PUBLISHER'S NOTE

Some of the pages in the following item are uncut. In order to make the Collection as complete as possible we have reproduced those pages which are available.

© Reproduced with the permission of Cambridge University Library by Darwin Online

The Action of Carbonate of Ammonia on the Roots of certain Plants. By CHARLES DARWIN, LL.D., F.R.S.

[Read March 16, 1882.]

MANY years ago I observed the fact that when the roots of *Euphorbia Peplus* were placed in a solution of carbonate of ammonia a cloud of fine granules was deposited in less than a minute, and was seen travelling from the tip up the root from cell to cell *. The subject seemed to me worthy of further investigation. Plants of the same *Euphorbia* were therefore dug up together with a ball of earth, and having been left for a short time in water, the roots were washed clean. Some of the finer transparent rootlets were then examined, and sections were made of the thicker roots, generally by my son Francis, who has aided me in many ways. All the cells were found to be colourless and destitute of any solid matter, the laticiferous ducts being here excluded from consideration. These roots, after being left for a few minutes or for several hours in solutions of different strengths, viz. from 1 to 7 parts of the carbonate to 1000 of water, presented a wonderfully changed appearance. A solution of only 1 part to 10,000 of water sufficed in the course of 24 hours to produce the same result. In well-developed cases the longitudinal rows of cells close to the tip of the root, with the exception of those forming the extreme apex, were filled with brown granular matter, and were thus rendered opaque. Long-continued immersion in water produced no such effect. The granular masses were square in outline, like the cells in which they were contained; but they often became rounded after a day or two; and this was apparently due to the contraction of the protoplasmic utricle. Above the dark-brown cells, which form a transverse zone close to the tip, and which apparently corresponds with the zone of quickest growth, the roots, as seen under a high power, are longitudinally striped with darker and lighter brown. The darker tint is due to the presence of innumerable rounded granules of brownish matter; and the cells containing them are arranged in longitudinal rows, while other longitudinal rows are destitute of granules. In a few instances the rows differed slightly in tint, and yet no

* 'Insectivorous Plants,' 1875, p. 64. The subject was at that time, 22 years ago, only casually investigated; and I believe that I erred greatly about *Lemna*, unless, indeed, some different species was then observed, or that the season of the year makes a great difference in the behaviour of the roots, which is not probable.

© Reproduced with the permission of Cambridge University Library by Darwin Online

were coloured blue. The tips of the roots of *Pastinaca sativa* turned dark brown by a similar immersion; but this was due to the formation of orange-brown balls of matter near the vascular bundle; higher up the roots there were no granules in the exterior cells. The tips of the roots of *Lamium purpureum*, after an immersion of 18 hours in a solution of 4 to 1000, were rendered brown, and the cells contained innumerable pale-coloured hyaline globules. The older roots of *Leontodon Taraxacum* and of a *Sonchus* had their tips turned brown by the solution. With *Lactuca sativa* the tips were rendered opaque; but much granular matter was not deposited except in that of one rather thick leading root, and here short longitudinal rows of cells containing dark-brown granular matter alternated with rows of colourless cells; the almost loose cells of the root-cap likewise contained brown granules. In the several following cases a much more strongly marked effect was produced by the solution.

Urtica.—This plant, the common nettle, shall be first considered, as it is distantly allied to the Euphorbiaceæ, though the roots are not so much affected as in succeeding cases. Several roots were left for 27 hours in a solution of the carbonate (4 to 1000). In one of them the exterior cells were plainly tinted of a brown colour in many longitudinal rows, but they contained no visible granules; and these rows regularly alternated with others formed of colourless cells. In another part of this same root all the exterior cells were coloured dark brown, and contained visible granules, which were generally collected into heaps at one end of the cell, or were fused together in some instances into small brown spheres. In a second, rather thick root, there was a space in which all the exterior cells had become brown; but at no great distance rows of brown and colourless cells regularly alternated. In a third, rather thick, and in a fourth, thin root the alternation was extremely regular. Near the tip of a fifth (thin) rootlet two rows of a brown colour ran alongside one another in many places; but when these and other single rows were traced up the root, they changed into colourless rows, and afterwards reassumed their former character. Whenever the root-hairs were traced down to their bases, they were seen to arise from colourless cells. Neither granules nor brown fluid were observed in the parenchyma-cells nor in those surrounding the vascular bundle.

Some roots which had been left in water for several days were

© Reproduced with the permission of Cambridge University Library by Darwin Online

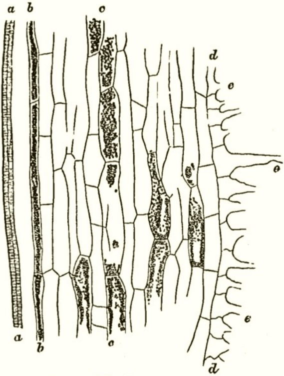
longitudinally striped with very faint brown lines; and one cell was observed which included granules; so that plain water produces some effect. These same roots, after being irrigated with a solution of 7 to 1000, were left for 24 hours; and now the longitudinal rows of brown cells had become much darker, and presented a much stronger contrast with the colourless cells. Several of the brown cells moreover included granules, which here and there were aggregated into small dark-brown rounded masses.

Drosera, *Dionæa*, and *Drosophyllum*.—The roots of the plants belonging to these three closely allied genera are strongly acted on by a solution of carbonate of ammonia. In the case of a young plant of the *Dionæa*, all the exterior cells of the roots, after immersion for 24 hours in a solution of 4 to 1000, contained almost black or orange, or nearly colourless spheres and rounded masses of translucent matter, which were not present in the fresh roots. In this case, therefore, the exterior cells did not differ in alternate rows. Near the extremity of one of these roots many separate cells in the parenchyma, as seen in transverse sections, contained similar translucent spheres, but generally of an orange colour or colourless. The cells surrounding the vascular bundle abounded with much smaller dark-coloured spheres.

Three main or leading roots of *Drosophyllum lusitanicum* were cut off and examined before being immersed in the solution, and no aggregated masses could be seen in them. Two were left for 22 hours in a solution of 4 to 1000, and they presented an extraordinarily changed appearance; for the exterior cells in many rows from the tips to the cut-off ends of the roots included either one large, or, more commonly, several spherical or oval, or columnar masses of brown translucent matter. The columnar masses had sinuous outlines, and appeared to have been formed by the confluence of several small spheres. The loose, or almost loose, oval cells composing the root-cap included similar brown spheres; and this fact deserves attention. Two rows of cells containing the just-described masses often ran up the root alongside one another; and sometimes there were three or four such adjacent rows. These alternated with others which were colourless, and contained either no solid matter, or rarely a few minute pale spheres. These roots were carefully examined; and all the many root-hairs arose from the colourless rows of cells, except in some few cases in which the cells on both sides abounded to an unusual

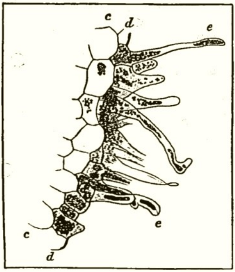
© Reproduced with the permission of Cambridge University Library by Darwin Online

Fig. 1.



Longitudinal section of root of *Cyclamen persicum* after immersion in a solution of carbonate of ammonia, and deposition in some of the cells of granules. *a*, part of vascular bundle; *b*, endoderm-cells; *c*, parenchyma-cells; *d*, exterior cells of the root, bearing root-hairs, *e*, with their tips cut off. Drawing made by aid of a camera, magnified 260 times, but here reduced to two thirds of original scale.

Fig. 2.



Transverse section of another part of the same root, magnified as before, showing the exterior cells *d*, together with the root-hairs *e*, here containing granules.

© Reproduced with the permission of Cambridge University Library by Darwin Online

coloured granules, unlike those in the same cells after immersion. Thick and thin roots were left for 22 hours in a solution of 7 to 1000, and the cells forming the exterior layer were filled over considerable spaces with green granules, while over other spaces they were empty. The granular and empty cells did not form regular alternate rows, as occurs in so many other plants; yet, as we shall presently see, there is occasionally some degree of alternation. The exterior cells with the green granules were so numerous in certain cases, that roots which had been pale brown before immersion were afterwards distinctly green. The green granules sometimes became aggregated into spherical, or oval, or elongated masses having a sinuous outline; and some of these are shown within the root-hairs in fig. 2. Many of the cells of the parenchyma, either standing separately or two or three in a row (as shown in fig. 1), contain similar green, or sometimes brownish, granules. Almost all the narrow elongated cells of the endoderm (*b*, fig. 1) likewise contain these granules, with merely here and there an empty cell. Although both kinds of cells often appear as if gorged with the granules, yet these really form only a layer adhering to the inside of the protoplasmic utricle, as could be seen when cells had been cut through. With some thick fleshy roots, after an immersion for 42 hours (and thick roots require a long immersion for the full effect to be produced) the green granules in the parenchyma-cells had become completely confluent, and now formed spheres of transparent green matter of considerable size.

The granules are not dissolved, nor is their colour discharged by sulphuric ether. Acetic acid instantly changes the green into a dull orange tint. The granules are not dissolved by alcohol. Their precipitation by the ammonia solution seems to depend on the life of the cell; for some transverse sections were examined and found colourless, as well as destitute of granules. They were then irrigated with a solution of 7 to 1000, and reexamined after 22 hours; and only a very few cells in two out of the five sections showed any trace of colour, which, oddly enough, was blue instead of green. The few coloured cells occurred exclusively in the thickest parts of the sections, where the central ones would obviously have had the best chance of keeping alive for some time. In these coloured cells a little very fine granular matter could be distinguished.

On most of the roots root-hairs were extremely numerous,

© Reproduced with the permission of Cambridge University Library by Darwin Online

and they generally arose from cells destitute of granules; yet in many places whole groups of cells abounding with granules gave rise to well-developed root-hairs. Therefore the rule which holds good with so many plants, namely, that root-hairs arise exclusively from colourless cells destitute of granules, here quite breaks down. The granules extend from the cells into the hairs which spring from them, as is shown in fig. 2; and they here sometimes become confluent, forming rounded or elongated masses of transparent green matter. This matter within the tips of some of the hairs seemed to pass into a brownish fluid. It was repeatedly observed that where many hairs rose close together from cells containing the green granules, the tips of the hairs were glued together by cakes or masses of orange-coloured translucent tough matter. This matter could be seen, under favourable circumstances, to consist either of very thin homogeneous sheets or of aggregated granules. It was not acted on by an immersion of two hours in absolute alcohol or in sulphuric ether. The smaller globules were either dissolved or destroyed by sulphuric acid, while others were rendered highly transparent. The formation of this orange-coloured matter is independent of the previous action of ammonia; and I have noticed similar matter attached to the rootlets of many other plants. It is probably formed by the softening or liquefaction of the outer surface of the walls of the hairs, and the subsequent consolidation of the matter thus produced*. Nevertheless some appearances led me to suspect that the brownish fluid which was seen within the tips of the hairs enclosing the green granules may perhaps exude through the walls, and ultimately form the cakes of orange matter.

A few other solutions were tried. Roots were left for from 20 to 43 hours in a solution of 7 parts of pure carbonate of soda to 1000 of water, and in no case were granules deposited in the exterior cells; but some of these cells in longitudinal rows became brown; these alternated with rows of colourless cells. In one instance several of these cells included oval or spherical masses of an apparently tenacious fluid of a brown tint. Single cells in the parenchyma likewise became brown; others were dotted, like a mezzotinto engraving, with barely distinguishable granules, which,

* See some remarks on this liquefaction of the outer surface of root-hairs by my son Francis and myself in 'The Power of Movement in Plants,' 1880, p. 69.

© Reproduced with the permission of Cambridge University Library by Darwin Online

however, in other cells were plainly visible; and, lastly, a few of these cells included spherical or oval masses of the same nature as those just mentioned in the exterior cells. Most or all of the endoderm-cells either contained a homogeneous brown fluid, or they appeared, from including excessively fine granules, like a mezzotinto engraving. In no case were any of the cells coloured green.

Some roots were immersed for from 20 to 44 hours in a solution of carbonate of potash of 7 to 1000; and these were affected in nearly the same manner as those in the soda solution. In the exterior cells, however, more granules were deposited; and these were oftener aggregated together, forming transparent orange-coloured spheres. The cells containing the granules or spheres were of a brown colour, and were arranged in longitudinal rows which alternated with rows of colourless cells. There were fewer granules in the parenchyma-cells than in the roots which had been subjected to the soda solution; and there were none in the endoderm-cells, even in roots which had been left immersed for 44 hours. A solution of phosphate of ammonia (4 to 1000) produced no effect on the roots after 43 hours' immersion.

Concluding Remarks.—The most remarkable conclusion which follows from the foregoing observations is that, in the roots of various plants, cells appearing quite similar and of the same homologous nature yet differ greatly in their contents, as shown by the action on them of certain solutions. Thus, of the exterior cells, one, two, or more adjacent longitudinal rows are often affected; and these alternate with rows in which no effect has been produced. Hence such roots present a longitudinally striped appearance. Single cells in the parenchyma, or occasionally two or three in a row, are in like manner affected; and so it is with the endoderm-cells, though it is rare when all are affected. The difference in aspect between sections of roots before and after their immersion in a proper solution is sometimes extraordinarily great. Of all the solutions tried, that of carbonate of ammonia acts most quickly, indeed almost instantaneously; and in all cases the action travels up the root from cell to cell with remarkable rapidity. With *Euphorbia Peplus* a solution of 1 part of the carbonate to 10,000 of water acted, though not very quickly.

When the action is very slight, the fluid contents of the cells are merely rendered pale brown. Nevertheless, judging from the gradations which could be observed, the brown tint is probably

© Reproduced with the permission of Cambridge University Library by Darwin Online

due to the presence of invisibly minute granules. More commonly distinctly visible granules are deposited, and these, in the case of *Cyclamen persicum*, adhered to the inner surface of the protoplasmic utricle; and this probably is the case with other plants. From granules we are led on to globules more or less confluent, and thence to spherical or oval or oddly shaped masses of translucent matter. These were coloured pale or dark blue or green in seven of the genera experimented on; but usually they are brownish. The granules or globules are not acted on, except as far as colour is concerned, by alcohol, sulphuric ether, a solution of iodine, or acetic acid; but they are slowly dissolved by caustic potash. It has been shown in a previous paper that in the leaves of certain plants carbonate of ammonia first causes the deposition of granules from the cell-sap, which aggregate together, and that matter is afterwards withdrawn from the protoplasmic utricle which likewise undergoes aggregation. Something of the same kind apparently occurs in roots, judging from the occasional difference in colour of the aggregated masses within the same cell, and more especially from what has been described as occurring in the root-cells of *Sarracenia* and *Pelargonium*.

Other solutions besides that of carbonate of ammonia induce nearly, but not quite, the same effects. Phosphate of ammonia acted more slowly than the carbonate on the roots of *Euphorbia Peplus*, and not at all on those of *Cyclamen*. With this latter plant and with the *Euphorbia* carbonate of soda was efficient, but in a less degree than the carbonate of ammonia. In one trial which was made, carbonate of potash acted on the exterior cells, but hardly at all on those of the parenchyma and endoderm. An extremely weak solution of osmic acid was highly potent, and the deposited granules were blackened. This acid is poisonous; but it must not be supposed that the mere death of a cell induces deposition. This is far from holding good; so that, judging from several trials, cells which have been killed are not acted on even by carbonate of ammonia, which is the most efficient of all known agents.

I have not sufficient data to judge how generally roots are acted on by the carbonate of ammonia in the manner described. Those of 49 genera, many of which belong to widely separated families, were tried. The roots of 15 were conspicuously acted on, those of 11 in a slight degree, making together 26 genera;

© Reproduced with the permission of Cambridge University Library by Darwin Online

while those of the remaining 23 genera were not affected, at least in any plain manner. But it should be stated that sections of all these latter roots were not made, so that the cells of the parenchyma and endoderm were not examined. We may therefore suspect that if various other reagents had been tried, and if sections had been made of all, some effect would have been observed in a larger proportional number of cases than actually occurred. I have elsewhere shown that the contents of the glandular hairs and of the epidermic and other cells of the leaves undergo aggregation in a considerable number of plants when they are acted on by carbonate of ammonia; and the roots of these same plants are especially liable to be affected in the same manner. We see this in 7 out of the 15 genera which had their roots conspicuously affected coming under both heads.

The question naturally arises, what is the meaning of matter being precipitated by a solution of carbonate of ammonia and of some other substances in certain cells and not in other cells of the same homologous nature? The fact of granules and spherical masses being formed within the loose exfoliating cells of the root-cap, as was observed in several instances, and conspicuously in that of *Drosophyllum*, apparently indicates that such matter is no longer of any use to the plant, and is of the nature of an excretion. It does not, however, follow that all the aggregated matter within the root-cells is of this nature, though the greater part may be; and we know that in the filaments of *Spirogyra* not only are granules deposited from the cell-sap which aggregate into spheres, but that the spiral chlorophyll-bands also contract into spherical or oval masses. The view that the granules consist of excreted matter is supported, to a certain extent, by their not being redissolved, as far as I could judge, in the roots of living plants of *Euphorbia Peplus*; and in this respect they differ in a marked manner from the aggregated matter in the leaves of *Drosera* and its allies. A larger amount of granular matter is deposited close to the tip of the root than elsewhere; and it might have been expected that where growth with the accompanying chemical changes was most rapid, there the largest amount of excreted matter would accumulate. It also deserves notice that there exists some degree of antagonism between the presence of these granules and of starch-grains in the same cells. On the other hand, it must be admitted that no excretion in the vegetable kingdom, as far as is at present known, remains dis-

© Reproduced with the permission of Cambridge University Library by Darwin Online

solved in the cell-sap, or, as in the present cases, is precipitated only through the action of certain reagents.

On the view here suggested the exterior cells in many rows, some parenchyma-cells, and many or most of the endoderm-cells serve as receptacles for useless matter. It will, however, at first appear highly improbable that so many cells should serve for such a purpose. But this objection has no great weight; for in certain cases a surprising number of cells may be found which, instead of containing chlorophyll-grains like the surrounding cells, are filled with crystalline masses of carbonate of lime and other earthy salts which are never redissolved. Many isolated cells or rows of cells likewise contain gummy, resinous, or oily secretions and other substances, which, it is believed, are of "no further use in the changes connected with nutrition or growth"*. We thus see that useless or excreted matter is commonly collected in separate cells; and we thus get a clue, on the view here suggested, for understanding why the deposited granules and spherical masses are found in isolated cells or rows of cells, and not in the other cells of the same homologous nature; and this is the circumstance which, as lately remarked, at first surprised me most.

In the roots of plants the endoderm-cells commonly separate those of the parenchyma from the vascular bundle. Very little is known about their use or functions; so that every particular deserves notice. They resemble the exterior cells in their walls partly consisting of corky or cuticularized matter†; and we have here seen that they likewise resemble the exterior cells by serving as receptacles for the deposited granular matter, which, in accordance with our view, must be excreted from the inner parenchyma-cells or from the vascular bundle.

The fact of the granules being deposited in the exterior cells in one, two, or more adjacent longitudinal rows, which alternate with rows destitute of granules, is the more remarkable, as close to the tip of the root all the exterior cells are commonly gorged

* Sachs, 'Text-Book of Botany' (Engl. transl.), 1875, p. 113. Also De Bary, 'Vergleichende Anatomie,' pp. 142–143. When odoriferous oils or other strongly tasting or poisonous substances are deposited in cells, and are thus thrown out of the active life of the plant, there is reason to believe that they are by no means useless to it, but indirectly serve as a protection against insects and other enemies.

† On the nature of endoderm-cells, see De Bary, 'Vergleichende Anatomie,' 1877, p. 129.

© Reproduced with the permission of Cambridge University Library by Darwin Online

with granular matter. It appears, therefore, that matter of some kind must have passed laterally from those rows which do not contain granular matter, after being acted on by the ammonia, into the adjoining rows. Why the useless matter should not pass out of the root through the outer walls of the cells, probably depends on the thickness and cuticular nature of the outer walls.

Pfeffer states that root-hairs are developed on the gemmæ, and apparently on the thallus, of *Marchantia polymorpha* from superficial cells which, even before the growth of the hairs, do not contain starch- or chlorophyll-grains; although these bodies are present together with matter of an unknown nature in the adjacent superficial cells. He has observed a nearly similar case with the roots of *Hydrocharis* *. No one else seems to have even suspected that root-hairs were not developed indifferently from any or all of the exterior cells. But it has now been shown that with many plants, with only one marked exception, namely that of *Cyclamen*, the root-hairs arise exclusively from cells in which granular matter has not been deposited after the action of certain solutions. This relation between the presence of hairs and the contents of the cells cannot be accounted for by matter, which would have been deposited if the roots had been subjected to a proper solution, having been consumed in the formation of the hairs; and this notion is wholly inapplicable to the cases described by Pfeffer. May we believe that cells filled with effete matter become unfitted for absorbing or transmitting water with the necessary salts, and do not therefore develop root-hairs? Or is the absence of hairs from the cells which contain the deposited matter due merely to the advantage which is commonly derived from a physiological division of labour? This and many other questions about the cells, in which granules or larger masses of translucent matter are deposited after certain solutions have been absorbed, cannot at present be answered. But I hope that some one better fitted than I am, from possessing much more chemical and histological knowledge, may be induced to investigate the whole subject.

* 'Arbeiten des botan. Instituts in Würzburg,' Band i. p. 79.

© Reproduced with the permission of Cambridge University Library by Darwin Online

Transverse sections of other immersed leaves presented various appearances. In one cell a central transparent sphere was surrounded by a halo of brown granular matter, and this again by a zone of the transparent matter, and such matter quite filled some adjoining cells. In the cells of another leaf there were, throughout its whole thickness, yellow, greenish, orange, pale or very dark-brown spheres. Some of these latter spheres had a dark centre, which was so hard that it was cracked by pressure, and the line of separation from the surrounding zone of paler matter was distinct. Two brown spheres were in one case included within the same transparent sphere. Gradations seemed to show that the opaque granular matter ultimately passed into dark-coloured transparent matter. In these same sections there were some colourless or yellowish highly-transparent small spheres, which, I believe, were merely much swollen chlorophyll-grains. One, two, or more of such grains, while still partly retaining their outlines, sometimes clung to the darker granular spheres. When there were only one or two of them thus clinging, they assumed the shapes of half- or quarter-moons. It appeared as if such swollen grains when completely confluent had often given rise to the pale zones surrounding the granular spheres. The pale zones were rendered still more transparent by acetic acid; and on one occasion they quite disappeared, after being left in the acid for 24 hours; but whether the matter was dissolved or had merely disintegrated was not ascertained. This acid produces the same effect on recently aggregated pale-coloured or almost colourless matter in the tentacles of *Drosera.*

In one leaf a good many unaltered chlorophyll-grains could still be distinguished in some of the cells; and this occurred more frequently in the thickest part of the leaf, near the midrib, than elsewhere. In one section the chlorophyll-grains had run together, and formed in some of the cells narrow green rims round all four walls. In many sections, more especially in those in which the process of aggregation had not been carried very far, there was much extremely fine granular matter, which did not resemble smashed or disintegrated chlorophyll-grains, such as may often be seen in sections of ordinary leaves. This granular matter occasionally passed into excessively minute, transparent, more or less confluent globules.

Judging from these several appearances, we may conclude that carbonate of ammonia first acts on the cell-sap, producing a gra-

© Reproduced with the permission of Cambridge University Library by Darwin Online

nular deposit of a pale brownish colour, and that this tends to aggregate into balls; that afterwards the grains of chlorophyll are acted on, some swelling up and becoming completely confluent, so that no trace of their original structure is left, and others breaking up into extremely fine greenish granular matter, which appears likewise to undergo aggregation. The final result is the formation of balls of brown, and sometimes reddish, granular matter, often surrounded by zones, more or less thick, of yellowish or greenish, or almost colourless transparent matter. Or, again, spheres, ovals, and oddly-shaped masses are formed, consisting exclusively of this transparent yellowish-green matter. As soon as the process of aggregation has been thoroughly carried out, not a grain of chlorophyll can be seen.

Drosera rotundifolia.—It is advisable to select for observation pale reddish leaves, as the dark-red ones are too opaque; and the process of aggregation does not go on well in the small completely green leaves which may sometimes be found. The tentacles, which are merely delicate prolongations of the leaf, are from their transparency well fitted for observation. In sections of the disks of fresh leaves, the cells of the epidermis are seen to abound with grains of chlorophyll, as well as those of the underlying parenchyma. The bases of the exterior tentacles and the part immediately beneath the glands are generally coloured pale green from the presence of chlorophyll-grains in the parenchyma; and some occur throughout the whole length of the longer tentacles, but are not easily seen on account of the purple cell-sap. Sometimes the epidermal cells of the longer tentacles include chlorophyll-grains; but this is rather a rare event. The footstalks of the short tentacles on the disk are bright green, and invariably abound with grains of chlorophyll.

A pale leaf, in which the basal cells of the exterior tentacles contained numerous grains of chlorophyll, was left for 24 hours in a solution of only 2 parts of the carbonate to 1000 of water; and now innumerable greenish spheres, resembling oil in appearance, were present in these cells, and the ordinary chlorophyll-grains had in most places disappeared. Nevertheless in several cells some swollen grains were still distinct. Other cells contained fine granular or pulpy green matter collected into masses at one end. In a few other cells the chlorophyll-grains had run together, forming a continuous green rim with a sinuous outline attached to the walls. In fresh leaves the guard-cells of the

© Reproduced with the permission of Cambridge University Library by Darwin Online

though slowly. In many, but not in all, of the cells of this leaf the grains of chlorophyll were still quite distinct. The several leaves were left both in the stronger and weaker solutions for 48 hours; and this caused the yellow spheres and masses to disintegrate into brownish granular matter. In this respect the aggregated masses in *Drosophyllum* differ from those in *Drosera* and *Dionæa.* Leaves were also left for 24 and 48 hours in an infusion of raw meat; but no yellow aggregated masses were thus produced, and the grains of chlorophyll remained perfectly distinct. This singular difference in the action of the infusion of raw meat on the tentacles, as compared with those of *Drosera*, may perhaps be accounted for by their serving in *Drosophyllum* almost exclusively for the secretion of the viscid fluid by which insects are captured—the power of digestion and of absorption being chiefly confined, as I have explained in my 'Insectivorous Plants' (pp. 332–342), to the minute sessile glands on the disks of the leaves.

As in the three foregoing genera the grains of chlorophyll tend to aggregate into moving masses under the long-continued influence of a weak solution of carbonate of ammonia, I thought that the grains would probably be similarly acted on in all insectivorous plants; but this did not prove to be the case. The immersion of leaves of the common *Pinguicula* in a solution of the ammonia and in an infusion of raw meat did not cause any aggregation of the chlorophyll-grains, though numerous transparent spheres were formed within the glandular hairs. Again, the immersion in carbonate of ammonia of pieces of young and old pitchers of a *Nepenthes* (garden hybrid variety) caused the appearance of innumerable more or less confluent spheres of various sizes in the glands on the inner surface of the pitcher and in the exterior epidermal cells. These were formed of translucent matter, either almost colourless or of a brown, orange, purple, or greenish tint; but the grains of chlorophyll were not acted on.

Sarracenia purpurea.—The pitchers of this plant are evidently adapted for catching and drowning insects; but whether they can digest them, or may have the power of absorbing matter from their decaying remains, is doubtful *. Many observations

* See an interesting account of the inner epidermal cells by A. Batalin, "Ueber die Function der Epidermis in den Schläuchen von *Sarracenia* &c." 1880. Reprinted from 'Acta Horti Petropolitani,' t. vii. (1880).

© Reproduced with the permission of Cambridge University Library by Darwin Online

were made; but one case will suffice. A piece of a pitcher was left for 24 hours in a solution of 4 parts of the carbonate of ammonia to 1000 of water, and for 24 additional hours in a solution of 7 to 1000. In the cells of the parenchyma, especially in those close to the vascular bundles, there were many spheres and aggregated masses of bright orange transparent matter. Spheres of the same and of various other tints were present in the epidermal cells, more especially in those on the inner surface of the pitcher; and some of these spheres were of exactly the same pale greenish colour as the swollen chlorophyll-grains which were still present in some places, being often collected together into rounded masses. In many of the epidermal cells which contained spheres no chlorophyll-grains could be seen, though they were abundantly present in the epidermis of fresh leaves; and it is this fact which chiefly leads me to believe that the chlorophyll-grains sometimes become so completely fused together as to form spheres, being often blended with the aggregated and coloured cell-sap. When a solution of iodine was added to these sections, the pale-coloured spheres and irregularly shaped aggregated masses became bright orange, and they were sometimes sprinkled over with blue particles of starch. The iodine did not cause their immediate disintegration and disappearance, nor did alcohol or acetic acid. In this respect they differ from the recently aggregated masses in *Drosera*; though in this latter plant the older and more solid aggregated masses are not acted on by these reagents. Many of the cells contained green granular matter, formed either by the chlorophyll-grains having been mechanically smashed or by their disintegration; and acetic acid sometimes caused this granular matter to change instantly into the same orange tint as that of the aggregated masses.

The orange spheres and variously shaped masses were seen in many sections of pitchers which had been exposed for different lengths of time to solutions of the carbonate of different strengths; and in many of them swollen grains of chlorophyll had become more or less confluent. The original nature of the latter could be recognized by the sinuous outlines and greenish tint. They were not seen to change their shapes spontaneously; but this could not have been expected in sections. Portions of a pitcher left in distilled water for nearly three days did not exhibit a single orange sphere or aggregated mass; but there were some colourless oil-globules which were dissolved by alcohol;

© Reproduced with the permission of Cambridge University Library by Darwin Online

alcohol they are subjected to the iodine solution, they soon almost disappear; but this, again, does not invariably occur. Acetic acid always caused their rapid disappearance, and without any apparent effervescence, a slight granular residue being sometimes left; and this occurred with leaves which had been kept so long in the solution that they were dead. The acid dissolved, of course with effervescence, the crystalline balls of carbonate of lime which occupy many of the palisade-cells. When sulphuric ether was added, the smaller spheres of transparent matter disappeared in the course of a few minutes, while the larger ones became brownish and granular in their centres; but this granular matter disappeared after a time, empty transparent bag-like membranes being left. Traces of similar membranous envelopes could sometimes be detected after the administration of acetic acid. Caustic potash did not act quickly on the spheres, but sometimes caused them to swell up. I do not know what ought to be inferred from the action of these several reagents with respect to the nature of the spheres and aggregated masses in which I never saw any movement.

On two or three occasions the palisade-cells of leaves which had been immersed in the solutions, instead of containing large transparent spheres, were gorged with innumerable, often irregularly-shaped, more or less confluent globules, many of them being much smaller than the chlorophyll-grains. This occurred with a leaf which had been immersed for only 18½ hours in a solution of 4 to 1000. After sections of this leaf had been cleared with alcohol, it was irrigated with the solution of iodine, and the globules rapidly ran together or became confluent, forming irregular amorphous masses.

It was difficult to ascertain whether the chlorophyll-grains ever or often became blended with other matter, and thus aided in the formation of the transparent spheres. The difficulty was partly due to the grains being easily acted on by water. Thus, in some sections made and placed in water, and then cleared in alcohol, no grains could be distinguished; while they were distinct in sections of the same leaf which had not been wetted before being placed in alcohol. Many grains were also found in a disintegrated condition in uninjured leaves which had been kept for 47 hours in water. It may be here added that not a single sphere could be seen in these leaves; nor were they present in leaves slightly injured by being kept for 24 hours in a very weak

© Reproduced with the permission of Cambridge University Library by Darwin Online

solution of osmic acid. Nor, again, in a leaf which had been immersed in an infusion of raw meat for 24 and for 50 hours; and in this leaf the chlorophyll-grains were still visible in many places, but were sometimes heaped together. Notwithstanding the difficulty of ascertaining the effects of carbonate of ammonia on the chlorophyll-grains, chiefly owing to the action of water on them, I am led to believe, from the gradations which could be followed, and from the absence of chlorophyll-grains in the cells in which one or two large spheres were present, that in the case of the palisade- and parenchyma-cells matter produced by the disintegration of the grains first aggregates, together with other matter derived from the cell-sap, into minute globules, and that these aggregate into the larger spheres. I will give a single instance:—A leaf was immersed for 22½ hours in a solution of the carbonate of 4 to 1000, and sections, after being cleared in alcohol, exhibited in many places distinct chlorophyll-grains, and in other places only very fine granular matter, and in a very few cells minute transparent globules. The leaf was left for 24 additional hours in the solution; and now sections cleared in alcohol exhibited numerous minute shining translucent globules, many of which were smaller than the few remaining chlorophyll-grains. There were also other much larger transparent spheres, more or less confluent, which, when irrigated with acetic acid, instantly disappeared.

A leaf was immersed in a solution of 4 parts of phosphate of ammonia to 1000 of water, and after 23 hours there was no trace of aggregation. It was left for 24½ additional hours in the solution; and now sections cleared in alcohol exhibited not only minute shining colourless globules, smaller than the few remaining chlorophyll-grains, but plenty of large spheres, more or less aggregated together; and in the cells containing such spheres no chlorophyll-grains could be seen. The spheres, both large and small, disappeared instantly when acetic acid was added, as in the case of those produced by the carbonate. It appears, therefore, that these two salts act in the same manner, but that the phosphate acts more slowly than the carbonate, as is likewise the case with *Drosera*. A leaf immersed for 45 hours in a solution of 2 parts of nitrate of ammonia to 1000 of water was a good deal infiltrated and darkened in colour; but no spheres were formed; some of the chlorophyll-grains had, however, become confluent while still adhering to the walls of the cells.

© Reproduced with the permission of Cambridge University Library by Darwin Online

the cell-walls were dotted over with chlorophyll-grains, there was at first some slight degree of aggregation, and then the grains all became disintegrated. In the second species, the filaments of which were extremely thin, the solution produced no effect. In a third the chlorophyll-bodies became aggregated into spheres. If the species in this family are difficult to distinguish, systematists might probably derive aid by observing the different actions of a solution of carbonate of ammonia on them.

Conclusion.—From the facts given in this paper we see that certain salts of ammonia, more especially the carbonate, quickly cause the cell-sap in various plants belonging to widely different groups to deposit granules apparently of the nature of protein. These sometimes become aggregated into rounded masses. The same salts and, in the case of *Drosera*, an infusion of raw meat tend to act on the chlorophyll-bodies, causing them in some few species to become completely fused together, either in union with the aggregated cell-sap or separately from it. Aggregation seems to be a vital process, as it does not occur in recently killed cells; and any thing which kills a cell causes the already aggregated masses instantly to disintegrate. These masses, moreover, display in some cases incessant movements. The process of aggregation is not rarely carried so far that the masses lose the power of movement; nor do they then readily disintegrate when subjected to any deadly influence. From these facts, from other considerations, and more especially from the action of carbonate of ammonia on the chlorophyll-bodies, I am led to believe that the aggregated masses include living protoplasm, to which their power of movement may be attributed. The most remarkable point in the whole phenomenon is, that with the Droseraceæ the most diverse stimuli (even a stimulus transmitted from a distant part of the leaf) induces the process of aggregation. The redissolution in the course of a few days of the solid aggregated masses and, especially, the regeneration of the chlorophyll-grains are likewise remarkable phenomena.

© Reproduced with the permission of Cambridge University Library by Darwin Online