Starch-Formation in *Hydrodictyon utriculatum*.

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With Plate XXXIV.
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WHILE the subject of starch-formation in the plant-cell has received a great deal of attention from botanists from the microchemical standpoint, very little has been done in the way of following, by the aid of suitable technique, the sequence of structural changes undergone by the cell in such a process. This is particularly true in the case of the green Algae where the formation of the starch is connected with the pyrenoid. The cells of *Hydrodictyon* furnish material suitable for working out the sequence of events in the manufacture of starch, and I have been able to determine in them a number of facts, heretofore undescribed, which help to explain some of the conflicting statements of previous investigators.

The observations of Schmitz¹, Meyer², and Schimper³ as

¹ Die Chromatophoren der Algen, 1882: Beiträge zur Kenntniss der Chromatophoren. Jahrb. f. wiss. Bot., Bd. xv, 1884, p. 1.

² Ueber Krystalloide der Trophoplasten und über die Chromoplasten der Angiospermen. Botanische Zeitung, Bd. xli, 1883, pp. 489, 505, 525.

³ Ueber die Entwicklung der Chlorophyllkörner und Farbkörper. Bot. Ztg., Bd. xli, 1883, pp. 105, 121, 137, 153, 809. Untersuchungen über die Chlorophyllkörper und die ihnen homologen Gebilde. Jahrb. f. wiss. Bot., Bd. xvi, p. 247.

to the nature and function of the pyrenoid are too well known to need more than a very brief review. Schmitz, to whom we are indebted for the first authentic description of the pyrenoid itself, described it as a spherical proteid body, forming a part of the chromatophore and bearing a morphological relation to the latter similar to that of the nucleole to the nucleus.

Chemically, the substance of the pyrenoid was considered to be similar to the chromatin of the nucleus. But the evidence for this view of the chemical nature of the pyrenoid was, as Schimper¹ afterwards pointed out, entirely insufficient. Schmitz believed that there was a genetic relation between the pyrenoid and starch in that the pyrenoid substance was transformed into starch, the latter, however, being laid down in the substance of the chromatophore, and not in the substance of the pyrenoid. But in addition to the starch thus formed around the pyrenoid, he described its formation in other parts of the chromatophore as well. According to Schmitz there are two types of starch in the chromatophores with pyrenoids, viz. that formed in connexion with the pyrenoid and that formed in other parts of the chromatophore without reference to the pyrenoid.

Meyer insisted upon the crystalloidal nature of the pyrenoid and that it has no special function beyond that of being reserve proteid material. He observed cases in which the pyrenoid was angular rather than spherical in outline.

Schimper agrees with Meyer in the view of the crystalloidal nature of the pyrenoid, but thinks that it has a function in the formation of starch, as Schmitz had maintained previously. But neither Schmitz nor Schimper were able to bring any direct evidence for this conclusion beyond the fact that, as they thought, part of the starch is formed in the immediate vicinity of the pyrenoid.

More recently, Chmielewski² has described a star-shaped

¹ Jahrb. f. wiss. Bot., Bd. xvi, p. 247.

² Ueber Bau u. Vermehrung der Pyrenoide bei einigen Algen. Ref., Bot.

pyrenoid in *Spirogyra* and *Zygnema* whose points project between the parts of the surrounding starch-layer. Chmielewski insists that the pyrenoid is a permanent cell-organ always arising by the division of a pre-existing pyrenoid, a view previously suggested by Schmitz and Strasburger¹; still Schmitz also described the origin of pyrenoids in *Nemalion*, *de novo*—and Strasburger described the disappearance of the pyrenoids prior to the formation of the swarm-spores in *Cladophora*.

In his paper on the formation of the reproductive cells of *Hydrodictyon* Klebs² attempted to establish, by certain experimental culture-methods, a difference between the so-called pyrenoid-starch and the stroma-starch. But in this work Klebs, like his predecessors, failed to find the real genetic relation between the pyrenoid and all of the starch.

From the brief *résumé* given above it may readily be seen that there has been very little contributed towards the solution of the problem as to the nature of the relation of the pyrenoid to the formation of starch. This has been apparently due to two causes, the failure to find stages showing active starch-formation, and inadequate methods of treatment in studying the early and most important stages in the process.

The material I have used was fixed in either Merkel's fluid or in one of the two iridium chloride and acetic acid mixtures whose formulas I have already published in another connexion. The material was embedded in paraffin and cut on a microtome into sections of from three to six μ in thickness. The triple stain of Flemming gives a very clear differentiation, staining the pyrenoid bright red and the starch blue, while the surrounding cytoplasm is stained orange³.

The general structure of the *Hydrodictyon* cell has long

Centralbl., Bd. lxi, p. 277. Die Pyrenoide. Bot. Centralbl., Bd. lxxvii, p. 108.

¹ Zellbildung u. Zelltheilung.

² Fortpflanzungszellen bei *Hydrodictyon utriculatum*, Roth. Bot. Ztg., 1891.

³ A more detailed account of the method of procuring and treating the material will be published later, in another connexion.

been known to be that of a cylindrical coenocyte, having on the outside a thick cell-wall, and within this a layer of protoplasm surrounding a large central vacuole. Seen in section the protoplasm is clearly bounded on the outside by a plasma-membrane and on the inside by a vacuolar membrane of similar appearance. Between these two membranes the protoplasm contains the nuclei and pyrenoids and vacuoles of various size. There is no indication of any differentiation into a layer containing the chlorophyll and one containing nuclei. *Hydrodictyon* contains no differentiated chromatophore. In *Chlamydomonas* Dangeard has recently sought to establish the presence of a distinct chromatophore by the fact that the protoplasm of the chromatophore shows an alveolar structure, while the surrounding cytoplasm is more like a network. An examination of Figs. 21 and 26 (Pl. XXXIV) will show clearly that no such differentiation can be made out in *Hydrodictyon*. The cytoplasm shows no differentiation, such as is always observed in *Spirogyra* cells fixed and stained in identically the same way. This agrees with what is observed in the living cells of *Hydrodictyon*, in which, in spite of the statement of Klebs and Artari, it is plain that the chlorophyll is distributed in the whole peripheral protoplasmic layer of the cell. Most striking evidence of the lack of a distinct chromatophore is also to be found in the distribution of the pyrenoids and nuclei. The pyrenoid, as Schmitz pointed out, belongs to that part of the protoplasm that contains the chlorophyll, i.e. to the chromatophore where it exists as a distinct part of the cell. If, then, there is a distinct chromatophore here we should expect to find its position indicated by the position of the pyrenoids. On the other hand, the nuclei would lie in a layer outside or inside the chromatophore. That this is not the case is shown clearly in figures where both pyrenoids and nuclei are seen to be scattered throughout the protoplasmic layer, neither being confined to any one region (Figs. 21 and 26). Moreover, the two bodies are often in immediate juxtaposition so that it is impossible that one should be considered as in the chromato-

phore and the other not (Fig. 27). Still another point of importance is the fact that in those cells containing an abundance of starch, practically the whole layer of protoplasm, from the plasma-membrane on the outside to the vacuolar membrane on the inside, is filled with the starch-grains, all of which, as I shall show below, have their origin in the pyrenoid, and are later transferred bodily to other parts of the cell (Fig. 26). If we are to think of a differentiated chromatophore we should expect the starch to be retained within its body, as is clearly the case, for example, in *Spirogyra*. It would seem that, then, *Hydrodictyon* is to be placed among those plants in which the chlorophyll is distributed generally in the cytoplasmic part of the protoplast. Klebs and Artari¹ have described a chromatophore in *Hydrodictyon* made up of a complicated network, which may, according to Artari, become a single slightly alveolar layer, or, according to Klebs, become divided into several layers having a net-like structure. Both of these observers worked, however, with surface-views of the cells, a condition under which accurate observation of the whole protoplast is quite impossible. The numerous vacuoles which frequently appear in the cells might easily lead to some such interpretation as that of Klebs or Artari, provided only surface-views were had.

It is an interesting question whether the condition above described for *Hydrodictyon* is a primitive condition in chromatophore-development. Prior to the appearance of Schmitz's work on the chromatophores of the Algae, it was not uncommon to have forms described as having the chlorophyll diffused throughout the cytoplasm. Schmitz first insisted upon the doctrine that the chromatophore in all Algae was a distinct cell-organ, and that there was no such thing as chlorophyll contained in undifferentiated cytoplasm. This doctrine of Schmitz was soon extended to all of the so-called plastids by Schimper, and is now the prevailing theory among botanists. Still it is not impossible that a more careful investigation of a large number of forms will reveal conditions

¹ Zur Entwicklungsgeschichte des Wassernetzes. Moskau, 1890.

in many like those in *Hydrodictyon*. It may not be without significance that whereas there is no differentiated body here which can be distinguished as a chromatophore, still there are well-defined centres (the pyrenoids) for the formation of starch.

If we turn now to the details of starch-formation it is to be noted that the whole process can be traced from certain structural changes occurring in the body of the pyrenoid. This body, while it is not undergoing such transformations, presents a deep red homogeneous appearance in sections treated with the safranin gentian-orange mixture. In outline it may present many variations in form depending largely upon its relation to the process of starch-formation as described below. The typical appearance, however, of the resting pyrenoid is, perhaps, that described by Schmitz, in which the pyrenoid shows a spherical form with a clear outline. In this condition it is often surrounded by a single layer of starch made of separate grains of a decidedly concavo-convex form (Fig. 21). This is a condition that is very often observed and forms the basis of most of the familiar descriptions of the pyrenoid. That it is a resting condition, however, is abundantly shown by the fact that during the formation of starch a complete series of stages may be found, leading from it to the conditions in which starch-formation seems to be most active, and by the further fact that it generally appears prior to the formation of swarm-spores, when starch-formation has ceased and the processes leading to the disappearance of both starch and pyrenoids have set in. The first indication of the changes leading to the formation of starch consists of a differentiation of the body of the pyrenoid into two portions, one of which is destined to become transformed into a starch-grain and the other to remain unchanged. The part that is to form the starch soon stains less densely. Instead of being stained red it now becomes a neutral grey, or faint orange. The portion so differentiated may include nearly half of the pyrenoid, but frequently does not include so much (Figs. 1-4 and 6-8). In connexion with the change in staining capacity,

there are some important structural changes to be noted. The dense homogeneous structure becomes more spongy, with denser and less dense regions. There is, however, no regularity in the form or distribution of such regions. Frequently there is simply a dense region toward the outside with the less dense toward the unchanged part of the pyrenoid (Figs. 3 and 8). Very often, as indicated above, the denser regions may be so distributed as to give the appearance of a roughly alveolar structure (Fig. 6). In any case the denser regions soon become more prominent and take up the blue stain. They apparently increase in size until nearly the whole part of the pyrenoid undergoing transformation presents a deeply blue stained homogeneous structure, the typical appearance of all the starch-grains in this plant. There is nearly always between the fully formed starch-grain and the unchanged remainder of the pyrenoid a thin zone of slightly stained material (Figs. 1 and 6), as if with increasing density there were a shrinkage in the whole mass. Such a zone may appear before the starch-grain has reached the homogeneous character of the mature grain. There is in effect a splitting off of a segment of the pyrenoid which is metamorphized into a starch-grain. When the grain of starch is fully formed it appears to lie in a vesicle or vacuole in the cytoplasm, but without being surrounded by a differentiated membrane. The vesicular appearance may of course be due to a slight contraction of the protoplasm in fixing, but this is a point that could not be settled definitely.

The mature grain has practically the shape of the segment of the pyrenoid from which it is formed. Even after it is widely separated from the pyrenoid, in the manner described below, the plano-convex or concavo-convex form is retained (Fig. 1). This is a point of much importance, in establishing the fact that all of the starch is formed from the pyrenoid.

When starch is being formed rapidly a second grain will be built at once before the pyrenoid regains its original form. The remaining part of the pyrenoid undergoes the same differentiation as was seen in the original body, and the

differentiation occurs in such a fashion that the long axis of the second grain is at right angles to that of the first: that is, a second segment is cut off from the pyrenoid with the plane of cleavage at right angles to that in which the first grain was cut off. This rule is not invariable, but I have never, for example, found a second grain formed between the first and the remainder of the pyrenoid. Of course it is impossible to establish, with any certainty, the sequence in which any great series of grains have arisen such as are shown in Figs. 1-4; but the general arrangement would seem to support the above statement. So far as the pyrenoid itself is concerned it is hard to understand why a second grain should not follow the first on the same side. Still the process as followed here is better adapted to secure an equal distribution of the starch through the cytoplasm, and this fact may have led to the establishment of a more or less fixed order in the formation of the grains as described above.

It is possible, too, that the formation of the starch depends upon the action of the surrounding cytoplasm as well as of the pyrenoid itself. In this case when a starch-grain is fully formed it would separate the two and thus, perhaps, lead to a shifting in the place of formation for the next grain.

When the process of starch-formation is going on very rapidly, the grains, as they are formed, are continually crowded outward by the later-formed grains, so that finally, as previously indicated, they are densely packed through nearly the whole protoplast (Figs. 1 and 26). This condition is undoubtedly what led to the distinction made by Schmitz, and later worked out in more detail by Klebs, between starch formed around the pyrenoid (pyrenoid-starch) and that formed in other parts of the chromatophore (stroma-starch). But that there are not two kinds of starch, at least in their origin, is made clear by an inspection of Figs. 1-5. It is to be noted in these cases that no distinction whatever can be made between the starch immediately surrounding the pyrenoid and that farther out in the cytoplasm. Moreover, the form and arrangement of the whole mass of starch-grains, as shown

in Fig. 1, is seen to have in a general way, but quite clearly, orientation about the pyrenoid as a centre. The concentric arrangement will be especially noted in Fig. 5. This arrangement seems to point clearly to the common origin of all the starch-grains from the pyrenoid in the manner described above.

The failure on the part of former observers to trace the origin of all the starch to the pyrenoid is due, as I have previously indicated, to the lack of material taken in a condition of active starch-development, coupled with methods of treatment inadequate to bring out the stages in the genesis of the individual grains. That various observers have drawn their conclusions from a study of isolated stages in starch-development is shown by the conflicting statements as to the form of the pyrenoid itself. Schmitz maintained that it is a spherical body, a conclusion doubtless drawn from a study of resting pyrenoids as described above. Meyer probably saw a single stage in starch-formation and was led to insist upon the angular form of the pyrenoid. It is even possible that the star-shaped pyrenoid described by Chmielewski was simply a condition similar to that shown in Fig. 4.

The size of the pyrenoids at the time they are forming or about to form starch is very variable. While in many cases the pyrenoids are of approximately the same size in any one cell during active starch-formation (Fig. 1), in many others there is a very wide variation. Two pyrenoids representing almost extremes in size are frequently found lying side by side in the same part of the cell, either or both of which may show the beginning of starch-formation (Fig. 21). Whether these represent pyrenoids of different ages with independent genetic histories, or whether they are sister organs formed as the result of the division of a pre-existing pyrenoid, is an important question, as yet unsettled. There is abundant evidence that the development of the starch may take place in such a way as to completely divide the pyrenoid into two parts. This is most often brought about by the equatorial region of the pyrenoid being transformed into starch in such

a fashion as to leave an undifferentiated portion on either side of the starch-grain (Figs. 10 and 15). In the latter figure the constricted appearance of the pyrenoid in the region of starch-formation is noteworthy. The explanation of this fact is not evident, but it may be due simply to the shape of the grains earlier formed, an hypothesis that seems to be supported by the shape of the grains on either side of the pyrenoid. Fig. 11 is quite striking in this respect, while Fig. 12 shows no such clear relation of the shape of the starch-grains to that of the pyrenoids. Note also that in Figs. 11 and 12 there is no evidence of starch-formation in the constricted region.

Whether the pair of pyrenoids in the central part of Fig. 6 is the result of the division of a single pyrenoid is not clear. The peculiar shape of the starch-grain lying between the two pyrenoids is uncommon, and may possibly be the result of a fusion of the parts of two closely adjacent pyrenoids that are being metamorphized into starch. But earlier stages indicating such a fusion have not been observed, and it is not impossible that the starch-grain simply represents a middle portion of the original single pyrenoid after it had the constricted appearance, as a result of the formation of the older grains on either side.

It is, I think, sufficiently clear that there may be a division of the pyrenoid due to a transformation of a certain part of it into starch. Whether all the cases of division of the pyrenoid that have been described by other observers are to be explained in this manner is an interesting question that needs further careful investigation. The apparent time-relations of the division of the pyrenoids in *Spirogyra* and *Zygnema* (in the one case immediately preceding and in the other immediately following the division of the cell), as described by Strasburger and Chmielewski, would seem to have little relation to the process as described above for *Hydrodictyon*. Still, a single pyrenoid in the condition shown in Figs. 10 or 18 might well suggest, if taken alone, a reproduction of pyrenoids rather than starch-formation. That the pyrenoids

of *Hydrodictyon* disappear prior to the formation of the swarm-spores or gametes has been known since Braun's classic researches on Rejuvenescence in Nature. This of course necessitates an origin *de novo* in the young cells. Hence division cannot at least be considered the only means for the multiplication of pyrenoids.

The steps in the disappearance of the pyrenoids and usually of the starch prior to cleavage are worthy of attention, and for the starch may be taken as typical for the process of starch-solution in the plant. Generally the first starch to disappear is, as might be expected, that farthest from the pyrenoids. The starch-grains seem to be dissolved uniformly over the surface instead of in furrows and by irregular corrosions as is often observed in the storage-starch of the higher plants. Very often a stage is seen where there remains but a single layer of starch around the pyrenoid, while the pyrenoid itself shows no indication of further starch-formation, but has the homogeneous structure and spherical form of the typical resting pyrenoid (Fig. 21: large pyrenoid). This stage is also often met with in cells that give no other indications of reproductive activity. It probably represents, as previously suggested, a quiescent condition of the cell so far as starch-formation is concerned, and the frequency of its appearance serves to explain Schmitz's account of the pyrenoid as a spherical body surrounded by a single layer of starch. Such stages must be carefully distinguished from that represented in Fig. 6, where there is manifestly development of starch taking place, but where also the solution of the starch seems to wellnigh keep pace with its formation so that there is but little more than a single layer of starch around the pyrenoid. Let the process of starch-formation cease in such a condition as this last, and we can very readily imagine the whole structure passing over into such a resting-stage as that shown in Fig. 21. As the solution of starch continues, the pyrenoid itself generally becomes smaller, although the starch is usually all dissolved before the pyrenoid has entirely disappeared (Figs. 22-24). Still, it is not necessary that either

the starch or the pyrenoid should wholly disappear before the spores are formed; for, as Klebs observed, and as many of my preparations show, in spores just formed, as well as during cleavage-stages, an abundance of starch may be seen, always appearing as if in a stage of solution (Fig. 27). Pyrenoids may also be present at these stages. Klebs thought the starch to be what he called the stroma-starch, as he failed to see the remaining pyrenoids here and there through the segmenting protoplasm, and even in some of the swarm-spores (Fig. 28). In such pyrenoids, however, I have never seen any indication of starch-formation. My preparations seem to show that the formation of starch has ceased, but that conditions were such that cleavage took place without the preliminary solution of the starch as well as the body of the pyrenoids. The relation of the pyrenoids and starch to spore-formation I hope to discuss more fully in connexion with an account, now in preparation, of the spore-formation. It should be especially noted that the presence of starch-grains in the young spores that contain no pyrenoids does not mean the formation of such grains *in situ*, but that they are grains of starch formed in the manner previously described, and simply remaining in the portion of the protoplasm forming the spore. In this connexion it may be further noted that under certain conditions the pyrenoid itself may entirely disappear, leaving the surrounding starch-layer or layers still intact (Figs. 19 and 20). This condition seems to arise in cells in which there is no sign of cleavage to be found, but it is possible that it may also occur just prior to cleavage, so that all the swarm-spores formed in any one cell may contain starch, but none of them show a pyrenoid. It should be borne in mind that, as Klebs has pointed out, prior to cleavage the number of nuclei in the mother-cell is far in excess of the number of pyrenoids, so that even should the pyrenoids persist during cleavage, as they in some cases do, many of the uninucleate spores would be without them, a fact that seems to point unmistakably to the *de novo* origin of pyrenoids in the young cells.

During the solution of starch around the pyrenoids there is

frequently an appearance indicative of the formation of a vesicle containing the dissolved substance of the grain. This presents often the appearance of a homogeneous layer, having a faint blue or grey colour surrounding the whole pyrenoid (Figs. 18 and 27). In it may often be seen portions of the partly dissolved starch-grains (Figs. 16 and 17). The significance of such a condition is not entirely clear, but it seems to suggest that the product of the solution of the starch may not be used as fast as formed. On the other hand, cases frequently occur where the grains, being dissolved, seem to lie imbedded in the protoplasm without any surrounding vesicle, and the pyrenoid itself, where the starch is entirely dissolved, is in immediate contact with the protoplasm (Fig. 25).

The condition just described, in which the products of the solution of the starch appear in a vesicle surrounding the pyrenoid, is probably quite distinct from that shown in Figs. 7, 8, and 9, where there is a clear space surrounding the young pyrenoids. Boubier's¹ recent attempt to establish the region of starch-formation in this space, including it in the pyrenoid, is entirely unsupported so far as the facts in *Hydrodictyon* go. In no case have I found either the net-like structure or the delimiting, distinct membrane that Boubier described. The outer sharp boundary of the vesicle in these stages is not to be taken as a permanent membrane, for, besides being entirely wanting in many cases where there is no starch present, it is also lacking in every case of rapid starch-formation.

CONCLUSIONS.

If we turn now to a brief consideration of the bearing of the facts described in the foregoing pages upon the general processes of metabolism in the cell, it is of most importance to note that the pyrenoid is directly the seat of the processes resulting in the formation of starch. Both the structural and microchemical changes shown by differences in staining indicate that the process is an exceedingly complicated one,

¹ La membrane pyrénoidienne; Bull. de l'Herbier Boissier, tome vii, p. 451.

involving many chemical transformations whose nature must be left to future investigations to determine. The fact that, as seems to be well established by various observers, the pyrenoid is of a proteid nature, and that a portion of it—at least in *Hydrodictyon*—is converted into starch, suggests that the process involves the breaking down of a proteid into a carbohydrate. Still, it is not to be thought that the substance of the pyrenoid is merely passive in the process, for on that assumption it would be hard to explain the differentiation of its body into two portions, one of which is transformed into starch, while the other retains the original pyrenoid character.

To be sure the pyrenoids themselves may totally disappear at certain periods in the life-history of the cell, notably prior to reproduction; but it is quite probable that active cell-organs may disappear when their work is not immediately needed, and be formed anew when their activities become necessary. (Cf. Wilson, *The Cell*, p. 305, as to the centrosome.)

Whether the pyrenoid represents an active, independent cell-organ whose function is the formation of starch, or whether it is to be thought of as a mere stage in the process of starch-formation whose real seat is to be found in the chlorophyll-bearing cytoplasm, is not certain from the facts thus far at hand. As pointed out above, the method of differentiation of the body of the pyrenoid preceding the actual formation of the starch indicates that the pyrenoid is more than a passive body representing a stage in the process. The genetic history of the pyrenoid itself, including its relations to the chromatophore, where a differentiated chromatophore exists, or to the cytoplasm in such a form as *Hydrodictyon*, would perhaps throw some light on the nature of the whole metabolic processes resulting in starch-formation.

In the paper previously referred to, Boubier developed the interesting hypothesis that the pyrenoid is comparable to the leucoplast of the higher plants, and that the method of starch-formation in it is similar to that in the latter bodies. While the suggestion may later prove to be a valuable one, still

the evidence in favour of it is not sufficient to be convincing. The fact that the pyrenoid is a temporary structure does not certainly disprove the truth of the suggestion, for, as Eberdt has maintained, it is possible that the leucoplast may arise *de novo* in the cell. It is perhaps more difficult to think of a leucoplast and chloroplast as associated together in the same cell. Such an association would certainly be out of harmony with the doctrine of the genetic relations of the two organs as maintained by Schimper. The most serious objection, however, to the comparison suggested by Boubier seems to me to lie in the fact of the difference in structure between the pyrenoid and leucoplast. So far as I have been able to observe, it is by no means easy to differentiate the leucoplast from the rest of the protoplasm by the ordinary methods of cytological research, such as those used in the present investigation; and when it is differentiated, it has a granular or reticulate appearance, while the pyrenoid appears homogeneous, dense, and sharply bounded.

The method of starch-formation in the pyrenoid as I have described it has, so far as I can determine, no analogy with that in the leucoplast—at least so far as its functional aspects are concerned—with the possible exception that, as maintained by Eberdt¹ and Schimper², there is a breaking down of a proteid substance to form a carbohydrate³.

Should the conclusions of Meyer⁴ and Salter⁵, that starch-formation in the leucoplast is a process of secretion within the body of the leucoplast itself, without involving any change in its structure, prove correct, Boubier's hypothesis would be

¹ Beiträge zur Entstehungsgeschichte der Stärke; Jahrb. f. wiss. Bot., Bd. xxii, p. 293.

² Untersuchungen über das Wachsthum der Stärkekörner; Bot. Ztg., Bd. xxxix, 1881, p. 185.

³ The difference between these two authors lies not in the method of starch-formation itself, but in the nature of the body in which it is formed. Schimper, as is well known, maintained that it is a permanent cell-organ, while Eberdt thought it to be a temporary body consisting of what he called starch ground-substance, which was entirely converted into starch.

⁴ Untersuchungen über die Stärkekörner. Jena, 1895.

⁵ Jahrb. f. wiss. Bot., Bd. xxxii, 1898, p. 117.

strongly negatived. I am inclined to the view that the pyrenoid is an active body, differentiated in the chlorophyll-bearing cytoplasm, which in co-operation with the latter acts as the base of the process of starch-formation. In just what the co-operation consists must remain to be determined by future investigation.

If we compare the method of starch-formation in *Hydrodictyon* as described above with starch-formation in chromatophores without pyrenoids, there is little to suggest a parallel, and the relations of the two must be regarded as uncertain for the present.

MADISON, WIS., May, 1901.

EXPLANATION OF FIGURES IN PLATE XXXIV.

Illustrating Mr. Timberlake's paper on Starch-Formation in *Hydrodictyon*.

All figures were drawn with the aid of the Abbé *camera lucida*, and with the Zeiss apochromatic objective 2 mm., num. ap. 1.30, and compensation oculars 4, 12, and 18.

Fig. 1. Part of surface-section of cell, showing condition of rapid starch formation; P. pyrenoid, S. starch. $\times 1500$.

Figs. 2, 3. Details of starch-formation in single pyrenoids. $\times 2250$.

Fig. 4. Shows peculiar angular form of pyrenoid. $\times 2250$.

Fig. 5. Pyrenoid surrounded by distinctly concentrically arranged layers of starch. $\times 2250$.

Fig. 6. Starch-formation, where solution of starch is going on nearly as rapidly as its formation.

Fig. 7. Young cell, showing two small pyrenoids in which no starch is forming, and a larger one in which a single grain of starch is being formed.

Fig. 8. Same as above.

Fig. 9 *a*. Cell, showing pyrenoid without starch, and surrounded by a colourless, clearly bounded area. Pyrenoid itself shows but a faint colour. $\times 2250$.

Fig. 9 *b*. Starch-forming in pyrenoid with colourless region as before. Small pyrenoid lying against nucleus. $\times 2250$.

Figs. 10-18. Different phases of starch-formation resulting in the division of the pyrenoid. For explanations see text. $\times 2250$.

Figs. 19, 20. Showing disappearance of pyrenoid without the starch being entirely dissolved. $\times 2250$.

Fig. 21. Vertical section, showing two pyrenoids of unequal size in the same cell. The larger pyrenoid in resting stage; smaller showing starch-formation. $\times 2250$.

Figs. 22-25. Disappearance of starch and pyrenoids prior to spore-formation. $\times 2250$.

Fig. 26. Portion of vertical section, showing distribution of nuclei, pyrenoids, and starch grains in the protoplast. $\times 600$.

Fig. 27. A pyrenoid and two nuclei lying very close together. Nuclei are in prophase of division. Pyrenoid surrounded by homogeneous substance formed by solution of starch. $\times 2250$.

Fig. 28. Early stage of spore-formation, showing a single pyrenoid remaining in the protoplasm. $\times 600$.

Fig. 29. Young swarm-spores containing starch-grains. $\times 1500$.

Fig. 30. Young cells, each containing a pyrenoid and several starch-grains. $\times 1500$.

Fig. 31. Pyrenoid showing unusual forms of starch-grains.

Timberlake, H. G. 1901. "Starch-formation in *Hydrodictyon utriculatum*." *Annals of botany* 15, 619–635.

<https://doi.org/10.1093/oxfordjournals.aob.a088841>.

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