would then be mainly remarkable only for their inordinate dimensions, and to form which would have required the cooperation of a very large number of individuals. But there was not a trace of pseudopodia, still less of "axes"; the whole had a certain degree of "flow," whilst the hyaline smooth margin here and there pushed out great convex (almost, when very much projected, hemispherical), wave-like expansions, like some Amœbæ, but with exceeding slowness. There seemed, however, to be evinced no tendency whatever to become encysted. This therefore still remains a problematic production.

In a future number I propose to continue the consideration of the newly-described Sarcodina, as recorded in the various memoirs.

The Process *of* Aggregation *in the* Tentacles *of Drosera rotundifolia.* By Francis Darwin, M.B. (with Plate XXIII.)

The term "aggregation" is applied by my father[1] to the changes which are produced in the tentacles of *Drosera*, and in certain parts of other plants, by the following agencies:—

I. The mechanical irritation of the glands by repeated touches or by prolonged contact with organic or inorganic substances.

II. Similar mechanical irritation transmitted from the glands on the disc to other glands on the same leaf.

III. The absorption of certain fluids such as solution of carbonate of ammonia, infusion of raw meat, &c.

IV. Heat.

V. Osmosis caused by immersion in glycerine, &c. I do not feel certain that this form of aggregation is identical with that due to the previously mentioned agencies, and it will not be further alluded to in the sequel.

The phenomena connected with these changes have been fully described by my father; the following remarks are merely intended to bring forward a few questions connected with this subject which have not at present been answered, and which are of importance to any one forming a judgment on the essential nature of process.

Aggregation occurs both in the pedicel of the tentacle and in the gland which surmounts it; the present paper

[1] 'Insectivorous Plants,' Ch. iii.

deals with the process as it occurs in the former of these parts, where it is far more easily observed.

The pedicel is composed of elongated cells of various lengths and of about ·016 mm. in breadth; the cells forming the upper and middle portions are filled with a crimson fluid (fig. 1), which is, however, absent from dwarf leaves grown in very shady places (figs. 9 and 10), and is absent from the lower cells of all the tentacles (fig. 8). The protoplasmic currents which may be observed in the cells vary much in complexity; either flowing round the cell-walls as described by my father;[1] or forming a complicated flowing network like that seen in the staminal hairs of *Tradescantia*, &c. This network is well seen in the tentacles of pale dwarfed leaves which grow almost hidden in moss; the current almost invariably flows from one chlorophyll body to another (when these are present), so that they are connected together by a most beautiful moving network. In other cells the currents are simpler as shown in figs. 1, 9, and 10. Neither my father nor myself have ever been able to make out a nucleus; and this forms a marked distinction between the flowing network of *Drosera* and that in the hairs of *Tradescantia*, *Cucurbita*, &c.[2] Well-formed chlorophyll bodies are found in the lower cells of the tentacle, in the middle cells they are dwarfed and yellow and do not contain starch, in the extreme upper cells close under the gland they are again found fairly developed. Highly refracting globules which appear to be oil-drops are occasionally seen floating in the coloured fluid and carried round by the currents.

The change from the normal state to one of aggregation is extremely striking; various forms and stages of aggregation are shown in figs. 1, 2, 3, 6 and 7. Instead of containing a homogeneous purple or crimson fluid the cells contain variously shaped masses of crimson matter suspended in almost colourless fluid. "The change is so conspicuous that it is visible through a weak lens or sometimes by the naked eye."[3] The crimson masses are now seen to undergo most remarkable changes both in form and position; the rapidity with which these changes occur varies with the nature of the exciting cause and the stage of aggregation in which the tentacle is; sometimes the changes are only to be detected by drawing a given mass and examining it after an interval of several minutes; in other cases they are so conspicuous as to

1 'Insectivorous Plants,' p. 38.

2 According to Sachs ('Handbook,' p. 38) the nucleus disappears when the streaming commences in the sacs of the Characeæ.

3 'Insectivorous Plants,' p. 39.

be seen at once. This was the case with the cell shown in fig. 6; the appearance of the object changed as it was being drawn, so that it would have been impossible to draw the whole cell. By whatever cause aggregation is induced, it commences in the gland and spreads from cell to cell *down* the tentacle. On the other hand, when, on the cessation of the stimulus which has induced aggregation, the masses of crimson matter gradually dissolve and the cells become once again filled with homogeneous crimson fluid, the process of dissolution travels from cell to cell *up* the tentacle.

The above brief description may serve as an introduction to a discussion in which the various features of the phenomenon will be considered in greater detail.

The first questions which arise are—What is the nature of the aggregated masses, and with what parts of a typical vegetable cell are they homologous?

In the course of his description of the aggregated masses, my father remarks:[1] "Their movements are rather slow, and resemble those of Amœba or of the white corpuscles of the blood. We may, therefore, conclude that they consist of protoplasm." And in his subsequent discussion he always speaks of the aggregated masses as protoplasm. My father's assumption will appear justifiable to many; nevertheless more than one eminent physiologist have expressed doubts on this point. Professor Ferdinand Cohn in his review of 'Insectivorous Plants'[2] always speaks of aggregation as a "massing together (Zusammenballung) of the red sap" and never calls the masses protoplasm. Any opinion even hinted at by so eminent an authority deserves the most careful consideration. And since the question whether the aggregated masses are condensations of protoplasm or precipitations of cell-sap is of the utmost importance in determining the true nature of the phenomena of aggregation, it seems worthy of discussion. Before entering on the question it must be premised that the phenomena cannot possibly be considered as purely a mechanical or chemical one, like the running together of oil-drops into larger drops; the minute and continuous changes of *form*, and the separation into secondary masses absolutely negative such a view. Moreover the changes are subject to the general conditions to which protoplasmic movements are subject. It follows therefore that the changes not being mechanical must be vital, *i. e.* due to the movement of living protoplasm. If, then, we accept Professor Cohn's view that the masses of aggregated matter are passive con-

[1] P. 40.
[2] 'Deutsche Rundschau,' ii, 9, 1876, p. 454.

densations of cell-sap incapable of originating movement, it follows as a necessary corollary that the movements are impressed on these passive masses by protoplasm of some kind external to them. We can therefore state the two opposing theories to be discussed, thus:—

I. My father's view, that the aggregated masses consist of protoplasm, and that their movements are simply due to their own contractility, excited by various external agencies.

II. Professor Cohn's view, which appears to be that the aggregated masses consist of condensations of cell-sap, and as a necessary corollary that the movements are impressed on the masses by some kind of protoplasmic action *external* to the masses.

The received notion of the structure of a typical adult vegetable cell is that within the cell-wall there is a sac of protoplasm enclosing the cell-sap, and sending prolongations in the form of "plates or threads," which traverse the cell-sap cavity in various directions; the nucleus may be described as differentiated protoplasm. The typical cell may, therefore, be said to contain two kinds of protoplasm.

As far as the present discussion is concerned Strasburger's classification of vegetable protoplasm[1] confirms this statement, although in reality he makes a third variety, his "couche membraneuse," which includes the peripheral or limiting portion of a protoplasmic mass.

A cell of Tradescantia obviously presents the two main varieties of protoplasm, namely, those which form the current and the nucleus, the rest of the cell being occupied by purple cell-sap. But if my father's view is correct, the Drosera cell not only differs in having lost its nucleus, but also in possessing another variety—a coloured protoplasm diffused throughout the cell, and distinct from the granular protoplasm which forms the current.

Professor Strasburger remarks "that the separation of the protoplasm into the granular plasma, the membranous layer, and the nucleus signifies a division of labour, in such a way that the nucleus governs the molecular phenomena connected with the genesis of cells, while the membranous layer is charged with the peripheral limitation of the whole, and the granular layer (or plasma) with nutrition."[2]

Now, the cells of the tentacle of Drosera, besides the vital functions common to all cells, have special ones to perform, namely, absorption of a special kind of food and the

[1] 'Sur la Formation, &c., des Cellules,' 1876, p. 263.
[2] Loc. cit., p. 263.

transmission of peculiar stimuli. It is not, therefore, surprising that a different form of protoplasm should be found in them. The origin of chlorophyll bodies in the vegetable cell is quite in accordance with Professor Strasburger's view; for, as Sachs remarks,[1] "the process may be supposed to take place by very small particles of a somewhat different nature originally existing in or being distributed through the previously homogeneous protoplasm, then collecting at definite places, and appearing as separate masses." It must be noted that this process accords strictly with the principle of "division of labour." In certain vegetable cells the chlorophyll is uniformly disseminated[2], and not collected into chlorophyll bodies. This appears to show that the power of junctioning in a given manner may, in some cases, be localised, in others disseminated.

The disappearance of a nucleus (in Peziza) by its substance being "distributed through that of the protoplasm"[3] seems to point to the same possibility. In some cases the protoplasm of a cell is "tinged by a colouring matter . . . . . which is not present in the cell-sap."[4] It is, therefore, not impossible that this should be the case in Drosera.

The following observation seems to show that in Drosera the crimson colouring matter is not in the cell-sap. Kühne states[5] that when a cell in a Tradescantia-hair dies, the dead protoplasmic network absorbs the purple cell-sap, and becomes in this way stained. Now, in the case of Drosera no such appearance has been observed either by my father or myself. When a cell dies the colour fades away from the crimson cell-contents, and nothing but a dingy mass of granular *débris* remains; this seems to show that the colour is not in the same state as in Tradescantia, but that it is in intimate connection with something living. It should be observed, however, that the colour is in no way essential to the process of aggregation, as it oocurs in almost colourless or light-green tentacles.

The general aspect of the phenomena in reference to the two theories above mentioned having been considered, a few of the details will be examined in the same manner.

The physical condition of the masses varies with the stage of aggregation in which they are. The process commences by the cells becoming "slightly cloudy from the for-

[1] 'Handbook,' p. 45.
[2] Sachs, p. 46.
[3] Sachs, p. 11.
[4] Sachs, 'Handbook,' p. 40.
[5] 'Das Protoplasma,' 1864, p. 94.

mation of numberless only just perceptible granules, which rapidly grow larger."[1] Ultimately, when the aggregation becomes intense, and especially when produced by a solution of carbonate of ammonia, the cell contains a single spherical mass or only one or two large spherical ones (fig. 3). These are of a deeper colour and refract light more strongly than the earlier formed masses. The change of colour connected with an alteration in the degree of condensation is shown in fig. 7; the masses here drawn were in process of dissolution; in A we have two dense and dark masses at one extremity of the cell (nearest the gland) and some lighter-coloured ones at the other end; the process of dissolution travels up a tentacle from the base towards the gland, so that in B, which represents the same cell drawn at a later period, we find the masses which were separate, and dark coloured in A, coalesced, and of a lighter tint.

In the early stages of aggregation the masses are extremely motile, and seem to be carried by the currents round and among each other in that peculiar plastic way that one sees in the coloured blood-corpuscles, where currents are set up among them.

In the strongly aggregated condition they become nearly or quite motionless, and seem to be of a fairly dense nature, and on pressing the cover glass they assume the star-like aspect with radiating fissures, described by my father[2] and shown in fig. 5. The change in motility might be due according to my father's views to the crimson protoplasm assuming a passive instead of an active condition; according to the second theory, it might be due to aggregated cell-sap becoming too dense to be influenced by the protoplasm which supplies the motive power. When tentacles die in an aggregated state, the masses usually become turbid instead of remaining clear, and gradually break down into a granular *débris*, which fills up a large part of the cell. The fact that the death of the cell affects the condition of the masses is more in favour of their being of a protoplasmic nature than of their being mere mechanical aggregates. Oil globules, for instance, are not affected in this way by the death of the cells in which they are contained. On the other hand, turbidity is a characteristic sign of the death of such protoplasmic structures as are transparent during life, *e.g.*, cornea corpuscles.

Effects of Reagents.—These effects are most advantageously studied on dense and firmly aggregated masses which

[1] 'Insectivorous Plants,' p. 45.
[2] 'Insectiv. Pl.,' p. 47.

withstand the action of strong reagents. In order that the tints produced by the reactions may be perceptible, it is essential that very pale leaves should be selected; the dwarfed plants growing hidden in moss answer this purpose well.

The masses are not soluble in absolute alcohol or in a mixture of turpentine and creasote; either of these would dissolve oils; the aggregated masses are therefore not purely oleaginous.

They are not coloured blue by iodide or by Schultze's solution, and therefore are probably not amylaceous. A pale aggregated tentacle placed in a solution of caustic potash assumes a yellow tint; the masses not being dissolved; on removing the superfluous alkali and adding strong nitric acid the masses assume the orange-yellow colour of xanthoprotein. If a tentacle is placed in syrup and afterwards in strong sulphuric acid, the masses become reddish brown.

The aggregated masses (in a tentacle killed by osmic acid) are stained yellow by iodine. The three last-mentioned reactions are given by Sachs[1] as tests for protoplasm. But we must not therefore conclude that the aggregated masses consist of protoplasm; for Sachs adds that the above-mentioned reactions are characteristic of "true albuminoids as caseine, fibrine, albumen. We are at least justified in assuming that the aggregated masses consist of albuminoid substances.

A tentacle was placed in strong solution of hæmatoxylin (which acted, I presume, as a poison); the staining fluid was absorbed at one of the minute papillæ and the aggregated masses in the neighbouring cells became brightly tinted with it. The power of absorbing colouring matter is mentioned by Sachs as a characteristic of dead protoplasm and other albuminoid bodies.

The action of osmic acid on the tentacles was not satisfactorily made out. The aggregated masses of tentacles placed in $\frac{1}{4}$ per cent. solution of this acid, are intensely blackened and are fairly well preserved from the degeneration into granular *débris* which is the usual result of the death of an aggregated cell. But in some cases osmic acid seems to alter the appearances of the aggregated masses before the death of the cell is caused. Thus in one instance I mounted in water a tentacle in which there were fine ropelike masses of aggregated matter. I then irrigated the preparation with about three drops of $\frac{1}{4}$ per cent. osmic acid; it was chiefly absorbed at the cut-off end of the tentacle, where it blackened the aggregated masses; but in cells further removed from the cut surface a distinct effect was produced although

[1] 'Handbook,' p. 40.

the aggregation masses were not blackened; the change was conspicuous and consisted in the transformation of the ropes of aggregation-matter into chains of delicate spheres, reminding one of rows of dewdrops on a spider's web;[1] this change occurred very generally throughout the tentacle. The same kind of formation of minute spheres has been observed in non-aggregated cells exposed to the action of osmic acid. In a leaf allowed to remain during the night in half a watchglass of water to which a few drops of $\frac{1}{4}$ per cent. osmic acid had been added, presented in some cells a similar formation of spheres, while a neighbouring cell was absolutely uninjured and contained *changing* aggregation masses. It appears from this that an extremely weak solution of osmic acid is sufficient to alter the appearance of the masses, for the amount absorbed by a tentacle of which *any* cells remain alive must be very small.

Relation of the aggregated masses to the flowing Protoplasm.—In a normal cell before aggregation has commenced the streaming protoplasm does not appear as colourless bands, but as lines of moving granules imbedded in a slightly refracting substance; when, however, the process has well begun, the currents show out clearly as a moving network of colourless granular substance. During the process of dissolution we find cells in which the aggregated masses are nearly dissolved, but which are still divided by narrow divisions into a few large masses, nearly filling up the whole cavity of the cell; and in the narrow spaces between the masses currents of protoplasm may sometimes be observed. Again, in the state of intense activity of movement which is produced by subjecting an aggregated tentacle to certain temperatures, one observes small masses of crimson matter forced by the currents to pass between larger masses. The network of flowing protoplasm is continually changing its form, so that it is possible that it may help to mould into shape or divide the aggregated masses. This possibility is obviously favorable to the theory that the changes in the aggregated masses are impressed on them from without. Nevertheless, it is not destructive to my father's hypothesis, for a plastic substance such as protoplasm would naturally be affected by a rapid current like that which streams in the cells of Drosera.

In *strongly* aggregated cells the current is invisible or

[1] This appearance is possibly similar to the "chains of small globular masses" ('Insectiv. Plants,' p. 207) produced by the action of a strong solution of cobra poison, which, however, is not a poison to Drosera, but merely an intense stimulant.

almost absolutely so; its disappearance is ascribed by my father[1] to the loss of the granules which ordinarily make its movement perceptible, and which, as he believes, are absorbed by the aggregated masses. But although the flowing protoplasm may be invisible, evidence of its existence may be found in the free particles which can be seen occasionally travelling round the cell. Moreover a protoplasmic network can sometimes be detected in a strongly aggregated cell after death. In one case an aggregated tentacle was mounted in water and cemented with dammar varnish,[2] with a view to watching the process of dissolution; this tentacle died before the process began, and Fig. 3 is a drawing of one of its cells. In consequence of death the protoplasmic network became visible, passing, as shown in the figure, from one chlorophyll body to the next, like the processes of connective-tissue-corpuscles, and just as the current does in many living cells (fig. 8). I have also seen the protoplasmic network in specimens killed in osmic acid and left in absolute alcohol for a day or two; they were then transferred to glycerine by Strasburger's method of adding a few drops of the latter to a quantity of alcohol, and allowing it to evaporate. In this way I clearly saw cells in which strongly aggregated masses coexisted with a protoplasmic network. These networks are more delicate than the currents seen in the living cell, I suppose owing to the shrinking caused by the alcohol.

Altogether the impression left on the mind by a study of the protoplasmic currents is that they are not capable of producing the changes that occur in the aggregated masses. This impression is not one that can be tested, for the question is obviously one of degree; a sufficiently abundant protoplasmic network containing small aggregated masses in its meshes *might* be able to produce changes in them similar to those of aggregation. There is another peculiarity of the aggregated masses which seems to shows that they are not passive condensations of cell sap, namely, that they frequently contain large vacuoles apparently filled with colourless, or very slightly tinted, fluid; a cell in which some of the cells are in this condition is shown in fig. 2. In many instances a single mass contains many vacuoles, giving it a far more complex aspect than that of the simple example of vacuolation here given. But what is of importance is, that in the alterations

[1] 'Insectiv. Plants,' p. 48.

[2] This otherwise useful varnish does not answer the above purpose, as it almost always runs under the cover glass after a time. Kühne recommends mastich dissolved in chloroform.

which the arrangement and appearance of the vacuoles undergo give us distinct evidence of *internal* change occurring in the aggregated masses, which is quite inconsistent with the view that their changes of form are impressed from without. On the other hand, both the presence of vacuoles, and the fact of internal changes occurring, are perfectly consistent with the view that the aggregated masses are protoplasm. An observation of my father's points to the same conclusion. He describes[1] how whilst one mass was rapidly increasing and another in the same cell rapidly decreasing, he "was able to detect a connecting thread of extreme tenuity, which evidently served as the channel of communication between the two."

It may be worth while here to sugest a third view combining in a certain sense my father's and the opposing theory; but it is more justly to be described as a modification of my father's view. It might be possible that a condensation of the colouring matter of the cell-sap should form the basis of the aggregated masses, while the power of movement would be supplied by particles of protoplasm separated from the flowing network. In favour of this view might be adduced the observations of Sachs[2] on the hairs of Cucurbita, and those of Kühne[3] on those of Tradescantia. Both of these observers saw small portions of protoplasm separate from the network under the influence of heat in one case and cold in the other, and undergo amœboid changes of form while floating freely in the cell-sap. Now, if such amœboid masses were to permeate the precipitated cell-sap, spontaneously moving crimson masses would be the result.

This view must not be dismissed at once. But the fact that an abundant protoplasmic current may be seen flowing, while numerous aggregative masses are actively changing their shape, seems to show that it is not the true explanation. It must be remembered that quantity of aggregated matter is very considerable; the granular *débris* produced by the death of an aggregated cell almost fills the whole cavity. Moreover, the motive power seems to be intimately distributed throughout the aggregation masses, for very minute threads undergo changes of form. If, then, this motive power (intimately distributed through a large quantity of matter) is supplied by the actual deduction of protoplasm from the flowing network, it is difficult to see how enough of the latter can be left to produce the strong currents which certainly coexist with active aggregation.

[1] 'Insectiv. Pl.,' p. 42.
[2] 'Physiologie Végétale,' p. 74.
[3] 'Das Protoplasma,' p. 103.

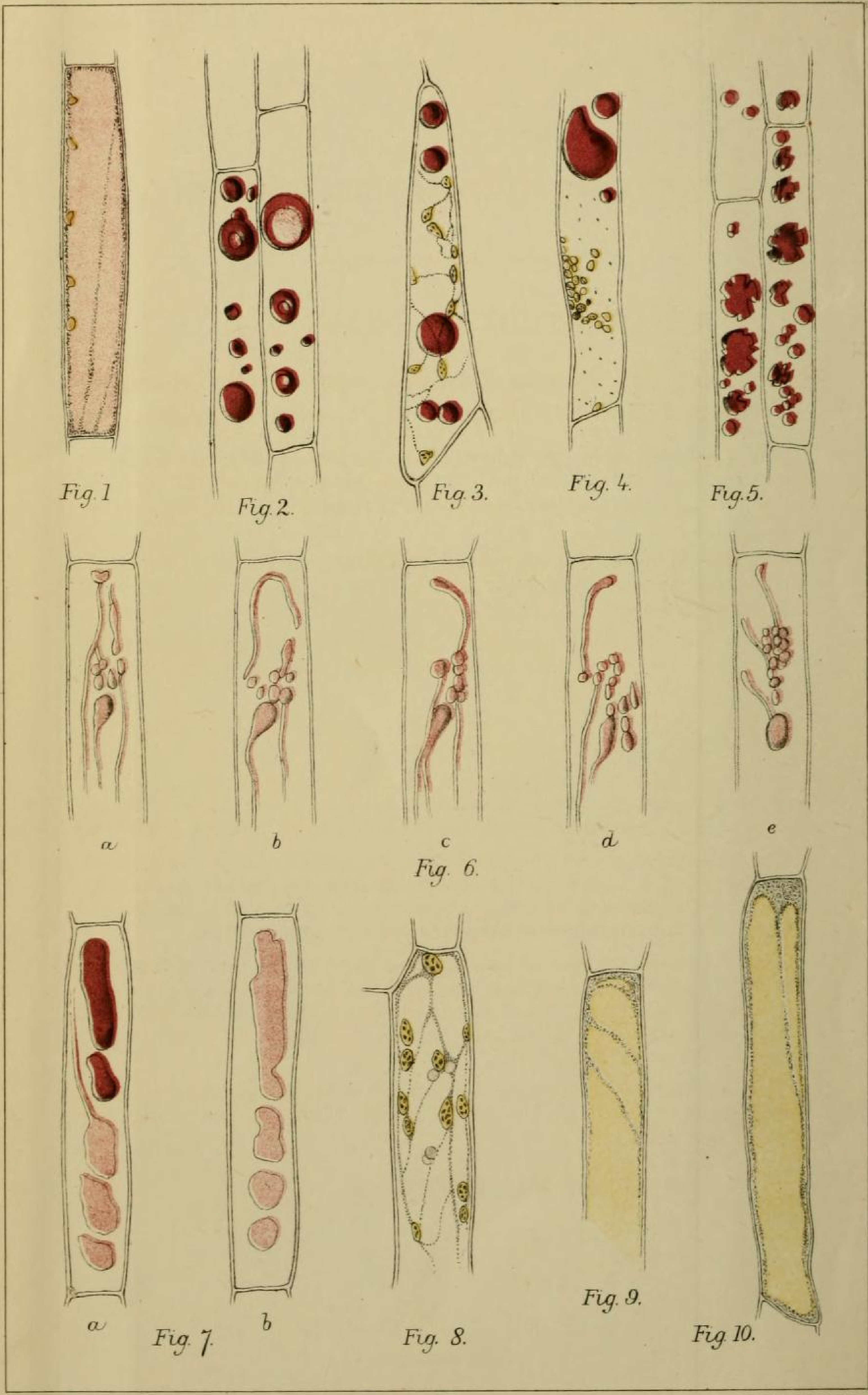
A few observations were made on the effects of different temperatures; but on testing the thermometer of my warm stage the graduation was found to be incorrect. I hope to repeat my experiments and also make some observations on electrical stimulation.

In conclusion, a phenomenon connected with aggregation will be touched upon. My father observed that in *Drosera* and in certain other plants such as *Erica tetralix* immersion in solution of carbonate of ammonia causes the chlorophyll bodies to collect into heaps, and, what is more remarkable, these heaps are converted into masses by the coalescence of the bodies. I have unfortunately been unable to attend to this subject and have only cursorily observed this coalescence; but I have frequently noticed the chlorophyll bodies in the cells of a *Drosera* tentacle collected into heaps, as shown in fig. 4. The subject is no doubt worthy of investigation. It is curious that a solution of carbonate of ammonia which is so potent in producing aggregation of the crimson matter in the cells of *Drosera* should also cause a somewhat similar movement in the chlorophyll bodies; and that this aggregation of chlorophyll should occur in other plants than *Drosera*. Sachs[1] describes the changes in position undergone by chlorophyll grains in various unfavorable external circumstances; "for instance, in small fragments of tissue, when respiration is defective, turgidity diminished, temperature too low, the cells too old, or—what is of most interest here —where light is cut off for a considerable time." Under these circumstances the chlorophyll bodies collect together in certain positions. Sachs considers it certain that they possess no power of independent movement, and that their change in position is due to the action of the colourless protoplasm in which they are embedded.

If this holds good with the chlorophyll bodies which collect together under the influence of carbonate of ammonia, the case might be adduced in favour of the view that the crimson aggregated masses are in like manner passive and only moved by protoplasm external to themselves.

Finally, I believe that a fair consideration of the arguments for and against my father's view, that the aggregated masses consist of protoplasm will incline the inquirer to accept it in preference to any opposing theory. I believe moreover that in this way the physiological significance of the process of aggregation is more likely to be justly determined.

[1] 'Handbook,' p. 672.



Mc Farlane & Erskine Lithrs Edinr