XLII.—*On the Development of* Chara. By C. Müller*.

[Continued from p. 262.]

§ 5. *The perfect Plant.*

The modifications of the development of the stem in *Nitella* have been traced with tolerable completeness. In *Chara,* however, the simple tubes are covered externally with utricular cells which are arranged spirally around them. These are not formed until after the germinating plant has become much elongated and its ramifications have acquired considerable increase. In a plant which has attained this stage of development several peculiarities are apparent. Such are the evolution of axillary cells to form axillary branches, and the formation of other more or less rounded cells in ascending and descending rows at the articulations of the stem; lastly, the formation on the stem itself of new papillary cells which are identical with those last mentioned, as in *Chara hispida* and *crinita,* where they sometimes again elongate into articulated tubes, and thus give the plants a very rough appearance. The cells themselves do not differ at all in their internal structure from those of the spore-sac. *How are these organs formed? How are the utricular (cortical) cells formed around the central utricle?* Here the process of development of the entire plant is far more complicated than that of the germ. Moreover the history of the development of all the organs, of the branches, shoots, and even of the stem itself, is intimately connected, and we must again commence with the stem.

To trace the formation of the *stem,* we must search for that point at which it is developed. This is *its apex, the terminal bud;* which consists externally of a single large cell forming the immediate continuation of the stem, and as such, terminating it in the form of a cupola (Pl. VI. fig. 11). The bud appears of the same form in the centre of the recently formed whorl of branches whilst still short. Its membrane is extremely delicate and therefore easily injured. It contains a reddish granular matter (cytoblastema), which too frequently renders it impossible to arrive clearly at the structure of the bud. If we succeed, however, in any way in removing it—which can only be effected by dissection, for iodine and acids render the contents still more obscure—we find the whole of the interior already covered with cells with exceedingly delicate walls (fig. 14). [In this figure the preparation fig. 11 is placed on its vertex, and we are supposed to look internally from above towards the vertex.] This cellular structure consists of a central cell, around which some other cells are de-

* Translated from the Botanische Zeitung for June 26, 1845.

posited. There are usually six of them, but sometimes seven, eight or more. Hence, according to their number, the central cell is six-, seven-, eight- or more sided, since they directly limit and compress it. They are themselves also naturally flattened at their points of contact, but are spherical towards the exterior. If at this time, as is usually the case, many layers of them are arranged together, the central cell becomes a short, six- or more sided column; the cells surrounding it therefore possess only four longitudinal surfaces—the external of which are thus spherical, the three inner flattened—and two transverse surfaces bounded by four sides. Each cell contains a cytoblast. There are four perfect cells also, having very delicate walls, on the apex of the bud. But exactly at that part where they meet in the centre of the axis they inclose a small and very pale body, which is the true vegetating point—perhaps it is the newly forming cytoblast of the central cell. This point is likewise of importance for the further formation of the cellular tissue of the axial bud.

The central cell is the commencement of the true stem, or, as it is usually called, of the central utricle in all *Charæ* provided with a cortical layer. But the cells which surround it do not constitute the commencement of this layer, but of the *branch*.

As the plant elongates, of course the central cells also elongate and form long tubes. At first lying close to the cells of the branch they continue to become more expanded, especially when they have acquired the cortical layer. Its cells then become rounded both externally and internally, and thus the central utricle appears distinct, whilst previously (fig. 18) it could only be made perceptible by iodine, which coloured the delicate intercellular spaces and the walls of the utricle blue. Thus it is transformed from the cytoblastema into an amylaceous substance, which subsequently becomes converted into membranous matter, and is then no longer coloured by iodine. As soon as this happens the central utricle becomes considerably thickened by the absorption of more nutriment, and it is very beautiful to see how this is deposited in layers. Hence the stem of the perfect plant differs considerably from that of the germinating plant in the manner of its development. In the former it is primary—an immediate expansion of the nucleus; in the latter secondary, but formed by a higher process.

When the branches are about to form, the cells which surround the central cell become expanded in the form of simple cylinders (figs. 11, 14). Internally they exhibit exactly the same structure as the axis, for we find the same central cells and external cells also with extremely delicate walls already formed in them (fig. 16). They also have a terminal bud, from which new cells are formed as in the case of the stem. The principal difference between it and the stem-bud is merely, *that this can be elongated to infinity, whilst the growth of the bud of the branch is very limited.* This limit is shown in the apex of a branch (figs. 12, 13), which no longer appears as a bud, but as a simple terminal cell. The explanation of this limited and unlimited development is to be sought for in the fact that the cytoblastema of the stem has to take a direct ascending course, whilst in the branches this is at first indirect, *i. e.* dependent upon the stem, devious, and limited by the amount of nutritious fluid. Thus the more the organs are removed from the centre of the individual, so much the more simple must they become, since enough nutriment is not present for a higher development. We also see this in a greater degree in the formation of the shoots. *This is effected by the simple cylindrical expansion of the external cells which are deposited upon the central cell of the branch*; consequently exactly in the same manner as the branches were formed from the stem. The difference between them is merely, that here the further formation ceases, so that we have no more to do with buds, but merely simple cells, exactly as at the apex of the branch. Hence both agree in not possessing the property, like the other parts of the stem and branch, which lie nearer to the axis of the individual, of forming a cortical layer. At the most they can only produce a few cells (articulations) in their interior.

The peculiarity of the branch, that its membranes rapidly become thickened, is opposed to another property of the axial bud. This always possesses closed branches, *i. e.* rolled up over it, which are only subsequently separated from it when the individual whorls of branches separate from each other by fresh increase. New whorls of branches, *i. e.* the most recently formed, appear in their place and surround the delicate terminal bud. Thus they defend it from accidental injury, since the oldest always cover the youngest, until the latter have become sufficiently strong to be able to undertake the same office for the younger branches. Of course this function does not occur in the shoots; they are therefore protected by becoming rapidly thickened.

If we now compare the formation of the branches and the stem in the germinating and the perfect plant, we find the following essential difference, that *in the former* these organs are formed *by intercalary or intermediate growth*, whilst *in the perfect plant they are produced directly*. In the germinating plant the whorls of branches follow the formation of the stem; in the latter the branches and stem go hand and hand in development. In the former there are at first always two cells where an internode is about to form; in the latter the whorls of branches mark the internodes from the very commencement. Hence the former, strictly speaking, are only accidental, the latter are essential.

Both however agree perfectly in being effected through cytoblasts. But I consider it hardly necessary again to bring forward proofs of this kind of formation. They lie however, in the absence of anything like a secondary membrane, in each cell containing cytoblasts, and in the existence of real, although very minute intercellular spaces; hence the formation of new cells by the contraction of secondary membranes, or from the deposition of membranous matter upon projecting portions, as in Unger's view, is here quite out of the question.

We shall now consider *the cortical layer*. In the further progress of the growth of the plant, the whorls of branches and of shoots become separated from one another (figs. 12, 13). Between each whorl an internode is formed; and if we trace this process in its earliest condition, we find the cortical layer already prepared, running from internode to internode: hence it must happen that the number of utricular cortical cells is constantly double that of the number of branches; thus if there are six branches, there are twelve cortical cells, &c. We do not find any instances in which there is any great variation from the above laws. *Thus, in each cell of the branches, which is immediately attached to the stem, two cytoblasts are usually transformed into two new cells*; there is rarely only one present (fig. 17). Although this process is very difficult to trace, I have directly observed it. It is most easily followed by making extremely delicate longitudinal sections through the axis of the plant; we then find the new cells with their cytoblasts, but always considerably turbid. In a transverse section I have also found two cytoblasts in a cell, which were on the very point of expanding into cells. These new cells which are thus formed from cytoblasts now expand longitudinally, and thus run in a longitudinal direction between the epidermoidal membrane of the apex of the stem and the central utricle; hence they do not run externally but internally, being inclosed by the membrane. In other respects their formation is similar to that of other cells, as already described in the germinating plant. Fig. 17. Pl. VI. represents a transverse section with the internode cut through beyond its margin. This section, and such may be frequently found, exhibits a central cell in its interior and seven placed around it. These are provided with separating walls, and divide the cavity of the stem into internodial cells. This is distinctly seen in a longitudinal section, as in fig. 19. Pl. VII., and in a transverse section when made distinct by iodine, as in fig. 18, also in fig. 15. Thus it is at the same time clear that the formation of the cortical layer occurs upwards, and consequently resembles the growth of the apex. The entire process however proceeds simultaneously with the development of the stem, the branches and the shoots. Its cytoblasts are formed at the same moment as the external cells are transformed into branches and shoots. The cortical layer is thus attached in its very earliest stage, subsequently attaining very great strength, and is readily separable from the central utricle. The intercellular spaces which are formed between its separate cells and the former become filled with a formative mass, from which new cells may arise according to circumstances.

The following question is closely connected with this development: *How is it that this cortical layer is wanting in the* Nitellæ? I cannot offer any history of the development of the *Nitellæ* which will solve this question; still it appears to me that it may be better answered here than from them alone. Moreover the structure of the *Nitellæ* does not differ from that of the germinating plant. We have therefore both stages in the *Charæ*: the simple stem of *Nitella,* and one surrounded with a cortical layer, the genus *Chara.* We must be able to draw some conclusion from the observation of the successive development of the two. If we expose a plant of *Chara* in a glass of water to the warmth of a stove and light, the stems are developed with uncommon rapidity into long thready utricles. If they are examined more closely, we observe not only that the above double relation of the cells in the cortical layer to those in the stem is disturbed, because generally speaking fewer must always be seen than would be necessary to completely cover *the central utricle, the stem*; but we find very frequently, in fact nearly always, that the cortical cells are completely wanting*. I think that we may directly solve the question from this observation. If this phænomenon is merely produced by the rapid growth of the articulations of the stem, when we apply this to the *Nitellæ,* we have the solution. First, the rapid growth is unfavourable to the formation of cytoblasts; secondly, the cytoblastema present is rapidly assimilated by the membranes of the stem; whence, thirdly, the very remarkable circumstance happens in the *Nitellæ,* that there are formed in the internodial cells a very considerable quantity of starch-cells, in which those cells abound. This has been already observed by several persons and may be readily repeated; it is an essential period in the formation of *Nitella* and of its structure in general, such as we find no instance of in *Chara,* at least as far as I know. The cytoblastema which is produced from the transformation of starch again becomes converted into starch, when the proper time arrives for its again acquiring the state of aggregation of starch. This also occurs in the internodial cells of the *Charæ* and of the papillæ of many species; but whilst in

* This fact appears also to have been observed by Quekett (see Jahresbericht, &c. von J. Em. Wickström, translated by Beilschmied for 1838, Breslau, 1843, p. 26) in *Chara hispida.* The author thinks it follows hence, that all *Charæ* are only modifications of one and the same species.

them the further development of starch ceases, in the *Charæ* it is converted into chlorophylle. Hence the above papillæ, for instance—in which this is very readily and beautifully observed—become at first deep blue, and subsequently brownish when treated with iodine. Thus the ready conversion in *Nitella* of the internodial cells into new plants is explained; and they have been distinguished as gemmæ, although they are not true buds, upon which the organs are situated, but rather mere formative cells. The step to the new formation of cells is certainly not great; if cytoblastema is formed from starch, then the power exists of forming cytoblasts and consequently new cells. We have not far to seek for its analogues, when we recollect the fermentation-cells. *Wherever formative matter is present, there cells may form; and as many are formed from them as correspond to the matter deposited in them.*

From what has been stated it appears to me to follow, that we must still separate *Chara* and *Nitella*; for the latter constantly forming simple stems only, proves *that the power of rapid growth must be inherent to them as a principle*; and although on the other hand the *Charæ* may exhibit the same phænomenon, it is only a deviation from their natural condition*.

As regards several accidental cells in the Charæ, for instance, the above papillæ, as also those which are developed beneath and above the internodes but still upon their cells, their formation invariably occurs by intercalary growth as stated above, or by the simple elongation of cells which are already formed. All the axillary branches are likewise formed by interrupted growth. The above-mentioned papillæ frequently or always fall off subsequently, for instance, in *Chara crinita*, leaving round brown spots at the points to which they were attached. They appear unsusceptible of further development. It is moreover remarkable, that frequently *three* cells are formed from a single articulation at the internodes. This great power of increase of the plant explains how the internodes are frequently surrounded with one or more whorls of cells (those *below* them assuming a descending direction, as they are prevented from ascending by the branches, those *above* an ascending direction).

The last phænomenon which requires notice is *the formation of new cells at the apex of the branches.* It appears to me *in this case* that the new cells are formed by subdivision. The first commencement of this process appears as a dark line surrounding the circumference of the cell. The secondary membrane then becomes constricted. [We saw above that the very apex of the

* Kützing (*l. c.* p. 319) has formed a third group "*Charopsis*" of *Chara Braunii, scoparia* and *barbata*.

branches and the shoots soon formed strong membranes.] The primordial utricle also follows this course until it is completely drawn in. This also agrees with Mohl's observations. We have some striking proofs of it in the preparations of Pl. VII. figs. 21, 22, 23, 24 and 26. In fig. 21 the preparation was treated with iodine, and the primordial utricle has become retracted from the walls of the cells; but it is not completely separated, for it is distinctly seen under the microscope to pass through both of the cells. The part which was not contracted appeared much more transparent and clear than the other portions. In Pl. VII. fig. 22 the apex is sliced longitudinally, and it is distinctly seen how the secondary membrane projects into the interior, but has not yet come into contact: I have observed this *once only*. In fig. 23 the apex is seen to be entirely separated by constriction: it appears exactly as if it passed into the previous articulation. The same occurs in fig. 24. The preparation was treated with nitric acid, and the primordial utricle of the lower cell has separated from the cell-wall, so as to resemble an open utricle, into which we can easily see. Fig. 26 exhibits the two extremities of the primordial utricle so remarkably torn, and agreeing in this particular so much, that we cannot help believing that they must once have been connected. Moreover I have frequently perceived this form. Are we now authorized to conclude from these transitions that there occurs a division of the cells? I think we are! and I could have added many more sketches of preparations which are in my manuscript. One thing appears to me remarkable, viz. that all other parts of the plants are certainly formed from cytoblasts, and in this instance we have a division of already formed cells. However, I see no other deduction from the observations than the division of the cells.

It is remarkable in the primordial utricle, that it (as in fig. 24) appears as completely separated as if it had been cut with a knife; it also seems not to be so important in these cells as in those formed from cytoblasts. *Double septa* formed from secondary membranes (one belonging to each cell) are in tolerably close contact and perfectly close the separate cells. Now as the primordial utricle is firmly attached to this, the interior of the cell is closed as regards the regular course of the circulation of the sap. And when it is completely absent in the old cells, as Mohl observed, this from its gradual absorption could not produce any interruption to the cell-life.

[To be continued.]