B. Panmeristic Cell-Division

Chapter I

THE ORGANIZATION OF PROTOPLASTS

§ *1. The Visible Organization*

Protoplasm is the vehicle of the phenomena of life, and therefore also of hereditary characters. Hence, any theory of heredity must start from a definite view in regard to the structure of this important substance. But anatomical investigation, in spite of its astonishing progress during the last decade, has in this very field not yet achieved a clear and generally accepted conception of this structure.

This is essentially due to the circumstance that the newer methods for the study of the nucleus and its division have disclosed a field so important, and so rich in surprising results, that attention has been directed chiefly, and frequently exclusively, to this organ. Often one even meets with views which put the protoplasm (cytoplasm) into the background with reference to the nucleus.

But the study of the nucleus is so much advanced at present that one may hesitate at this one-sided treatment. The researches of Flemming, Strasburger, and so many other investigators, have disclosed the structure of the nucleus and the changes of this structure during division, and have, in the main, brought our knowledge to a definite conclusion. Now, especially in botany, the investigation of cell-division itself comes again to the front. And it is not only a question of establishing the relation of the nucleus to the cytoplasm; it is just as essential a problem to find out the attitude of the individual organs of the latter and especially of the vacuoles, the granular plasm, and the plasmatic membrane. For the knowledge of cell-division will be complete only when all the organs of the protoplast have been equally considered.

The described course of investigation makes it clear why even a practical and simple designation of the living cell-contents has not yet gained general recognition. Such a designation was suggested by Hanstein, in his well-known lectures, by the word "protoplast."[1] The word "protoplasm" was coined by Mohl for the semi-liquid nitrogenous substance "which furnishes the material for the formation of the nucleus and the primordial utricle," and from which originate the first solid structures of the future cell.[2] The formed body, built up from this substance, has frequently been called protoplasmic body, plasm-body, sometimes even protoplasmic globule or drop, expressions which are obviously inadequate to create a clear conception in the minds of readers and hearers.

Compared with these designations, Hanstein's word clearly and distinctly describes the individuality of the living cell-contents. This individuality has long been recognized by the best investigators. As early as 1862 Brücke said that protoplasm was an organic body; not a drop of fluid, but an elementary organism.[3] But the lack of an appropriate name obscured the clearness of the conception, and it was Hanstein who supplied this want. Klebs and others have accepted his designation and

[1]Hanstein, J. von. *Das Protoplasma als Träger der pflanzlichen und thierischen Lebensverrichtungen.* 1 Theil. 1880.

[2]Mohl, H. von. *Bot. Zeit.* 4: 75. 1846.

[3]Brücke, E. Die Elementarorganismen. *Sitzungsber. Kais. Akad. Wiss. Wien.* 44^2: 381. 1861.

through their influence it will doubtless be more generally adopted.*

Protoplasts are elementary organisms in the true sense of the word. They consist distinctly of individual organs, which are more or less sharply distinguished from each other and which possess a high degree of mutual independence. In the greatest number of plants this structure is clearly evident, but in the lowest organisms this differentiation is entirely wanting, or at least it is limited to a great extent. Sometimes one meets with the expression "unorganised plasm," even for organisms which by no means lack differentiation. But doubtless this expression must be understood to mean that the methods so far employed have not yet revealed any insight into the organization, and not that the want of any kind of organs has been thoroughly studied and definitely proven.

*As is well known, the term is now in common use. *Tr.*

CHAPTER II

HISTORICAL AND CRITICAL CONSIDERATIONS

§ 2. *The Neogenetic and the Panmeristic Conceptions of Cell-Division*

Only a few decades back it was generally believed that individual organs, such as the nucleus and the chlorophyll grains, could always, or at least very frequently originate from the undifferentiated protoplasm through differentiation. However, in recent years, investigations have not confirmed this neogenesis in a single instance. Wherever the origin of an organ has been thoroughly and comprehensively studied, with the present means of investigation, the organ has been shown to originate by a division of differentiated members already present.

The organization of the protoplasts is not periodical, nor evident only in grown cells. It is permanent, inherent in all cells, and in all stages of their development. The assumption of formation *de novo* gives place everywhere to the recognition of divisions; the neogenetic conception gives way to the panmeristic.[4]

It is of interest to glance over the course of development of our knowledge. In his "Lehre von der Pflanzenzelle," Hofmeister describes the nuclei according to the knowledge of that time. They appear in the protoplasm as drops or masses of a transparent homogenous substance, either in cells with few nuclei, of a definite

[4]The view that all the organs of protoplasts, as a rule, multiply only by division I call *panmeristic*. This assumption was maintained for plant-cells for the first time in my plasmolytic studies. Cf. Vries, H. de. *Jahrb. Wiss. Bot.* **16**: 489. 1885.

size from the beginning, or in cells with many nuclei, first as small formations which increase through growth. Sometimes they contain granules as soon as they become visible, but frequently they occur at first without any internal solid structure, and attain this only later. Every cell-division is usually preceded by a disappearance of the nucleus, which is then followed by the appearance of two or more new nuclei.[5]

The comprehensive investigations of Strasburger and Schmitz have proven this assumption to be erroneous, at first for isolated and then for an increasing number of cases, and wherever a disappearance and subsequent reappearance of nuclei was assumed, the origination of the new nuclei through division of the original ones could be proven. Exceptions to this rule are no longer known.

The history is exactly the same for chlorophyll grains. Even in the last edition of his text-book[6] Sachs said: "The chlorophyll bodies originate in young cells through the separation of the protoplasm into clearly distinct colorless portions that are becoming green. The process can be conceived to mean that, in the originally homogenous protoplasm, most minute particles of a somewhat different nature are distributed or originate for the first time and then accumulate at various points, appearing as differentiated bodies." That the green bodies which had formed in this way could multiply through division, and that the chlorophyll bodies of many algae are usually cut through at every cell-division by the forming wall, can easily be observed and was not unknown at that time.

But it was Schmitz who first showed that, in the algae,

[5]Hofmeister, *Die Lehre von der Pflanzenzelle*. p. 79. 1887.
[6]*Lehrbuch der Botanik*. 4. Auflage, p. 46. 1874.

division is the only way in which the chromatophores are newly formed.[7] Following up this idea with the phanerogams, Schimper discovered the colorless organs of the youthful cells, which in these cells are exclusively charged with the formation of starch and through whose assumption of green color the real chlorophyll grains are formed. In all cases that have been observed those amyloplasts multiply only through division, and Schimper, as well as Arthur Meyer, has accumulated such a number of observations on this manner of development that the former view has been abandoned by all botanists. Some special cases, it is true, still await explanation, but as long as they have not been thoroughly investigated, there is no reason for regarding the old conception more plausible than the new one.

It is similar with reference to the vacuoles. Until about four years ago they were generally regarded as a new formation in the protoplasm, caused by the secretion of superfluous water of imbibition. In my *"Plasmolytische Studien über die Wand der Vacuolen,"* I have established the claim that, for them as well, the mode of origin of nucleus and trophoplast[8] must be the only real one.[9] I supported this claim by showing that all vacuoles are surrounded by a living wall, which, according to the method suggested by me, can always be easily and convincingly demonstrated, and which I believe may be regarded as an organ of the protoplast, with as much right as the nuclei and the chromatophores.

This conclusion, drawn from my panmeristic conception of cell-division, has been completely confirmed by

[7]Schmitz, F. *Die Chromatophoren der Algen.* Bonn, 1882.

[8]By this name Arthur Meyer designates the amyloplasts and their derivatives (chlorophyll grains, chromoplasts, etc.)

[9]*Jahrb. Wiss. Bot.* **16**: 489-505. 1885.

Went's investigations.[10] Thereby, to my mind is proven the correctness of this conception as opposed to that of neogenesis. Now the situation is reversed. While up to the present time the condition with reference to the nucleus and the chromatophores could be regarded as peculiar, there is now great probability that the different members of a protoplast have the same mode of origin, and therefore that they can claim the rank of independent organs only in so far as they follow this rule.

Now that the mode of origin for nucleus, trophoplasts and vacuoles has, in the main, been established, and that the works of Wakker[11] have taught us to recognize the crystals, most of the crystalloids, and the aleurone grains as contents of the vacuoles, the problem is chiefly concerned with the plasmatic membrane and the granular plasm.[12] In regard to their behavior during cell-formation our knowledge is essentially the same as at the time of Mohl and Hofmeister. Our insight into the process of cell-division has indeed become deeper, chiefly through Strasburger's work; but the very point in question, the beginning of the dividing wall, which for some time, seemed to be decided neogenetically, has again become extremely uncertain through the discovery (to be discussed later) of the cell-ring by Went[13] as well as through the objections of other investigators.

[10]Went, F. A. F. C. *De jongste toestanden der vacuolen.* Amsterdam, 1886. Les premiers états des vacuoles. *Arch. Neerl.* 1887, and Die Vermehrung der normalen Vacuolen durch Theilung. *Jahrb. Wiss. Bot.* **19:** 295. 1888.

[11]Wakker, J. H., Studien über die Inhaltskörper der Pflanzenzellen. *Jahrb. Wiss. Bot.* **19:** 423. 1888. Preliminary contributions are found in *Maandblad v. Natuurwetensch.* 1886, Nr. 7. 1887, Nr. 5 and 6, and in *Bot. Cent.* **33:** 360, 361. 1888.

[12]Cf. § 6 below. p. 150.

[13]Cf. § 7 and 8, pp. 157 and 160.

For these reasons I believe that a critical review of our knowledge in this field will be of substantial usefulness. It will then be shown how, in almost all cases, the attitude of the plasmatic membrane and of the granular plasm, during cell-formation, is in fact unknown. At least in all the cases which seem to contradict the panmeristic conception.

It is not a question of whether this latter conception is correct or not. This seems to me to have been proven above any doubt by the researches of the investigators that have been quoted. *The question is whether, with this* conception, we are to regard the granular plasm and the limiting membrane as two intrinsically different organs, *which pass over into one another as little as the nuclueus* and the chromatophores, or whether they stand in a similar relation to each other as the amyloplasts and the chlorophyll-grains. As long as it was thought that the granular plasm had the power of producing the other members by a process of differentiation, it was natural to assume a like mode of origin for the plasmatic membrance. It is therefore not astonishing that, even at present, this view is still regarded as the one that actually obtains. The instance described by Mohl as a type of cell-division, and which involved the historically noteworthy discussions of the question as to whether the protoplasmic body played a passive or an active rôle during this process is well known to all. Like Mohl's type of the filamentous algae, *Cladophora, Spirogyra* is in more recent times preferred for this study. At the future plane of division the limiting membrane and granular plasm fold into a ring which, growing inwards, apparently simply cuts in two *the remaining part of the cell-contents. For the* daughter-cells the two new parts of the limiting membrane originate as a continuation of the old membrane. According to Klebs's[14] descriptions the Euglenidae also offer a beautiful example of panmeristic cell-division.

It is very unlikely that in the case of such a fundamental process, the higher plants should behave differently from the lower ones. That there are differences in minor points is self evident, and everybody knows that there are important distinctions, especially in the relative duration of the individual steps in the process. And the same holds for the manner in which it is provided that every daughter-cell gets its own nucleus. But, that the completion of the plasmatic membrane should take place through the insertion of a quite newly formed piece is so much at variance with the rest of our knowledge, that one cannot by any means accept it on the basis of the older investigations. At any rate it must be held in doubt until supported by direct observation.

Such, however, is not the case at present, as I shall try to show in the last Chapter of this Section. On the contrary many facts already speak in favor of the complete autonomy of the membrane, although not with sufficient certainty to serve as conclusive proof.

However that may be, whether the limiting membrane can develop from the granular plasm, or whether both are mutually autonomous, it is certain, at any rate, that on the one hand these two, and on the other the nucleus, the trophoplasts, and the vacuoles are independent organs, which, in the normal course of things, multiply only by division.

Hence, the organization of the protoplasts is hereditary, and this not in the sense that the organization of the higher organisms is reproduced in each individual through

[14]Klebs, G. *Arbeiten Bot. Institut.* Tübingen. **1:** 282.

the development of invisible hereditary units, but through the direct passage, from the mother-cell to the daughter-cells, of all the organs which compose the organism.

The significance of this law for our hypothesis of intracellular pangenesis will be discussed in the last division of this Part. Here we will familiarize ourselves more thoroughly with the actual basis on which it is founded.

§ *3. Cell-Division According to Mohl's Type*

The *"Grundzüge der Anatomie und Physiologie der Vegetabilischen Zelle,"* by Hugo von Mohl,[15] was for a long time the chief source from which beginning botanists got their knowledge. It is only Hofmeister's *Pflanzenzelle* (1867) and Sachs's *Lehrbuch* (1868) which put an end to its reign, but many illustrations and statements from the *"Grundzüge"* are still vividly remembered by older botanists.

The multiplication of cells through division is described in the following manner in this book of Mohl's.[16] It "is introduced by changes which the primordial utricle of the dividing cell undergoes, in consequence of which the dividing walls develop, growing gradually inward from the periphery of the cell, and separating the cell-cavity into two or more cavities." We have to distinguish those cases where the cell-division is preceded by a doubling of the nucleus, from those in which this is not the case (our present poly-nucleate cells). This latter, less frequent, but simpler case occurs in *Conferva glomerata,* and therefore Mohl begins his description with this alga. But even where the formation of two new nuclei precedes the formation of the dividing wall,

[15] Published in Wagner's *Handwörterbuch der Physiologie,* 1851.
[16] *Loc. cit.* p. 211.

this latter process takes place in the same manner as in the *Conferva* above mentioned. And this as well among the algae as in the higher plants. According to Mohl, then, the plasmatic membrane is always produced by new parts growing out of old ones.

As to the historical aspect, it needs only to be emphasized that this law for the algae, which Mohl put into the foreground, has been confirmed by all later investigators.[17] Here its correctness is beyond any doubt, and can be easily controlled by anybody. Who, therefore, on theoretical grounds, is inclined, to assume that, in cell-division, the same principles are valid for the entire plant-world, must with Mohl, still regard the case in question as a type.

In the uni-nucleate cells there are usually present very peculiar structures, the function of which is to make the new dividing wall pass exactly between the two new nuclei. From our present conception of the significance of the nucleus this cannot be wondered at, for what would a cell be without its hereditary characters. In the higher plants these structures are not cleared up in every respect, though with the spirogyras this is, to a large extent, the case, especially through the repeated publications of Strasburger. We shall therefore describe the process in this plant, making use of the last description of this investigator as far as this serves for our purpose.

At the time[18] when the nucleus approaches the end of the prophase, the protoplasm collects around it and

[17]Cell-division through constrictions is widely distributed among the lower algae. Cf., e. g., Klebs, *Arbeiten Bot. Inst.* Tübingen. **1:** 336-343.

[18]The following is taken from Strasburger, *Ueber Kern- und Zelltheilung im Pflanzenreich.* pp. 9-23. Jena, 1888.

assumes, in the region of the poles of the nucleus, a structure of parallel fibres. It soon becomes clear that we have to do with the first signs of the spindle-fibres. These develop quickly and continue through the interior of the nuclear cavity, until they come into contact with each other. There is no valid reason for the eventual assumption that the spindle-fibres developing in the interior of this cavity are of a different origin from the external ones. On the aequator of the spindle the chromatic substance accumulates, touching the individual fibres at their circumference.

Next occurs the formation and longitudinal splitting of the nuclear skein, followed by the separation and moving apart of the two halves of the segments. During this period one sees clearly that not all the spindle-fibres have succeeded in uniting with the opposite ones. Only those that were successful in this are retained as connecting fibres between the two young nuclei which move apart. The space forming between them is surrounded by a protoplasmic mantle toward the outside, and apparently there collects in it a substance with osmotic action which enlarges this space and drives the young nuclei apart. In the meantime the number of the connecting fibres on the mantle of this space is lessened more and more, the mantle itself is made to bulge more and more in a transverse direction, and becomes correspondingly thinner. Yet it remains sharply and plainly visible. The space has assumed now the well-known barrel-shape, its wall is called the connecting cylinder, and remains for some time as an extended vesicle, closed in on all sides. Finally, by being strongly distended in an aequatorial direction, this vesicle reaches the protoplasmic accumulation at the margin of the protruding dividing wall. It unites with the latter, and is now gradually flattened by it, and finally constricted.

According to the principles of the theory of the vacuoles ascertained by Went and myself, it is probable that the space containing osmotic substance and surrounded by the connecting cylinder is a vacuole, which, contrary to Strasburger's conception,[19] must have penetrated from the outside between the two younger nuclei. It is just as evident that this vacuole must be surrounded by a wall of its own, and that this also forms the inner layer of the connecting cylinder. The latter is also separated from the other vacuoles of the cell-space, by a wall, and between the two walls there lies, at least in the beginning, granular plasm. The changes of that vacuole which forms the interior of the barrel during the whole process require, of course, special investigation, made on living material.[20]

But there can be no doubt about the correctness of Strasburger's conception, where he places the whole process of cell-division, with the one exception of the division of the nucleus, in the protoplasm itself. The daughter-nuclei are passive in this, the cytoplasm alone is the active element.

The chlorophyll-bands, the vacuole, and the granular plasm are simply constricted by the plasmatic membrane growing into the interior. The membrane itself finally separates in the same manner, after having entirely closed up the space remaining in the middle of the ring.

In those poly-nucleate algae, the nuclei of which are evenly distributed over the entire lining layer of proto-

[19]*Loc. cit.* p. 17.

[20]Zacharias, in his discussion of Strasburger's work *(Bot. Zeit.* **46**: 449. 1888), emphasizes also "that, on the living object, things may exist which can be better recognized and interpreted there than by fixing and staining."

plasm, no particular devices have been observed for assuring the possession of one or more nuclei at the cell-divisions of each daughter-cell. Moreover they do not seem necessary, owing to the great number and regular distribution of the nuclei. Nuclear spindle and nuclear barrel have therefore lost their significance in this case, and accordingly they are probably not present, at least not as a rule. Cell-division is essentially performed by the plasmatic membrane and the granular plasm only.

For the correct understanding of the processes of normal cell-division, one law, which has been ascertained by experiments on artificial division of living protoplasts in former and more recent times, is of extreme importance. I do not mean the adaptive processes of regeneration after wounding (these will be discussed in the next paragraph), but the constriction of the uninjured cell-contents in entire cells, and the division of the protoplasts into two or more pieces during plasmolysis. The respective cases I have put together in my *"Plasmolytische Studien über die Wand der Vacuolen."*[21] They teach that, in artificial constrictions of a protoplast, the limiting membrane, the wall of the vacuole, and the granular plasm close their edges, apparently without any difficulty, and round off to form a new unit. In plasmolytic experiments this is easily verified. Here one sees also, how upon the restoration of turgor, the parts flow together again, their members uniting with the corresponding organs of the other parts of the same protoplast.

This power of combining with homologous parts seems to be universally inherent in the three mentioned organs of the plant-protoplast. The walls of the vacuoles show it wherever the numerous vesicles of cell-sap

[21] *Jahrb. Wiss. Bot.* **16**: 501-505. 1885.

in young tissue-cells combine into one large vacuole during the rapid growth in the transition to the adult condition. When two or more like protoplasts unite to form a so-called symplast, something similar takes place in their walls, at least in some cases, as in the plasmatic membrane and the granular plasm. The ontogeny of the latex-vessels teaches this more clearly than anything else. A fusion of like parts in the "feet" of many rhizopods has also been repeatedly observed and described.

As far as we know, only simple contact is needed for this fusion, besides the required degree of homogenity. We may, therefore, regard it as a mechanical process and use it as an element in the explanation of normal cell-division. In *Spirogyra* it evidently accomplishes the fusion of the spindle with the inward growing ring, and later determines the final closing up of the opening that was left in the ring.

§ 4. *The Regeneration of Protoplasts after Wounding*

Even though, in the normal course of development, the individual organs of a cell multiply by division, this does not necessarily imply that this rule must be without exception, and that there cannot be cases where nature tries to achieve its ends in another way. Especially where, through outward interference, such as wounding and mutilations, individual members of a protoplast are completely lost, it might be expected that a regeneration in another way might be possible.

To be sure observations now available do not warrant the assumption that such cases actually occur. But this does not, by any means, exclude their possibility. And on this possibility I want to lay great stress in this connection, for the hypothesis of intracellular pangenesis allows us to regard as possible an occasional neogenesis of such organs out of pangens proceeding from the nucleus.

Judging from the facts published up to the present time, however, the phenomena of regeneration after wounding are closely connected with the normal processes. Nobody, at least recently, has maintained that in such a case there is a new formation of nucleus and chromatophores. There have been only few investigations in regard to a possible occurrence of new vacuoles. These were made by Went for the very purpose of testing the point in question, and teach at least one thing with certainty, that so far, wherever it had been thought necessary to assume a formation *de novo* of normal vacuoles, such does not really take place. For the vacuoles which have been observed originate partly through constriction from the large sap-vesicle of the cell, and partly through the swelling of the smaller ones which are suspended in the granular plasm. Especially in the case of the *Vaucheria*, which was studied first by Hanstein, and later by so many investigators, there surely can no longer be a well founded doubt on this point.[22]

Since the time when, in my *"Plasmolytische Studien,"* I expressed the opinion and sought to establish the fact that the plasmatic membrane is a separate organ of the protoplast[23] no decisive facts on this subject have been published. Klebs is opposed to my assumption on the ground of an observation made on *Vaucheria*.[24] For the study of these processes this investigator introduced a new method, which makes it possible to demonstrate, easily and with certainty, the beginnings of the formation

[22]Went, F. A. F. C. *Jahrb. Wiss. Bot.* **19**: 330-341. 1888.
[23]*Jahrb. Wiss. Bot.* **16**: 493. 1885.
[24]*Arbeiten Bot. Inst.*, Tübingen. **2**: 510.

of a cell-membrane around exuded masses of protoplasm. He stains the water or the diluted solution in which the threads are cut through, with Congo-red, which is stored up with great avidity by these young cell-membranes.

Nevertheless this method does not yet decide the question raised by me, because, as Klebs also says, there is no means of deciding the presence or absence of a plasmatic membrane on a portion of the mutilated protoplast that forms a cell-membrane. "Among the free swimming balls of protoplasm there are always a number of such that are quite large and rich in contents which live several days but without forming a cell-membrane." In the case of most of them, however, the beginnings of the formation of a cell-membrane are very soon evident.[25] Wherein the difference in the behavior of these two kinds of fractional parts consists, was not further investigated by Klebs. My assumption that the former lacked the limiting membrane, while the latter got a part of this organ when cut off, has not been at all disproved.

Nor does the great extensibility of the plasmatic membranes during the enormous swelling of the vesicles which later form the cell-membrane seem to me by any means improbable or even surprising. Plasmolytic experiments teach us at every step that the extensibility, not only of the plasmatic membrane, but also of the wall of the vacuoles and perhaps even of the granular plasm is very considerable. And Went has comprehensively demonstrated that the swollen spheres of *Vaucheria* contain only such vacuoles as have originated by the enlargement, and mostly also by division of the sap-vesicles present in the uninjured plant. The assumption of an extensibility of the plasmatic membrane which need not be much greater than the proven elasticity of the wall of the vacuoles can-

[25] *Loc. cit.* p. 507.

not seem very surprising. The phenomena of regeneration of *Vaucheria* demand renewed investigation in this respect also. As long therefore, as there is no actual proof of a neogenesis of this organ, independently of the old one, we cannot recognize such great significance in this instance as some authorities attribute to it.

Here also the observations by Haberlandt[26] on the same phenomenon are important. This investigator directed his attention chiefly to the nuclei, and familiarized himself with their behavior during regeneration. The nuclei accumulate near the wound in the plasma deprived of chlorophyll bodies, and are evidently more important than the latter for the growth of the new cell-membrane. In the exuded globules of protoplasm which remained alive, Haberlandt succeeded almost always in demonstrating the presence of one or more nuclei, but never the absence of any. In spite of this, not all of them formed a new cell-wall. "At times there occur cell-forms devoid of a membrane and rich in plasm. If the sap-cavity is lacking, the chlorophyll-bodies aggregate in the center, and the nuclei lie in the peripheral, colorless plasma. In case a cavity for cell-sap is present, the chlorophyll-grains lie in the innermost layer of the plasma-body the nuclei more toward the outside."[27] The possession of nuclei is therefore, in itself, not sufficient for the formation of a cell-membrane. It would be important to find out whether the parts of plasma referred to are perhaps the very ones that did not get part of the old limiting membrane.

It seems to me to be of great interest to regard the whole pending question from another point of view, and one which has already been considered by Haberlandt.

[26]Haberlandt, G. Ueber die Beziehungen zwischen Funktion und Lage des Zellkernes. pp. 83-97. Jena, 1887.

[27]*Loc. cit.* p. 92.

Regeneration is obviously an adaptation to guard against the results of injuries which occur frequently in nature. In such cases the higher plants usually give up the affected cells; the large-celled algae and fungi, especially those that have been designated by Sachs as non-cellular, evidently cannot do that. Therefore one generally finds in them the power of closing up wounds. That it would, however, be of particular importance to keep escaped globules of protoplasm alive is the less probable, as it is only possible to do so in solutions which are quite a little more concentrated than those in which the respective plants naturally live. Therefore, the closing up of the wound is primary, the processes in the escaped plasma secondary. From the adaptive characters available for the first, it ought to be possible to explain the latter. And as long as the first can be explained without the hypothesis of an independent neogenesis of the plasmatic membrane, this assumption must be regarded as at least improbable for the latter.

This consideration leads us to include in the field of these studies even the closing up of wounds in latex-tubes. The investigations of Schmidt on the latex-vessels, and of Schwendener on the latex-cells may serve as important points of departure in this.[28] For they teach that in parts of latex-tubes which adjoin the wound of the cut, a closing up of the tube can be accomplished in the same way as in some Siphoneae (e. g., *Bryopsis, Codium, Derbesia)* and in many pollen-tubes the injured part of the cell-cavity is separated from the uninjured one.[29]

[28]Schmidt, E. Ueber den Plasmakörper der Geliederten Milchröhren. *Bot. Zeit.* **40**: 462. 1882. Schwendener, S. Einige Beobachtungen an Milchsaftgefässen. *Sitzungsb. Kais. Akad. Wiss.* Berlin. **20**: 323. 1885.

[29]Schmidt, E. *loc. cit.* p. 462.

Chapter III

THE AUTONOMY OF THE INDIVIDUAL ORGANS OF THE PROTOPLASTS

§ 5. *Nucleus and Trophoplast*

A review of our knowledge concerning the anatomy of the nucleus can be regarded as superfluous in this connection. This knowledge is to be looked upon at present as an established achievement of science, the significance of which for the theory of heredity can hardly be doubted any longer. Flemming in the zoological, Strasburger and Schmitz in the botanical field have broken the way, and their observations have been verified and extended in the main by numerous other investigators.

It does not seem to be quite fully decided whether the amitotic nuclei, which have originated through constriction and scission, are of significance in questions of heredity, or whether they occur in somatic cells only, and not on the germ-tracks. In *Chara* the nuclei in the apical cells divide, according to Johow's investigations, according to the usual scheme of indirect nuclear division; the smaller cells of the grown plant, for example in the nodes, remain forever uni-nucleate, while the larger ones become multi-nucleate through constriction. This kind of nuclear formation, however, is never followed by cell-division.[30] According to Zimmermann direct nuclear division in the plant-world "is limited to only those cases in which the nuclear division is not accompanied by cell-division."[31]

[30] Johow, F. Die Zellkerne von *Chara foetida*. *Bot. Zeit.* **31**: 729. 1881.

[31] Zimmermann A. *Morphologie und Physiologie der Pflanzenzelle*. p. 34.

In the multi-nucleat cells of *Valonia* Schmitz[32] has frequently observed division, and always observed it to take place by constriction. It does not seem to be established with certainty, for all cases, how the nuclei of the swarm-spores originate here and in the case of the other Siphonocladiaceae, whether through direct or indirect division.

In this connection it should be mentioned that, according to Van Beneden and Julin, direct and karyokinetic nuclear divisions alternate in the spermatogenesis of *Ascaris megalocephala.*[33] Thus we see that this subject is not yet ripe for theoretical use.

The amyloplasts, with all their derivatives, among which the chlorophyll bodies are the most important, Arthur Meyer calls trophoplasts. In the lowest plants they are not yet differentiated, and, as far as these belong to the Phycochromaceæ, the whole non-nucleated protoplasm of the cell, according to Schmitz, is stained.[34] But later Hansgirg demonstrated nuclei and chromatophores in some algae of this group.[35] From the Chlorophyceæ upward they are universal in the green plants. In the higher plants, where they were discovered by Schimper,[36] they are usually colorless in young cells. As a rule they remain so in the underground parts, which are normally not exposed to light.

Phylogenetically, therefore, plants with undifferentiated colored protoplasm are probably older than those

[32]Schmitz, F. *Die vielkernigen Zellen der Siphonocladiaceen.* p. 27. 1879.

[33]Van Beneden et Julin, *La spermatogénèse chez l'Ascaride mégalocéphale,* Bruxelles, 1884.

[34]Schmitz, F. *Die Chromatophoren der Algen.* p. 9. 1882.

[35]Hansgirg, A. *Ber. Deut. Bot. Ges.* **3:** 14. 1885.

[36]Schimper, A. F. W. Ueber die Entwickelung der Chlorophylkörner und Farbkörper. *Bot. Zeit.* **41:** 105, 121, 137, 153. 1883.

which possess special chromatophores. Hence we must imagine them to have originated from the others through differentiation. A further step in the differentiation is then the development of colorless conditions of these chromatophores. These are still lacking in the lower Algæ, occur first in the highest groups of this class, and attain their full significance only in the higher plants. In other words, we must regard the amyloplasts, although they are generally the young condition from which chlorophyll bodies develop, as the consequences of a higher differentiation and assume that they have developed phylogenetically from the latter. This discussion is important for the reason that it brings nearer to our understanding the not infrequent changes of form of the trophoplasts on the germ-tracks. On the whole, the cells of the germ-tracks of the higher plants are, as many authors emphasize, of an embryonic nature, and such cells probably always possess colorless trophoplasts. But according to our definition of the germ-tracks, there are many exceptions to this rule. Thus, to name only one instance, the prothallia of ferns, in their youthful state, consist of green, dividing cells, with well-formed chlorophyll-grains, from which later the amyloplasts of the egg-cells will originate. Also in the callus-formation of cut petioles of *Begonia, Peperomia,* and other species, a reversion of green trophoplasts into colorless ones may take place, especially in the case of the production of adventitious buds. And, since generally the amyloplasts occur in young cells and their derivates in grown protoplasts, these and similar cases would be illustrative of a pronounced rejuvenation.

On the germ-tracks the amyloplasts usually take on a simple roundish form, on the somatic tracks they change their shape considerably, and with it the structure and size of the starch-grains produced by them.

Among the most peculiar characters of the chromatophores in connection with the organization of the protoplasts, belong their autonomous movements. Since the researches of Sachs on this subject, we know that the chlorophyll grains of some plants are moved about by streams of the granular plasm in such a way that, under the influence of light, they take up positions which are favorable for the assimilation of carbon dioxide. But in this process they are passive. The beautiful researches of Stahl, however, have disclosed independent movements of these structures under the influence of the same stimulation. They consist chiefly in changes of shape, through which the organs in question either approach a more or less globular shape, or that of a flat, circular disc. Thus it is brought about that, in direct sunlight, they present a smaller, in diffuse daylight, a larger surface for receiving the rays. And to us they afford an insight into the high degree of their inner differentiation such as we could never have attained by the simpler study of their chemical activity.

According to Weiss, the yellow and orange chromoplasts at times also make autonomous movements, which, according to the descriptions of this author, resemble the changes of form of the amœba and the white blood-corpuscles.[37] These structures, therefore, may also be more highly organized, and play a more important rôle, than that of the simple task of giving their color to the respective plants.

I wish to lay quite particular stress here on these

[37]Weiss, A. Ueber spontane Bewegungen und Formänderungen von Farbstoffkörpern. *Sitzungsb. Kais. Akad. Wiss. Wien.* **90**: 1884.

phenomena, for up to the present time they have probably not been utilized for the theory of heredity. But the more plainly we see the independence of the individual organs of the protoplasts, and the more clearly our conviction grows that they require a high inner differentiation for exercising their functions, the more will we be inclined to give them their due place in our theory, and especially will we try to investigate the more thoroughly their relation to the hereditary factors accumulated in the nucleus.

Wherever, hitherto, we have succeeded in demonstrating with complete certainty the origination of trophoplasts, we have found that they arise through a division of those already present. That the chlorophyll grains, in the higher plants as well as in the algæ, can multiply through constriction and scission has long been known. But it was Schmitz who showed that this process is the only form of their multiplication in the algæ.[38] In the Characeæ he discovered, in the apical cells, the colorless bodies from which the green organs of these plants are derived in the same way. These investigations are now so generally known that it would be superfluous to describe them here in detail. I shall only emphasize, as especially important, the fact that the swarm-spores also possess only such chromatophores as they have received from their mother-cell, a fact that was especially mentioned in the case of *Cladophora* and *Halosphaera*.[39]

The investigations by Schimper and others, who discovered this same law for the phanerogams, have already been discussed in one of the preceding Chapters.

Special consideration is still due to the rarer forms derived from the more general chromatophores. In the

[38]Schmitz, *Die Chromatophoren der Algen.* 1882.
[39]*Loc. cit.* pp. 135, 136.

first place we must mention the eye-spot[40] observed in many swarm-pores, and which, according to the opinion of those investigators who have examined it more carefully, is probably a metamorphosed chromatophore, the same as the chromatic bodies of the higher plants studied by Arthur Meyer.[41] In the Euglenæ its origin has been more carefully studied by Klebs. Here it always originates by division, the organs being always preserved in the resting cells.[42] It is not yet definitely decided whether or not the pyrenoids in the chorophyll bodies of *Spirogyra* and other algæ are to be regarded as specially differentiated parts of these organs. But it seems certain that, at least in isolated cases, they multiply through division.[43]

On the origination of oil in plant-cells little is known with certainty. Pfeffer has demonstrated that the oil does not form in the vacuoles, but lies imbedded in the granular plasm. Special organs which accumulate it within themselves have lately been described by Wakker for *Vanilla planifolia,* and have been called elaioplasts. Although it has not been possible to find out their mode of origin, the most natural assumption is that they are metamorphosed chromatophores.[44] In some cases, as for example in the diatomes, the oil-drops of the Algæ evi-

[40]Cf. Zimmerman, *Die Morphologie und Physiologie der Pflanzenzelle.* p. 71. 1887.

[41]Meyer, Arthur, *Das Clorophyllkorn.* 1883.

[42]Klebs, Ueber die Organisation einiger Flagellatengruppen. *Unters. Bot. Inst.* Tübingen. **1:** 233.

[43]Schmitz, F. *Die Chromotophoren der Algen.* pp. 42 and 65. 1882. Schmitz, F. Beiträge zur Kentniss der Chromatophoren. *Jahrb. Wiss. Bot.* **15:** 142. 1884. Strasburger, E. *Ueber Kern- und Zelltheilung.* p. 26. 1888.

[44]Wakker, J. H. De Elaioplast. *Maandbl. v. Natuurwetensch.* No. 8. 1887.

dently do not lie in the chromatophores, and this, according to Schmitz, is a general rule.[45] But in the higher plants this seems at times to be the case.[46]

Last to be mentioned here are the microsomes. In most cases it seems to be unknown what they are. Small oil-droplets, starch-grains, inactive vacuoles, amyloplasts, protein bodies formed by fixation[47] through the coagulation of the protein dissolved in the protoplasm, and perhaps many other formations are frequently all classed under this name. Very justly has Strasburger claimed "that not the microsome but the hyaloplasm is to be considered the active substance."[48] At any rate it ought never to be forgotten that the word microsome stands only for a question mark, and that we can talk of an insight into the significance of these structures only after the question concerning their nature in the cases concerned shall have been answered.

§ 6. *The Vacuoles*

Vacuoles were formerly regarded as empty spaces in the interior of the protoplasm. This accounts for their name, and explains the small interest shown in them, until recently, in the study of the anatomy of the cell. It is only since Sachs discovered that the turgidity of growing cells is not due to an imbibition of water in their walls, as was previously assumed, but to an osmotic tension between the wall and the cell sap, that attention was directed to the significance of the vacuoles.[49]

[45]Schmitz. *Loc. cit.* p. 164.

[46]*Cf.* Meyer, Arthur. *Das Chlorophyllkorn,* pp. 14 and 31. 1883.

[47] i. e. artifacts caused by the "fixing" fluid. *Tr.*

[48]Strasburger, E. *Neue Untersuchungen.* p. 107. 1884.

[49]Sachs, J. von. *Lehrbuch der Botanik,* 3 Aufl. 1872; 4. Aufl. 1874, p. 757.

This was still more the case through the demonstration furnished by the same author, that the tension to which growing cell-membranes are subjected by the cell-sap is one of the most essential mechanical causes of the surface growth of these membranes. For with this demonstration Sachs laid the foundation still valid, for the whole mechanical theory of growth in length.

Building on this foundation, many investigators have enlarged our knowledge of the mechanical causes of growth in various directions. Some have especially measured and analyzed the degree of extensibility of the cell-membranes and the amount of force supplied by the cell-sap. Others have studied the causes governing the variations of extensibility of the wall in one and the same cell, and which occur in different spots and in different directions, and have explained them, as due, with great probability, to local differentiations in the protoplast itself, which might regulate this elasticity through the secretion of certain enzymes. Others again have attacked the doctrine of intussusception, which was the prevailing one at the time of the discoveries mentioned, have proven it to be incorrect, and have tried to ressuscitate in its place, in a new form, the old "apposition theory."

Although subject to misunderstandings from some sides,[50] Sach's theory has acquired a prominent position in plant-physiology, and, since the two decades of its establishment, it has become, in ever increasing measure,

[50]In my "Untersuchungen über die Mechanischen Ursachen der Zellstreckung" (p. 3, 1877.), I have distinctly emphasized the fact that there are also phenomena of growth independent of turgor, and that therefore this turgor is neither the only, nor even the first reason for growth. Krabbe and Klebs arrived later at the same conclusion. Cf. *Arbeiten Bot. Inst.* Tübingen. **2**: 530. 1888.

the starting point of new investigations. It has been, without doubt, one of the most fruitful thoughts for the development of our science.

The further study of the cell-sap and the vacuoles, suggested by this theory, has led in regard to the morphological aspect, which alone interests us here, to the proof that the wall of the vacuoles is an essential, never wanting part of the plant-protoplast.[51] The method which made it possible always to demonstrate the presence of this wall consisted in the treatment of the living cells with a 10% solution of potassium nitrate, which has been stained with eosin. Directly, or after a shorter or longer period, the outer protoplasm dies in the reagent, while the wall of the vacuoles remains living for a while. It is then visible as a distended bubble, more or less completely separated from the dead parts, and entirely preventing the penetration of the eosin. In colorless cells, therefore, the bubble carries contents as clear as water, while the remaining protoplasm is stained red or brown by the eosin. Frequently the original vacuole separates into several smaller ones; and not infrequently one can follow this process directly under the microscope.

The wall of the vacuoles is to be regarded as a special organ of the protoplast, which regulates the secretion and accumulation of the substances which are present in the cell-sap in solution, and because of this function, it has been given the name *tonoplast.* But frequently the sap-spaces together with their walls are now designated as vacuoles.

In living cells the tonoplasts are, as a rule, not visible, because they consist of translucent vesicles of an extreme

[51]Vries, H. de. Plasmolytische Studien über die Wand der Vacuolen. *Jahrb. Wiss. Bot.* **16**: 465. 1885.

thinness. But they are clearly and distinctly visible in the tentacle-cells of some insectivorous plants, especially of the *Drosera rotundifolia* and *D. intermedia.* The process of aggregation, discovered by Darwin,[52] taking place here during the digestion of the prey, belongs to the most interesting phenomena that the life of a cell presents for our admiration. In the resting tentacle-cells there lies usually a large vacuole containing red cell-sap. Under the influence of stimulation it separates into several, and soon into numerous smaller ones. These contract, while secreting part of their contents, and are now carried through the cells by the currents of the granular plasm, with great rapidity, and in the most various directions. Thus they lie as red vesicles in unstained substance, and can therefore be seen very distinctly. During these movements they undergo striking changes of form; sometimes they are drawn out into long tubes, and thereupon split into numerous small globules, sometimes two or more unite to form larger vesicles. Toward the end of the phenomenon this last process has the precedence, and finally all the sap-bubbles have again united into one, of the original volume.[53]

The above mentioned phenomena of aggregation, and the division of the vacuoles, as it is so frequently observed in plasmolysis placed the ability of these organs to multiply by this process beyond any doubt. From the analogy of these structures with the chromatophores I then deduced the assumption, that "like the amyloplasts, they can be produced in no other way than by division."[54]

[52] Darwin, C. *Insectivorous Plants.* Chap. III. 1875.

[53] Vries, H. de. Ueber die Aggregation im Protoplasma von *Drosera rotundifolia. Bot. Zeit.* **44:** 1, 17, 33, 57. 1886.

[54] Vries, H. de. Plasmolytische Studien über die Wand der Vacuolen. *Jahrb. Wiss. Bot.* **16:** 505. 1885.

This supposition has since been completely confirmed by Went.[55] He showed first, that, contrary to the prevailing opinion, vacuoles are present even in the youngest cells of the meristem. These multiply continuously through division, and observation teaches that during cell-division one-half of the vacuoles present goes to one daughter-cell and the other half to the other. Sometimes it was possible to observe the constriction and afterwards the transmission of the two sap-vesicles, formed in this way, to the daughter-cells. From the vacuoles of the meristem all the vacuoles of the entire plant can thus be derived. Divisions of these structures are to be found everywhere; formations *de novo* nowhere. In the same way, in the cryptogams that grow with an apical cell, all the vacuoles originate from the original vesicles present in these cells.

According to these investigations the vacuoles behave exactly in the same way as the chromatophores, and are just as independent cell-structures as the latter. And through the demonstration of this independence, the panmeristic conception of cell-division has been definitely proven as correct, in opposition to the former neogenetic one.

According to later communication by the same author, he succeeded also in observing the formation of vacuoles in some special cases which had not been studied before. Here should be emphasized the formation of these organs in the swarm-spores which, according to a communication by letter from Went, comes about by a division of the sap-vesicle in the mother-cell in such a way that every

[55]Went, F. A. F. C. Die Vermehrung der normalen Vacuolen durch Theilung. *Jahrb. Wiss. Bot.* **19**: 295. 1888.

swarmer receives into its body a portion divided off from this bubble.

In the literature, an origination of sap-cavities in nuclei, chromatophores, or even in the granular plasm, outside the vacuoles already present, has repeatedly been described. But, on investigating these cases, it was found that here one had to deal, not with normal vacuoles, but with pathological formations, which occur with the ageing or dying of the cell. Frequently they are also due to the influence of the water in which the preparations are examined.[56]

From the theory that the vacuoles originate only through division, it may be concluded that the sap-vesicles of germinating seeds are derived from those present in the ripening ovules, and that, therefore, in the ripe condition, the vacuoles must indeed be dried out, but cannot be entirely lacking. Following up this thought Wakker arrived at the noteworthy discovery that the aleuron-grains are the dry states of the vacuoles in the seed.[57] During the process of ripening, the amount of protein matter dissolved in the cell-sap gradually increases until the fluid becomes of a thick, slimy consistency. In drying, some of the protein bodies crystallize and form the well known crystalloids, while the remaining protein hardens into an amorphous mass around them. When soaking the seed, these masses soften gradually and are later utilized as nourishment. By using a solution of one part nitric acid in four parts

[56]Went, F. A. F. C. *De jongste toestanden der vacuolen,* pp. 45-65.

[57]Wakker, J. H. Aleuronkorrels zyn vacuolen. *Maandbl. v. Naturw.,* Nr. 5. 1887. Over kristalloiden en andere lichamen die in de cellen van zeuvieren voorkomes. *Bot. Cent.* **33**: 138. 1888, and *Jahrb. Wiss. Bot.* **19**: 423. 1888. Since that time this result has been confirmed by Werminski, *Ber. Deut. Bot. Ges.* **6**: 199. 1888.

of water, one can bring about at will this hardening in the still liquid cell-sap, and in this way artificially produce the formation of aleuron-grains under his very eyes.

It is important that, in some seeds more, in others less, the vacuoles divide during the process of ripening into several smaller, frequently into very numerous extremely minute vesicles, which gradually fuse again into one large vacuole at the beginning of germination.

The processes in the seed, therefore, fit beautifully into the conception that the vacuoles originate only by division.[58]

Just as the chromatophores can differentiate into the most various organs, so also can the vacuoles, although to a lesser extent. Went observed how, in different cells, there lie vacuoles which remain separated throughout their existence, and are distinguished by their different contents.[59] Frequently some of them are stained, others are colorless, or some contain tannin, which is lacking in others. In such cases the latter are called by that author adventitious vacuoles.

The contractile or pulsating vacuoles form a special system. In the swarm-spores of the algæ they probably originate from the other vacuoles[60] through further differentiation, but in the Euglenæ, according to the investi-

[58]In Müller's bodies of the ant-plant, *Cecropia adenopus,* Schimper illustrates formations in the cell-contents which, at first glance, look like vacuoles, and which, on account of their semi-fluid contents, he compares with the aleuron-grains. Their origination from vacuoles can hardly be doubted. Schimper, A. F. W. *Die Wechselbeziehungen zwischen Pflanzen und Ameisen.* 1888. Cf. especially Taf. II, Fig. 11. Also Wakker, *Jahrb. Wiss. Bot.* **19**: 467. 1888.

[59]Went. *loc. cit.* pp. 65-91.

[60]Or have the turgor-vacuoles possibly originated phylogenetically from the pulsating ones?

gations of Klebs, they multiply by division.[61] They possess here a wall of their own which resembles the walls of ordinary vacuoles in its great power of resistance. Klebs observed how the pulsation may continue for a long time after the rest of the protoplast has been killed by some mechanical interference. The view that, in systole, the contents of these vacuoles are expelled into the surrounding tissues, while, in diastole, fluid is taken from the protoplast, is probably generally accepted for rhizopods and flagellates. My own observation convinced me of its correctness in *Actinophrys Sol.* The same opinion may also apply to the pulsating vacuoles in the plant-world.[62]

§ 7. *The Relation Between the Plasmatic Membranes and the Granular Plasm*

While the investigations of the last two decades have thrown a clear light on the organs of the protoplasts just discussed, the relation between plasmatic membrane and granular plasm is still completely in the dark. In our knowledge of the mode of origin of the nuclei, trophoplasts, and vacuoles, the theory of heredity, as I have tried to explain in this Section, finds its indispensable basis. On the mutual relation of the two other mentioned parts of the protoplast, no facts have yet been found, which might be utilized for the theory.

As already mentioned, what the nature of that relation is, is certainly not of essential importance for the hypothesis of intracellular pangenesis. Yet it remains an important question whether granular plasm and plasmatic membrane are mutually as independent as the granular

[61] Klebs, G. *Arbeiten Bot. Inst.* Tübingen, Bd. I. p. 250. ff.
[62] Pfeffer, *Pflanzenphysiologie,* pp. 399-401.

plasm and the wall of the vacuole, or whether they stand in the same genetic relation as amyloplasts and chlorophyll-grains. As long as this question remains undecided, the application of my hypothesis to the plasmatic membrane and therewith to the surface growth of the cell-membrane and all the formative processes of the cells, is rendered very difficult. For this reason may I be allowed to subject the respective phenomena to a critical revision in order to encourage further research. I think it will then be seen that the prevailing opinion that the plasmatic membrane originates in every case from the granular plasm is, for the present, not supported by certain and closely observed facts, but is adhered to only from habit. This, however, it seems to me, ought not to be allowed in view of the newer knowledge in regard to the origin of the wall of the vacuole. For, as long as no special wall was assumed for the vacuoles, it was natural not to regard the plasmatic membrane as a special organ. Since the independence of the former has been established, such is obviously most probably the case for the latter also.[63]

Besides the incompleteness of the observations, which is to be demonstrated in the next paragraph, the whole course of the development of our knowledge in the field of cell-anatomy on the one hand, and the already repeatedly described differentiations of the plasmatic membrane and the granular plasm on the other hand, controvert the prevailing opinion. The latter does not form at all, as

[63]A method by which the plasmatic membrane could be artificially separated everywhere from the granular plasm, just as strong plasmolytic reagents separate the wall of the vacuole, is particularly desirable. Such a method could also render great service in judging the hypothesis mentioned on page 160, Note 2, on the growth in thickness of the cell-membranes.

the old conception would have it, a ground-substance of protoplasm, mixing constantly by its movements, and therefore not organized in the ordinary sense. This is most clearly seen in the Characeae. Here it consists, first of all, of a moving portion and of a resting part that contains the chlorophyll grains. When, sometimes the green plastids are torn from their position, and carried away by the current, one sees that they did not adhere separately to the plasmatic membrane, for they are not carried off singly, but in bands and groups, while within these the grains retain their mutual position and distance. Neither does the moving part form a whole, for the rapidity of the current is not at all everywhere the same on a cross-section. It is greater near the chlorophyll-grains than next to the wall of the vacuole, and furthermore it increases from the two indifferent zones toward the center of the green areas which are separated by them. With declining vital energy the more torpid currents are the first to suspend movement, while the more rapid ones continue to move, and with decreasing rapidity the width of the current diminishes at the same time.

Quite generally speaking, the granular plasm seems to consist, in the plant-world, of moving and of resting parts, the limits of which can be shifted by more or less favorable life-conditions, or can also shift spontaneously in the course of development, adapting themselves to changing needs.

The latter condition is illustrated by the beautiful investigations by Dippel, Crüger, and Strasburger on the relations between the plasma-currents and the internal sculpture of the cell-wall.[64] For along those places where

[64]Dippel. *Abhandl. Naturf. Ges., Halle.* **10**: 55. 1864. Crüger, H. Westindische Fragmente. *Bot. Zeit.* **13**: 623. 1855. Strasburger, E. *Jenaische Zeitschr. Naturwiss.* **10**: 417. 1876.

ledges, jutting into the interior, are in the process of formation there generally run strong currents which evidently bring and distribute the requisite food. But this *differentiation in the granular plasm is, to all appearances,* controlled by a corresponding differentiation in the plasmatic membrane. For, according to Dippel, the bands which form the layers of cellulose, consist of an outer hyaline band, which is thicker than the rest of the plasmatic membrane, and, like the latter, cannot be stained with iodine, together with an inner, moving layer of the granule-bearing plasm, which takes a deep yellow tint when treated with iodine.[65] The hyaline band is evidently a differentiated part of the plasmatic membrane which, on its inside is covered and nourished by the current, and on its outside forms the ledges of the cell-membrane.[66]

In naked protoplasts the cilia also bespeak an inner organization of the plasmatic membrane. These are described by Strasburger[67] for the swarm-spores of *Vaucheria.* Here all the cilia adhere to a denser part of this layer; they appear to be embedded in it by a thick root.

§ 8. *The Question of the Autonomy of the Limiting Membrane*

While in cell-division, according to the type described by Mohl, the multiplication of the limiting membrane by

[65]*Loc. cit.* pp. 57, 58.

[66]Strasburger's hypothesis that the growth of the cell-wall is accompanied by a transformation layer by layer of the outermost strata of the limiting membrane into cell-wall can, without difficulty be combined with the assumption of the autonomy of this organ with reference to the granular plasm, and therefore need not be discussed in detail here.

[67]Strasburger, *Studien über das protoplasma,* p. 400. 1876.

division and growth is generally recognized, the insertion of a new layer and its connection with the old membrane is usually assumed for cell-formation in the higher plants. In addition to this, there are some cases of cell-formation which seem to argue quite directly in favor of a formation of the limiting membrane *de novo* from the granular plasm.

All these cases seem urgently to demand renewed investigation. It is only with the intention of encouraging it that I shall briefly discuss them here.

In regard to the ordinary mode of cell-division the situation has greatly changed during the past year through a discovery by Went[68] which has been confirmed by Strasburger.[69] This discovery concerns the nature of the so-called cell-plate, which, when nuclear division is completed, forms at the equator of the now barrel-shaped figure. As the name indicates, the cell-plate is regarded as a layer which, cutting across the figure, later divides into two layers, and between these secretes the new cellulose lamella. These two halves of the layer are the two complementary pieces of the plasmatic membrane; as the barrel becomes flattened and extends laterally toward the cell-walls, they increase until they reach the old limiting membrane of the mother-cell and blend with it.

Went succeeded in loosening this whole division figure from the cells after they had been fixed and stained, and allowed it to float around in the fluid of the preparation. In this way it became possible, by turning the cell-plate, to study a polar view of it, while hitherto only the side-view had been studied and figured. As long as

[68]Went, F. A. F. C. Beobachtungen über Kern-und Zelltheilung. *Ber. Deut. Bot. Ges.* **5**: 247. 1887.

[69]Strasburger, *Ueber Kern-und Zelltheilung.* 1888.

the cell-plate is smaller than the daughter-nuclei, this view, of course, does not teach anything, because it has not been possible to remove the nuclei. But as soon as the cell-plate protrudes sideways from betwen the nuclei, it can be seen that it is not, by any means, a continuous plate, but only a rather thin ring. This ring lies in the connecting tube that separates the interior of the figure from its surroundings and has probably the same significance as in *Spirogyra.*[70] This "cell-ring," as we must now call the cell-plate, enlarges until it unites, first on one, then gradually on all sides, with the peripheral protoplasm of the mother-cell.

That the plane of the cell-ring is the place where the dividing wall forms, is certain, and agrees essentially with the previous conception of the cell-plate. But it has not yet been possible to discover whether or not the secretion of cellulose in the cell-ring begins before the latter has joined the wall of the mother-cell at least on one side. As soon as its presence can be proven by reagents, the new membrane is already joining the wall of the mother-cell, at least on one side.[71] Likewise it has not been decided, whether, in the plane of the ring there is extended a membrane which crosses the vacuole situated there and separates it into two separate sap-vesicles. But this is not probable.

It is clear that, with the discovery of the cell-ring, the old conception of cell-division that contradicts the autonomy of the plasmatic membrane, is weakened. For its final refutation, however, further researches are necessary, especially such as will include the wall of the vacuoles in the figures of division.

[70]Cf. pp. 132-134.

[72]Strasburger, E. *Bot. Praktikum,* p. 597. 1884, and *Ueber Kern- und Zelltheilung.* p. 171 ff. 1888.

I agree here with Zacharias[72] who, from observation on *Chara*, is of the opinion that the cell-plate elements originate from the cytoplasm surrounding the nuclear figure. I wish also to recall here an opinion of Flemming's, according to which, cell-division in plants and animals generally begins with a constriction of the protoplast. This constriction has not been observed in many preparations for the only reason that it is frequently unilateral, and therefore requires a special position of the cell under the microscope in order to be seen.[73]

Platner's view that the spindle fibers are currents of the granular plasm requires further investigation. For this purpose direct observation on the living object is necessary. Obviously the plasma-currents have, until now, been sadly neglected in the study of cell-division.

There are still left for us to consider the instances of so-called free cell-formation, which probably represent the most striking exceptions to the rule of the autonomous origination of the plasmatic membrane. By free cell-formation is meant those cases in which not all of the protoplast of the mother-cell is used in the formation of the daughter-cells.[74] The new cells were thought to have originated in the interior of the mother-cell, and therefore without any contact with the limiting membrane.

[72]Zacharias, E. Ueber Strasburger's Schrift Kern-und Zelltheilung im Pflanzenreiche. Jena. 1888. *Bot. Zeit.* **46**: 456. 1888.

[73]Flemming. *Zellsubstanz, Kern-und Zelltheilung.* p. 243. 1882.

[74]In the most recent interview of the pertinent literature, Zimmermann suggests that the name free cell-formation be not used for these phenomena, but for the formation of free cells, i. e., of such that lose their connection with the mother-cell. If it should be discovered that a free cell-formation in the old sense, does not exist in the plant-world, this suggestion would certainly be acceptable. Cf. *Die Morphologie und Physiologie der Pflanzenzelle,* p. 160. 1887.

Hence it was clear that their limiting membrane must have been derived from the granular plasm.[75]

In the formation of the endosperm a new plasmatic membrane seems to be formed only in contact with that of the mother-cell. In small embryo-sacs, where each nuclear division is followed by a cell-division, the conditions are, evidently, not essentially different from those in vegetative cell-division. And, for those embryo-sacs which continue to grow after fructification, I am not able to find, in the literature in question, any proof against the correctness of this assumption.[76]

In a number of algæ *(e. g., Acetabularia, Hydrodictyon, Ulothrix)* the swarm-spores arise from only a part of the protoplasm of the mother-cell. In such a case this part is always the peripheral layer, and every swarm-spore receives, as far as the present literature allows us to judge, not only a nucleus, chromatophores, and vacuoles,[77] but also a part of the limiting membrane of the mother-cell. Similar conditions seem to exist among the fungi, e. g., in *Protomyces macrosporus*.[78] In the case of *Hydrodictyon,* Pringsheim states that the colorless, ciliated, anterior end of the swarm-spores represents the maternal membrane.[79] In the Saprolegniaceæ also, the

[75]At this point in the original occurs a discussion of the processes of cell-division within the embryo-sac in their relation to the question of the autonomy of the limiting membrane. Since the points there considered are now definitely settled and agreed upon, the two paragraphs are here omitted with the author's approval. *Tr.*

[76]See especially Hegelmaier, Zur Entwickelungsgeschichte endospermatischer Gewebekörper. *Bot. Zeit.* **44**: 529, 545, 561, 585. 1886.

[77]According to the communication by Went mentioned on p. 154.

[78]Cf. de Bary. *Vergleichende Morphologie und Biologie der Pilze, Mycetozoen und Bacterien,* p. 86. 1884.

[79]*Monatsbericht Kais. Akad. Berlin,* p. 246. 1871.

oöspores are formed in such a way that each takes up in itself a part of the maternal membrane.[80]

We meet with a greater difficulty in the ascospores. But their origin has not been carefully studied in late years. Thus, though we know that divisions of the mother-nucleus always precede their formation, the question as to how they acquire their other organs has not yet been studied. It is clear that every spore must get one or more vacuoles through the division of the maternal sap-vesticles, but how this comes about, nobody has yet investigated. The consideration of the other question also as to whence the spores obtain their plasmatic membrane, must be most urgently recommended.

In the same way the origination of the egg-cell in the oogonium of the Peronosporales awaits study by means of modern methods. In this case, too, nothing definite can be said for the present in regard to the origination of the plasmatic membrane. Concerning the membrane of the spermatozoids, consult the following Section (pp. 174-176).

As a final result of this review, we may therefore say that, in all cases in which the arising of a new plasmatic membrane is supposed to take place without contact with the old one, this assumption is chiefly due to investigation by the older and imperfect methods. Exceptions to the rule are not at all known with certainty, although, according to the hypothesis of intracellular pangenesis, they must not be considered, *a priori*, as impossible.

[80]De Bary. *Abh. Senckenb. Naturf. Ges.* **12**: 261. 1881.