it

* Poirault, 'Ann. Sci. Nat. (Bot.),' vol. 18, 1893.

† Kienitz-Gerloff, 'Bot. Zeit.,' 1891.

appears generally necessary to bring about a definite swelling of the cell wall. Thirdly, that it is not easy to stain and isolate the threads, even when they are known to be present.

These facts place many obstacles in the way of successful research. The difficulties attending the manipulation of fresh tissue are sufficiently obvious, and are apt to be so increased by the subsequent swelling as to render any really refined investigation well-nigh impossible, and as long as the threads cannot be stained so as to stand out clearly from the rest of the wall their identification is out of the question. These difficulties, which are sufficiently pronounced in the case of peculiarly favourable material such as that of endosperm, are only magnified when the investigation is concerned with ordinary or young tissue. In addition to the drawbacks already mentioned, the existing methods of research hitherto in use make no provision for the preservation of tissue.

It became obvious, therefore, that if the inquiry into the relations of the cell wall and the connecting threads was to be prosecuted with success, a more refined method must be devised, which could be reduced to terms of the usual procedure, viz., killing, fixing, hardening, preserving, cutting section, staining, and mounting, and that the methods heretofore in use were too coarse for so delicate an investigation.

I do not propose in the present paper either to give an account of the discovery of my method, or to go into elaborate technical details. It is sufficient to say that, expressed in the simplest terms, the method appears to depend upon the use of two principal reagents, viz., the osmic-acid-uranium-nitrate mixture of Kolossow as a fixative, and safranin as a dye. As a preservative I have used thymol water, and have obtained excellent results with material which has remained in it for as long a period as three years. Sections may be cut by hand or with the freezing microtome.

The fixing and staining reagents must be introduced and employed in different ways, the exact manner of procedure depending upon the character and age of the particular tissue under observation. This can be best illustrated by means of definite examples, and since the whole method is somewhat complicated, it will be expedient to consider under separate heads (1) the killing and fixing, with which is also associated the swelling, and (2) the staining.

In material such as that of young endosperms (*e.g.*, the endosperm of *Tamus communis*), no swelling is required, and the tissue, cut into small pieces, may be both killed and fixed at one and the same time by Kolossow's reagent, and then preserved in thymol water for future use. Where only slight swelling is necessary, treatment with water may precede that of Kolossow's reagent. In certain classes of tissue, where the walls are swollen with comparative ease, such as

that of the ordinary vegetative tissue of *Phaseolus vulgaris*, *Tamus communis*, *Nerium oleander*, *Salisburia adiantifolia*, &c., small pieces are killed and swollen in an aqueous solution of picric acid, and then fixed in the Kolossow's reagent and preserved in thymol water. Finally, where the tissues are more resistant, as, for instance, in *Robinia pseudacacia*, *Prunus laurocerasus*, or *Aucuba japonica*, treatment with picric acid may be followed by more severe swelling by means of solutions of zinc chloride or sulphuric acid, to be succeeded as before by fixing, hardening, and preserving. The blackening of the cell contents caused by osmic acid may be removed by bleaching.

From such preserved material sections may be cut when required.

The process of staining is no less complicated than that of killing and fixing, and is best considered under two heads, viz. :—(1) The methods applicable to certain endosperms and tissues of similar character. (2) The methods applicable to the majority of tissues.

In certain special cases it is possible to stain the threads directly either with safranin alone or by introducing safranin by means of a somewhat intricate substitution method such as that which I used with excellent results in the case of the endosperm of *Tamus communis*, where the sequence of staining was Hofmann's blue (or soluble water blue), methylene blue, safranin, and in which moreover the Hofmann's blue was dissolved in dilute picric acid or uranium nitrate, and the methylene blue in dilute salt solution. Once stained with safranin, all sorts of modifications are possible. Thus, the safranin may be succeeded by gentian violet or by eosin, and with gentian violet Gram's method is applicable and most advantageous. As safranin forms a precipitate with chromic acid, sections stained with safranin may be treated with this reagent, and then with silver nitrate, thus effecting a silver staining of the threads. Silver nitrate itself also forms a precipitate with safranin. In all cases the staining is practically limited to the threads.

When the above methods of direct selective staining are applied to ordinary tissue they are found to fail, for it usually happens that the whole of the wall becomes deeply stained, so that the threads are no longer visible. I was for some time completely baffled by this circumstance, but I ultimately adopted the well-known method of staining and washing out, using for the purpose orange G or acid fuchsin. With ordinary tissue the staining appears to be more easily accomplished than with the thick mucilaginous walls of endosperm cells, and the method may be somewhat more syncopated. Excellent results may be obtained by staining at once with safranin and washing out with orange G. This may be followed by staining with gentian violet, succeeded by treatment with acid fuchsin, or the sequence of staining may be safranin, gentian violet, acid fuchsin. Substitutions in which safranin, gentian violet, and eosin are included

give good results. The method of staining indirectly by washing out may also be applied to endosperm tissues generally. The stained sections are best examined either in water or in very dilute glycerine. I have as yet given little attention to the question of making permanent preparations, although I have initiated certain experiments which may possibly lead to a satisfactory result.

It is probable that the method in its present form will not be found to be available for the study of adult lignified or suberised cells, though up to the present I have made no observations upon such tissues. The investigation of young tissue will, however, doubtless give good results, and will establish that in them, also, the general structure prevails. I am strongly convinced that the above method, or similar methods, based upon "non-alcoholic" treatment, will be found to be peculiarly suitable for the investigation of the tissues of plants, and will in the future lead to observations of interest and importance.

A summary of results may now follow.

As my new method owes its origin to a study of the cells of endosperm I propose to deal with that tissue first.

The present investigation entirely confirms and extends the results I obtained in 1883.* At that time, however, many important problems still awaited solution. In the first place, what was believed to be the typical and universal structure could only be demonstrated with particularly favourable material and in a limited number of plants. Further, even in ripe seeds, where treatment with iodine proved that connecting threads were present, special difficulties were experienced when attempts were made to stain them with aniline dyes. Lastly, for young seeds the methods were quite unsuitable. With the present methods we are in a position to investigate the structure of endosperms generally, and to follow their development even from the earliest stages. The more refined method also gives more sure and satisfactory results.

It is possible to make certain general statements concerning the connecting threads of endosperm cells. In the first place, the histological structure of endosperm establishes a point of great importance which is only emphasised by the study of tissues generally, viz., that in pitted cells the pit-closing membrane is invariably traversed by threads. For descriptive purposes these may be called "pit-threads." Threads may also be present which traverse the general wall, and these may, similarly, be called "wall-threads." In the somewhat exceptional cases of unpitted cells the thread system is necessarily composed of "wall-threads" only. In many pitted cells both "pit-threads" and "wall-threads" are present; but in the majority of cells the threads appear to be limited to the pits,

* Gardiner, 'Roy. Soc. Proc.,' No. 225, 1883.

though it is not improbable that even in such cases other threads traversing the general wall will be found to occur.

Where both "pit-threads" and "wall-threads" coexist in one and the same cell, the former are stouter and more readily stainable than the latter. In pitted cells the pit-threads are necessarily in groups, and it is a point of some interest that the wall-threads also are usually in groups—as though a pit were present. This is especially striking in such cases as the unpitted cells of *Tamus communis* (fig. 1) and *Hordeum vulgare*. In *Tamus communis*, while the side walls exhibit the usual arrangement of isolated groups of threads, the end walls are traversed by a single large group, as in sieve-tubes. The structure of the endosperm cells of *Lilium martagon* (fig. 2) is

FIG. 1.

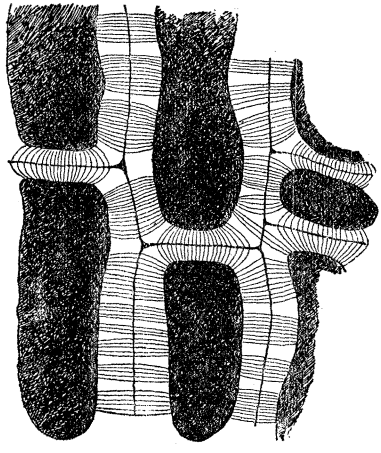
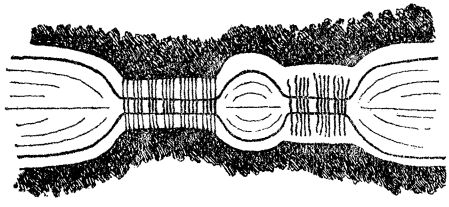


FIG. 2.

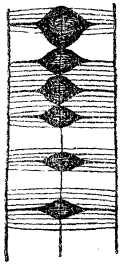


of some interest. The cells are pitted and each pit has its group of "pit-threads." In any given group the threads are arranged in bundles, recalling the similar arrangement of achromatin fibres, which Strasburger and others have found to accompany nuclear divisions in this plant. The mode of development of the threads

and the phenomena which accompany germination were investigated in the endosperm of *Tamus communis* only. The threads are found to be present at a very early stage, and can be detected even in the very youngest and thinnest walls. They are at first uniformly distributed over the cell membrane. In the case of the side walls, as surface growth proceeds, small groups of threads become separated from one another by intervening areas of clear membrane, while in the end walls, where the extension of surface is less, this segregation into groups does not take place. In the early stages the growth in thickness of the wall is not uniform, and pits are formed on the side walls at those points where thread groups occur; but they have only a transient existence, and ultimately disappear. It is, however, interesting to note that the vegetative tissue of *Tamus communis* consists for the most part of pitted cells.

In germination the ferment, in the first instance, appears to be conducted into the wall by means of certain of the threads, but when once an entrance is effected the corrosive action rapidly spreads quite independently of the threads, becoming the more potent as it reaches the neighbourhood of mucilaginous and less resistent middle lamella. In a given wall the penetration commences simultaneously at several centres, and at each centre the affected areas assume the general form of small cones with their apices directed towards the cell lumen (fig. 3). Moreover, since the action of the

FIG. 3.



ferment soon extends from cell to cell in two adjoining cells, where the common wall is affected on both sides, each side having its cone, the base of one cone appears opposed to that of the other. At this stage, by appropriate staining, the threads may still be seen shining through the disorganised mucilage of the affected areas. As the ferment action proceeds the boundaries of the several areas continually extend and at length unite when the whole of the wall is involved. The disorganisation of the wall is accompanied by marked stratification. The sphere of influence of the ferment action

is curiously limited and hardly extends beyond the immediate neighbourhood of the absorbent foot of the young embryo. The mode of disappearance of the wall by the coarse corrosive action of the ferment, and the manner of its proceeding with so little relation to the threads, seems to indicate that the structure of endosperm cells has more relation to the conduction of impulses and of food supply than to the needs of germination.

Satisfactory as are the results derived from the study of endosperm cells, the real success was achieved when the method was so modified that it could be applied with certainty and success to the investigation of ordinary tissue, and to specialised forms of it, such as pulvini and tendrils.

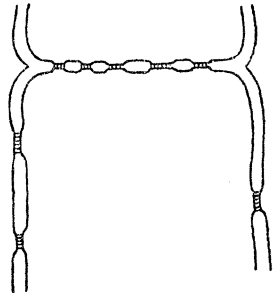
The unsatisfactory character of the somewhat meagre results hitherto obtainable have not, I think, succeeded in generally establishing a conviction that the structures postulated have necessarily an actual existence. Botanists have, on the contrary, been inclined to look askance at results depending upon such statements as "a stained area" or "a doubtful striation," and there lurked in the minds of many, the suspicion that the histology of endosperms was possibly exceptional, and peculiar to that tissue.

For the future such doubts need no longer be entertained, since it is now easy to demonstrate that the structure exhibited by endosperm tissue is in all respects entirely typical of plant tissue generally.

In these days of active investigation, it is not often given to one to be the medium for the criticism of his own research. This good fortune, however, now falls to me, and I hasten to say that in the light of the present investigation it is quite clear that apart from endosperms, and with such exceptions as *Aucuba japonica*, my earlier work on the continuity of the protoplasm, in pulvini and other tissues, does not afford absolute proof that a communication between cells actually occurs, but for the most part only brings forward strong evidence that such connection is exceedingly probable. At this juncture, also, I note with satisfaction, that the results then obtained were able to save me from the error of a belief in the existence of a system of open pits which have since then been repeatedly figured and described, and against which I have persistently spoken.

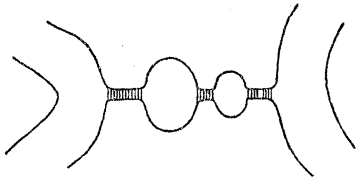
As I have already remarked, my new method makes it possible to establish with certainty that the structure of all kinds of plant tissue is precisely similar to that of endosperms, and that exactly the same modifications are exhibited. In pitted cells every pit-closing-membrane is traversed by its group of threads, and in unpitted cells similar groups also occur. In square-ended tubular cells, such as those of the leaf stalk of *Lilium martagon* (fig. 4), the

FIG. 4.



numerous small isolated groups of wall-threads are present on the side walls, and a large group occupies each end wall. In certain cells both "pit-threads" and "wall-threads" may simultaneously be present. The method gives equally good results with thin or thick-walled tissue, and in the case of the thinnest walls an "*en face*" view can be obtained where a sectional view fails. The threads vary in size. They are, for instance, exceedingly thick in *Viscum album*, where they are seen with the greatest ease; they are well developed in *Phaseolus multiflorus* and *Lilium martagon*, and they are fine and delicate in *Aucuba japonica* (fig. 5). The connecting threads may be

FIG. 5.



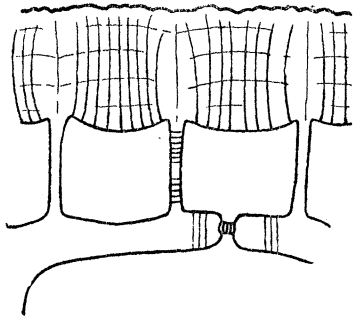
observed equally in the epidermis and cortex, and in all the living cells of the tissues of the central cylinder. In a section of any given tissue, it can be readily seen that all the cells are placed in communication with one another. One is tempted to expatiate upon the wonderful beauty of the appearance presented by the walls studded with their pits, each pit with its closing membrane traversed by the thread complex after the manner of sieve-tubes. It is indeed a sight which cannot fail to convey a lasting impression of wonder and surprise.

The pit-threads of such pulvini as were examined presented no striking difference, either in appearance or distribution, to the similar structures in ordinary tissue, and the same appears to be true of the sieves of tendrils. The important point, however, is this,

that in all these tissues the threads perforating the pit-closing membrane can be readily seen, and even counted.

A point of interest was observed in the epidermal cells of *Tamus communis* and *Lilium martagon* (fig. 6), viz., that the external or free walls are penetrated by a system of threads which radiate from the cell lumen and extend to the cuticle, so that the latter is the only structure intercalated between the protoplasm and the environment. The important bearings of this observation are obvious.

FIG. 6.



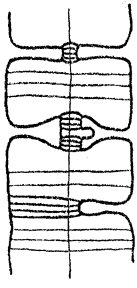
The following is a list of the tissues which have up to the present been investigated, together with the date of observation. In each case connecting threads were observed.

In 1894. The young and developing endosperm of *Tamus communis* and the young endosperm of *Asperula odorata.*

In 1895. The cotyledons of *Tropœolum majus.* The endosperm of *Lilium martagon* and *Fritillaria imperialis.* The root of *Ranunculus asiaticus.* The leaf stalks of *Tamus communis*, *Viscum album*, and *Marattia elegans.*

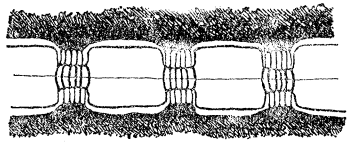
In 1897. The leaf stalks of *Aucuba japonica*, *Prunus laurocerasus*, *Nerium oleander*, *Lilium martagon*, *Lilium candidum*, *Salisburia adiantifolia*, and *Asplenium rutœfolium* (fig. 7). The flower stalk of

FIG. 7.



Taraxacum dens-leonis. The perianth-leaves of *Lilium martagon.* The pulvini of *Mimosa sensitiva* and *Robinia pseudacacia* (fig. 8). The tendril of *Cucurbita pepo.* The endosperm of *Hordeum vulgare.*

FIG. 8.



Although the research is still in its preliminary stages, since my attention has been chiefly directed to the elaboration of the new method, yet the results already obtained are sufficiently numerous and suggestive to enable one to make certain remarks and general statements.

It is impossible to resist the conclusion that the connecting threads consist either wholly or partly of protoplasm, and this view is largely confirmed by the staining reactions. It is, however, not improbable that the protoplasmic filament may be surrounded by a mucilaginous sheath. Osmic acid induces no blackening as it does in the threads of many sieve-tubes, and I am inclined to believe that in ordinary tissue the threads consist for the most part of ectoplasm, and are to be regarded in fact as extensions of it.

The threads appear to be present *ab initio.* This fact, coupled with the surface-growth of the cell wall, furnishes a sufficient explanation of the "barrel figures" so generally assumed by the various thread groups. The resemblance to the similar figures which accompany nuclear division is therefore superficial. Nevertheless it seems certain that the threads do, as a matter of fact, arise from that part of the cytoplasm which at the period of formation of the cell plate forms the fibres of the so-called "nuclear spindle," and that these fibres become, so to speak, partially imprisoned in the young wall.

My results appear to indicate that in a given cell the whole system of connecting threads arise at this early stage, and that no subsequent development occurs. This statement will, however, require careful confirmation, and has certain bearings on such interesting questions as the theory of grafts and "sliding growth."

In dead cells, such as those of ripe endosperm, the threads appear to degenerate into mucilage, and this is possibly also the case in adult lignified and other cells.

In the particular tissues which I have investigated, the threads can be shown to be present in all cells which still retain their cellulose character, and although I have not actually succeeded in

observing them in adult lignified and suberised tissue, it seems certain that they will be found there also. There can be little doubt that they occur universally in the cells of all the tissues of all plants.

From this arises the fundamental conception that the plant body must be regarded as a connected whole, and that the cell walls occupy only a subservient position. Thus our views as to the ultimate histology of tissue must be considerably modified. A new vista also opens to cytological research in the direction of the accurate determination of the distribution and orientation of the threads in the various tissues, which can hardly fail to lead to important results.

Should the structure presented by the external walls of the epidermal cells of *Tamus communis* and *Lilium martagon* be found to be of general occurrence, we shall be prepared for most interesting results when the examination is extended to secreting gland cells: to such non-cellular organisms as certain algæ and fungi, and to such unicellular bodies as spores and pollen grains.

Two important functions are, doubtless, performed by the connecting threads, viz., the conduction of impulses and the conduction of food. As to the first, there can be no question; and as to the second, one cannot but reflect that it must be of the greatest advantage to the plant to be able to transmit from cell to cell as occasion requires, and in a definite and determinate direction, *highly organised* food supplies and even protoplasm itself. It is, of course, possible that in the threads themselves a definite division of labour may occur as regards the transmission of food and the conduction of stimuli.

The consequences which arise from our more perfect knowledge of plant histology are obvious and far reaching.

In the first place we learn that the structure exhibited by sieve-tubes, which in the past was regarded as peculiar, is shown to be typical of cells generally, with this slight difference, however, that in sieve-tubes a secondary enlargement of the pores appears to occur. All the cells of a tissue must be regarded as being connected together by delicate groups of protoplasmic threads, which traverse either the general wall or the pit-closing membrane, just as the sieve-tube threads traverse the so-called "sieve plate," a fact which at length enables us to do tardy justice to the dominant position occupied by the protoplasm of the plant body, and to understand how the deep-seated cells of a tissue can telegraph their needs to those at the periphery, cell after cell taking note of the wording of the message, or how the peripheral cells may communicate to the interior their sense of gravity, light, heat, or touch, to which the whole organ may reply as its peculiar organisation directs.

As an integral part of cell structure, the connecting threads con-

stitute a factor, which cannot fail to have an important bearing in all general questions, such as the growth of the cell wall, the conduction of food, the ascent of water, the process of fertilisation, the penetration of fungi into their host, the process of secretion, and the transmission of the impulses which determine growth and movement of plant organs.

Concerning certain of these problems, I should like to make a few concluding remarks.

As to the passage of water from the root hair to the vessel, the presence of connecting threads in the cells of root tissue makes it possible to imagine that the ordinary laws of osmosis may be profoundly modified, and that the filaments which establish protoplasmic continuity may conduct stimuli, leading, for instance, to a difference in reaction of the proximal and distal halves of any given cell. Similarly, it is conceivable that a definite polarity is established, which helps to determine the direction of the flow. As to the larger question of rapid water movement, although this is neither the time nor the place to enter into theory, yet I cannot refrain from remarking that it is not impossible that the threads, doubtless present in large quantities in dead vessels, may, if they suffer mucilaginous change, have some bearing on the question, *e.g.*, by assisting to sustain the water at any given level or attracting water in the immediate environment. In any case, I am strongly of opinion that the part played by mucilage and the force of hydration have not as yet received sufficient attention.

As to movements generally, I am still unable to accept Pfeffer's view of the subsidiary part played by the protoplasm in connexion with turgidity,* and I am still of opinion that the ectoplasm is the master factor which determines the condition of the cell. The present research demonstrates among other things that there are fixed points in the ectoplasm, and this may have some bearing on the possibility of establishing the periodic or sudden contractions and dilatations which I believe are associated with turgescence, and of which such a phenomenon as the effusion of water from the cells of a stimulated *Mimosa* pulvinus is but an abnormal instance.

"On the Viscosity of Hydrogen as affected by Moisture." By Lord Rayleigh, F.R.S. Received September 8, 1897.

In Sir W. Crookes's important work upon the viscosity of gases† the case of hydrogen was found to present peculiar difficulty. "With each improvement in purification and drying I have obtained

* Gardiner, 'Roy. Soc. Proc.,' vol. 43, 1887.

† 'Phil. Trans.,' 1881, p. 387.

FIG. 1.

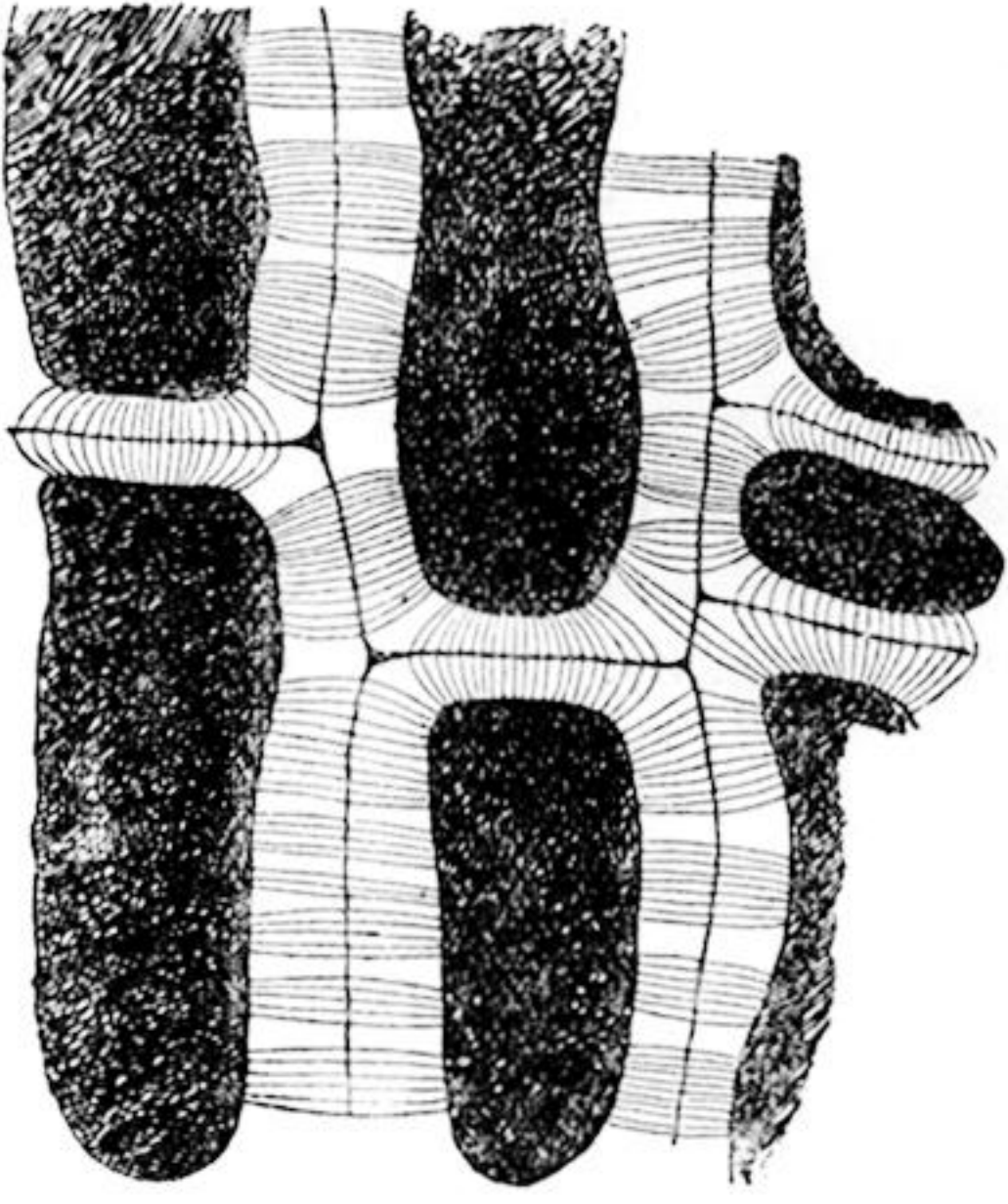


FIG. 2.

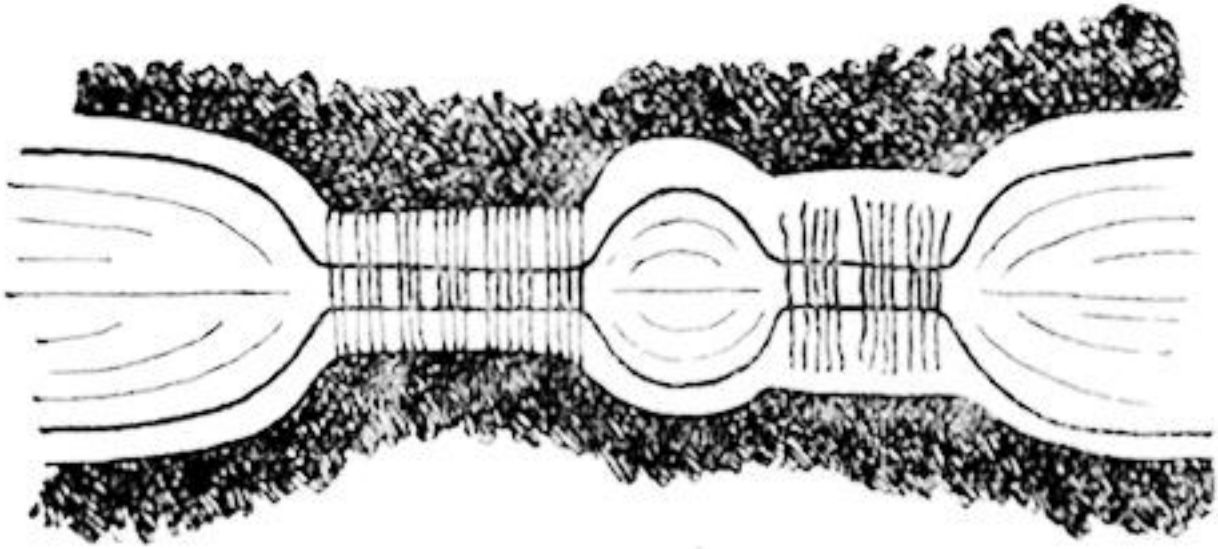


Fig. 3.

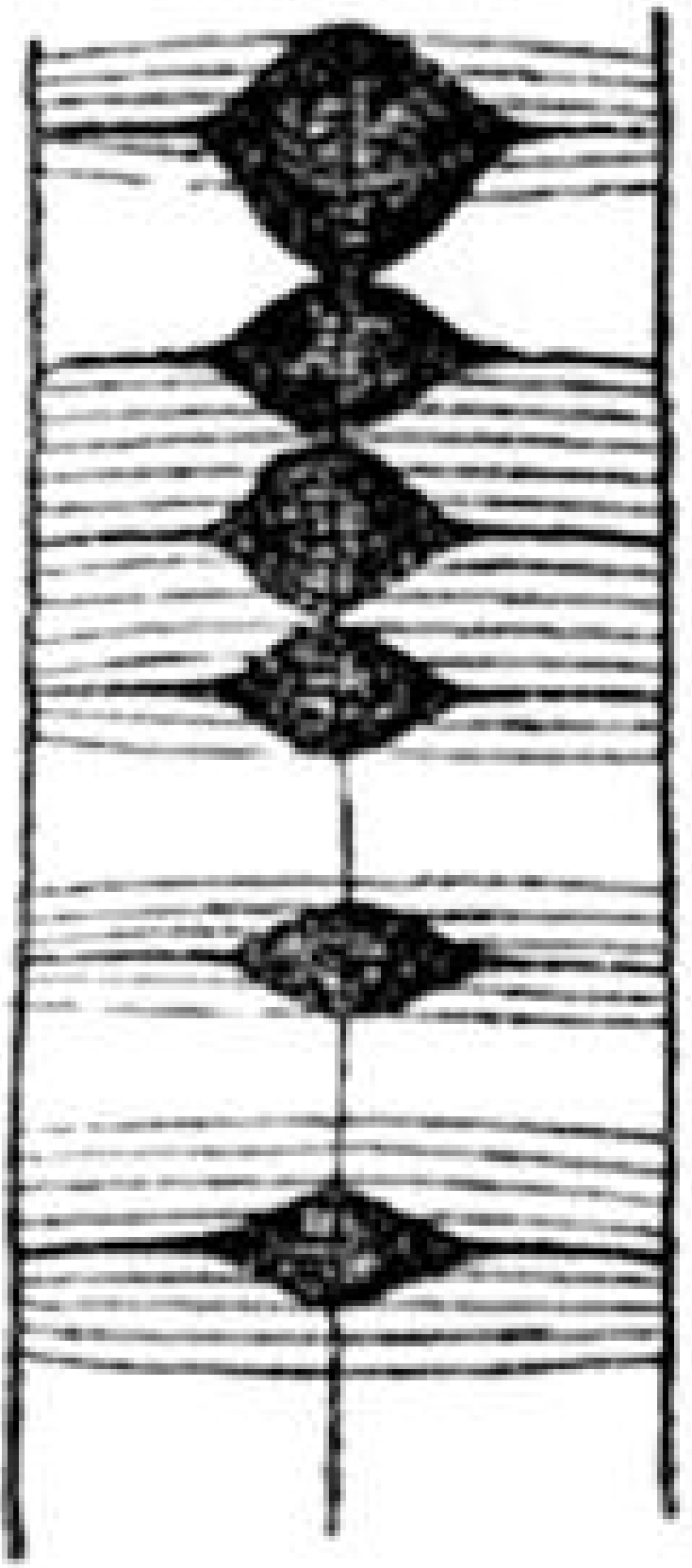


Fig. 8.