Spermatogenesis in certain Hepaticae.

BY

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With Plate XXV.

DURING recent years considerable interest has been shown in the study of spermatogenesis in the Bryophytes, Pteridophytes, and other cryptogams, as evidenced by the contributions on this subject. It has been difficult to arrive at correct conclusions in regard to the true nature and history of spermatogenesis in some of these forms, especially in the Bryophyta. Two difficulties in particular have, as a rule, been encountered. In the first place the cells are small, and certain important details of their contents are hard to differentiate. In the second place the process of spermatogenesis involves such a complete, and at times rapid, transformation of the individual cell that the origin and fate of certain parts are difficult to trace, and the nature and function quite as hard to determine. These difficulties are evidenced by the various opinions in regard to the process presented by the different investigators.

It was with the hope of obtaining further facts which might clear up somewhat the problems encountered in tracing the development of the sperm in some forms of the Hepaticae, that a study of spermatogenesis in *Porella* and other Liverworts was undertaken.

The first detailed account in recent years, treating of spermatogenesis in the Hepaticae, was given by Ikeno ('03) for *Marchantia polymorpha*. He discusses the early divisions in spermogenous cells, the last or oblique division, and the development of the spermatozoids. In all divisions of spermogenous tissue he finds a body which he calls a centrosome occupying each pole of the spindle. This centrosome he describes as separating from the chromatic mass in the nucleus, moving towards the nuclear membrane, and passing through the latter into the cytoplasm. Here it divides and the daughter bodies move to opposite sides of the nucleus, where they apparently take part in the formation of the spindle. They occupy the poles of the spindle until late anaphase or telophase, when they disappear, but reappear in the prophase of each successive division. In the last spermogenous division, the spindle lies diagonally, and the cell is consequently separated

into two triangular sperm cells, which lie in pairs, and are not separated by a cell-wall. During this division the centrosome does not disappear, but persists to function as the blepharoplast. It remains, at first, in the corner of the sperm cell originally occupied by the pole of the spindle, then it moves into one of the acute angles of the cell. Shortly thereafter, a large body (*Nebenkörper*) appears in the cytoplasm for a short time, and then, during the formation of the blepharoplastic band, disappears. Whether, like the centrosome, this body is of nuclear origin or not, Ikeno did not determine with certainty.

After the centrosome has taken up its position in one corner of the sperm cell, it lengthens slightly, keeping in contact with the plasma membrane, and soon develops two cilia. Then the nucleus moves so as to come in contact with the plasma membrane on the same side along which the centrosome has stretched out to function as a blepharoplast. In this position, the nucleus changes both in form and structure. It becomes homogeneous in staining capacity, and begins to flatten or draw out in a bow-like form. In the meantime, a protoplasmic projection has grown out from the nucleus in the direction of the blepharoplast until the two are united. The nucleus continues to lengthen until it becomes a narrow band, and the cilia long slender threads. At the same time the cytoplasmic mass decreases in size until only a small vesicle is left at the posterior end of the nucleus, while from the anterior end of the latter a very narrow band extends forward to the diminished blepharoplast. The latter grows only a short distance towards the nucleus, and then becomes smaller, and practically disappears as the cilia develop, so that the forward end of the sperm projecting beyond the nucleus consists of a short, scarcely perceptible blepharoplast, bearing two cilia, and connected to the nuclear portion by a band of cytoplasm. Posterior to the nuclear portion is a cytoplasmic vesicle. The whole body of the sperm is more or less loosely coiled; it is formed of three metamorphosed elements of the cell—the centrosome, which has become the blepharoplast, the nucleus, which forms the main body of the sperm, and the cytoplasm, which forms the posterior vesicle and the band connecting the nucleus with the blepharoplast. The cilia may consist partly of material derived from the blepharoplast, and partly from the cytoplasm or plasma membrane of the sperm cell.

Bolleter ('05) described briefly the spermatogenesis of *Fegatella conica*. This description agrees mainly with that of Ikeno for *Marchantia*, except that Bolleter failed to see the centrosome-like body at the poles of the spindle in the prediagonal divisions of the antheridial cells. However, since he found such a body occupying the region of the spindle pole of the last division, and also observed its nuclear origin, he concludes that it must be present also in the earlier divisions.

Lewis ('06) found centrosome-like bodies at the poles of the spindles

in the earlier, as well as in the diagonal, divisions of the spermatogenous tissue of *Riccia natans*. These bodies, which he is not inclined to consider as true centrosomes, do not have genetic continuity, but disappear after each cell-division, except the last, when they persist in the sperm cells and function as blepharoplasts. He finds no evidence of their nuclear origin, but considers them as arising *de novo* with each cell-division. In the formation of the spindle, cytoplasmic fibres radiate from the nucleus and converge at the centrosome-like body. While the behaviour of this body seems more like a blepharoplast than a centrosome, the fact of its occurrence in so many cell generations before the formation of the sperm leads to some difficulty in determining its nature.

Escoyez ('07) describes the behaviour of the chromatin and the formation of the spindle in diagonal and prediagonal divisions of *Marchantia polymorpha*. Contrary to Ikeno's observations, no centrosome-like body, according to Escoyez, is apparent until during the prophase of the diagonal division. Then in each corner of the sperm-mother-cell a darkly stained body appears, which will lie at the pole of the spindle. A body of similar appearance may occur also in each corner of the cell, which will be in the plane of the cytoplasmic division. He found no evidence of the nuclear origin of either of these bodies, but states that the body, or corpuscle, which occupies the pole of the spindle, persists in the sperm cell and functions as the blepharoplast.

In early divisions of antheridial cells of *Fegatella*, he found no centrosome-like bodies. The last division of the spermatogenous tissue was not studied.

Escoyez concludes that centrosome-like bodies are present in the spermatogenous tissue of *Marchantia* in the last division only, and that these corpuscles are the blepharoplasts; that they are not true centrosomes, functioning secondarily as blepharoplasts, but organs *sui generis* of the antheridial cells. They are the 'cilia bearers' and play no rôle in karyokinesis. If they were true centrosomes they should also be found in the earlier divisions. The mere fact that they occupy the poles of the spindles does not imply that they take part in the spindle formation.

In his studies, Schaffner ('08) found bodies, which he called centrosomes, occurring in all spermatogenous divisions of *Marchantia*. These bodies, he says, persist through the last divisions and function as blepharoplasts. The latter are modified centrosomes, and their ontogeny can be traced back through the preceding cell-divisions of the antheridium. He does not state the origin of these bodies, nor whether they persist during all phases of karyokinesis.

Miss Black, while working on *Riccia Frostii*, Anst., during the winter of 1909-10, found no centrosome-like bodies in any phase of the prediagonal divisions or immediately after the diagonal division. But at a later stage,

in the cytoplasm of the sperm cell, a dense body appears which grows out into a densely staining slender cord around one side of the cell. This densely staining cord is the blepharoplast and bears very near one end—the anterior end of the mature sperm—two cilia, while the nucleus draws out into a narrow band along the posterior region of the blepharoplast.

So far I have not found that any work has been done on the spermatogenesis of *Porella*. The observance of large nuclear figures in this Liverwort suggested that it might be suitable for such a study. Accordingly material was fixed at various times in the chromic-osmic-acetic killing fluid as formulated by Mottier ('97). Antheridial branches were fixed entire. At almost any time of the year, especially when cool and damp, various stages in the development of the antheridia were found in acropetal succession on the same branch—from very small antheridia to almost mature sperms. But only occasionally were nuclei found in the process of division. Resting conditions may be of long duration, but the process of division seems to occur very rapidly. This fact is no doubt correlated with the ability of *Porella* to dry up for long periods and then revive with rapid cell-division. Along with *Porella* careful studies were made in the spermatogenesis of *Marchantia* and *Fegatella*.

The material, after fixing ordinarily for about twenty-four hours, was washed, brought up through the various grades of alcohol, embedded in paraffin, and sectioned, for the most part two microns thick. The following combinations of stains were used: anilin safranin and gentian violet, usually without the orange G; Heidenhain's iron alum-haematoxylin; anilin safranin, iron alum-haematoxylin, and gentian violet; iron alum-haematoxylin and gentian violet; and iron alum-haematoxylin and Bismarck brown.

In what follows I shall discuss the mitosis in spermogenous tissue, the last, or oblique division, and the development of the sperm.

MITOSIS IN SPERMOGENOUS TISSUE.

As already suggested, the spermogenous cells of *Porella* are usually quite large. Compare Fig. 3, Pl. XXV (*Porella*), with Fig. 27 (*Marchantia*) and Fig. 46 (*Fegatella*). In an early stage of the antheridium, the nucleus presents a large nucleolus surrounded by a clear space, and, nearer the nuclear membrane, an open linin network with scattered chromatin granules (Figs. 1 and 2). The cytoplasm is more or less alveolate or fibrillar in nature, and quite granular. The granules vary in size, number, and disposition, as do also the vacuoles if present (Figs. 1 and 3).

In early prophase the chromatin lumps are larger and more drawn out along the linin threads. More than one nucleolus may be present at this stage (Fig. 3). If the latter figure be compared with Figs. 4, 5, and 6, it is evident that the chromatin continues to collect along the linin threads until a more or less irregular spireme is formed, which lies near the nuclear mem-

brane. The details of the formation of the chromosomes from this condition were not observed. Fig. 7 shows a cell in which the nuclear membrane has disappeared and the chromosomes are differentiated. This count shows only six, which is two less than the gametophytic number for *Marchantia* and *Fegatella*. However, this was the only cell found cut in such a plane through a dividing cell that the chromosomes could be counted. I am not certain, therefore, that six is the exact number.

The formation of the spindle was not observed, but many sections were found showing metakinesis (Fig. 8). The cytoplasm is a little coarser in texture than in preceding stages, but otherwise apparently the same. Fig. 8 shows numerous granules in the cytoplasm quite similar to those shown in Fig. 3. There is nothing to warrant the conclusion that any one granule, or body, differs from the numerous other ones, except in size and density. All are scattered promiscuously throughout the cytoplasm, some in vacuoles, others embedded in the cytoplasmic structure (Figs. 3 and 8). Nothing was found in the cytoplasm or at the pole of the spindle which resembled a centrosome. The spindle (Fig. 8) stands out clear and distinct. The fibres are collected into rather dense, irregular strands which are attached to the chromosomes at the plate, while they come to a sharp point at each pole. Sometimes one of the irregularly placed granules happens to lie near the pole, but no body similar to those shown by Ikeno ('03) and Schaffner ('08) for the same stages in *Marchantia*, and by Lewis ('06) for *Riccia*, was found. Neither were there any radiations from the region of the poles.

Fig. 9 represents an anaphase of one of the earlier spermatogenous divisions. The cells in one half of the antheridium have reached this stage simultaneously. Sharply defined spindle fibres are still attached to the ends of the retreating chromosomes. The cytoplasm is more coarsely granular, but the largest granules are doubtless oil-drops. The number of chromosomes apparent here would indicate that the previous count was correct. Fig. 10 represents a telophase following one of the divisions shortly before the last. The cells and nuclei are smaller than in earlier stages of the antheridium. However, too much significance must not be placed in size alone, as this varies considerably for cells of corresponding stages in different antheridia. It will be observed that the nucleus which shows most nearly the median section has a chromatin network quite similar to that in Fig. 3, save that a nucleolus is not visible.

In *Marchantia* the resting condition (Fig. 25 A) shows an evident linin network containing relatively large lumps of chromatin in a clear nuclear sap without any nucleolus. The nucleus seems to pass this stage very quickly, while those stages represented by Figs. 25, 26, and 27 persist for a longer period. Fig. 25 A is quite similar to Escoyez's Figs. 7 and 8. Ikeno ('03) does not figure the chromatin in this condition (Fig. 25 A), neither

does he find at this, or a later stage, the irregularly granular nature of the cytoplasm. The latter's figures all show a very smooth homogenous cytoplasm. At times (Fig. 28 A) I found the cytoplasm quite finely granular, but, as a rule, the structure was coarser and more irregular, the smaller granules tending to collect into larger masses.

The chromatin then collects into one or more lumpy, irregular masses in the centre of the nuclear cavity. This latter now stains rather densely, as if filled with some homogenous or very finely granular substance. This appearance of the nucleus continues during the formation of the chromosomes and the early stages of spindle formation. The two latter processes were not observed in detail, but Fig. 27 shows a chromatin mass, shortly before the differentiation of the chromosomes, still surrounded by the same darkly staining substance. This homogenous material around the chromatic mass seems to indicate a preparatory step in the formation of the spindle. The spindle fibres (Fig. 28) apparently occupy the former nuclear area and stain quite similarly to the homogenous nuclear content, so that it seems quite possible that the fibres have had their origin in this latter substance. If this be so, it agrees with the origin of the spindle in the spore-mother-cell of *Pellia epiphyllia* as described by Farmer ('95), with the exception that there are no centrosomes or centrospheres present (Figs. 28 and 28 A). There is nothing that indicates the presence of centrosomes, as claimed by Ikeno ('07) and Schaffner ('08) for similar spermatogenous stages in the same plant. Fig. 28 shows two granules, or bodies, in the region of one pole, but the latter is broad, a condition that does not obtain when true centrosomes are present. Even when these bodies do chance to be in such a position, it seems quite arbitrary to designate them as bodies specially differing from others which occur just as frequently in various places throughout the cell. Usually no special body of any sort occupies the spindle pole (Fig. 28 A). So far my observations agree with those of Escoyez ('07), except that I did not find the spindle fibres forming definitely outside of the nuclear membrane as represented by his Figs. 13-15.

The earlier mitotic conditions in the spermatogenous cells of *Fegatella* are much the same as in *Marchantia*, but as metakinesis approaches there are some differences, so that a brief description will be in place. The nuclear figures are, as a rule, larger and more distinct. The nucleus in a resting condition (Fig. 45) shows a linen network with small lumps of chromatin quite evenly scattered throughout, and one or more relatively large, centrally placed bodies. Fig. 45, which is typical of this stage, shows one body which resembles a nucleolus, and another body which has the appearance of a mass of chromatin. The number of the larger bodies may vary from one to several. During prophase the chromatin becomes scattered rather evenly in the nuclear network as irregular lumps of nearly uniform size (Fig. 46). From this condition an irregular spireme develops which is

evidently of a double nature (Figs. 47 and 48). Since this is not a reducing division the double nature is certainly the result of a split. The chromosomes are now quickly differentiated. Whether the large loops and enlarged portions of the spireme (Fig. 48) separate as individual chromosomes must be left for further investigation. In sections cut in the plane of the equatorial plate the chromosomes are easily counted, and, as already stated by other investigators, the number is clearly eight.

During the prophase the nuclear cavity is not so often found to be filled with the homogenous or very finely granular substance as observed regularly in *Marchantia*. This condition, however, prevails to some extent (Fig. 48). But in this figure the nuclear membrane has practically disappeared, and there are indications in the surrounding cytoplasm that spindle fibres are beginning to form. These are at first very fine and evenly and closely distributed; later they collect into coarser strands attached to the chromosomes at the equatorial plate (Figs. 50 and 51). The appearance of the chromosomes in this position is quite similar to that in *Marchantia* (Fig. 28 A) and *Porella* (Fig. 8). The individual chromosomes cannot always be distinguished. The spindle fibres are evidently active in laying down the cell plate (Fig. 52, *Fegatella*, and Fig. 10, *Porella*), as is the case in the higher plants.

The cytoplasm is quite similar to that of *Marchantia*. At no time is there any evidence of a centrosome-like body. This agrees with Escoyez's ('07) observations on the same plant. Bolleter ('05), however, concluded that, since he saw bodies in the sperm cells resulting from the diagonal division, and that, following Ikeno's conclusions for *Marchantia*, these bodies were centrosomes, therefore they must surely be present in the preceding spermatogenous divisions, although he did not observe them.

In *Asterella* my studies were less extensive, but some sharply defined spindles were found (Figs. 61 and 62). Very minute dots were sometimes seen occupying the poles of the spindle, but these were very insignificant as compared to the size and appearance of granules lying in various places throughout the cytoplasm. Nothing was found in this study as far as pursued which resembled centrosomes in appearance or behaviour.

THE OBLIQUE DIVISION.

Fig. 11 shows two cells from an antheridium of *Porella*, which is evidently ready to enter the last division, as indicated by the size of the organ and by the number and size of the cells. The nuclear network is quite coarsely granular, with small chromatin particles, and contains a small nucleolus. The cytoplasm is rather finely and evenly granular. The last division was not found, although hundreds of sections were examined for this particular stage. Pairs of sperm cells, however, just succeeding the last division were found, but whether these are the result of a diagonal division

is not shown by their position, as the rounded pairs lie variously placed within the antheridial cells, whose walls are also somewhat rounded off (Fig. 13).

As already described by Ikeno and others, the last division of the spermatogenous tissue, in *Marchantia*, is a diagonal one which separates the cell contents into a pair of triangular sperm cells not separated by a cell-wall. The oblique position of the spindle has also been accurately described. In some respects, however, my observations differ from those of the other observers. Cells that are ready to enter upon this division have usually a more coarsely granular cytoplasm than those in early stages of spermatogenous development. The cytoplasm becomes more vacuolate, and the denser areas tend to collect in aggregations of granules, or in definite bodies (Fig. 30). There are usually two rather larger, dense bodies of about equal size, lying on opposite sides of the nucleus, and usually opposite the angles of the cell-wall. Smaller ones, which apparently differ only in size, are also found in the cytoplasm. Fig. 29 shows spindle fibres beginning to form from the immediate region of one of these larger bodies. Comparing Figs. 31 and 32, we see that the fibres reach down around the nuclear membrane, which soon disappears, and the fibres will enter the nuclear cavity and become attached to the chromosomes. Fig. 32 shows an intermediate stage in the formation of the spindle, in which the nuclear membrane is evidently gone, but where the chromosomes are neither fully differentiated nor attached to the spindle fibres. During this stage the bodies occupying the poles of the spindle are quite conspicuous. In Figs. 33 and 34 the chromosomes, while each one cannot be distinctly made out, are evidently arranged in an equatorial plate. Nearly every spindle has at least one body at the pole. Sometimes (Fig. 34) more than one body occurs with the spindle fibres attached to each. On the other hand, a single body may be present with few of the fibres attached to it, while the rest evidently extend beyond, often forming a broad pole (Fig. 33). These bodies as a rule do not stain as deeply in metakinesis as during prophase. Figs. 29-37 were drawn from the same slide, and consequently had received the same treatment in staining. After metakinesis these bodies do not persist with any regularity. Sometimes they may be seen in anaphase and telophase, but more frequently they cannot (Figs. 35, 36, and 37). I could find no evidence that the body, which occupies the pole of the spindle during the diagonal division, persists as an individual organ in the resulting sperm cell, as stated by Ikeno ('03), Escoyez ('07), and Schaffner ('08) for *Marchantia*, Bolleter ('05) for *Fegatella*, and Lewis ('06) for *Riccia natans*. At a later stage a definite body is found in the sperm cell, but no certain connexion can be traced between this body and the one which earlier occupied the spindle pole in the diagonal division, further than the fact that they both appear in the cytoplasm of the cell concerned. The cytoplasm

often shows bodies of various sizes and collections of granules (Fig. 30). Some of these seem to be merely dense globular masses of cytoplasmic material and the two which eventually occupy the spindle poles are apparently similar. There has been no evidence produced which would indicate their nuclear origin as claimed by Ikeno ('03). The drawings of the latter do not confirm his opinion that the intranuclear body seen in his Figs. 1 or 2 is identical with the body seen outside the nucleus in his Fig. 3.

The oblique division of the spermatogenous tissue in *Fegatella* occurs in essentially the same manner as in *Marchantia*. The spindle fibres extend from a small point or body in the cytoplasm towards the nuclear membrane (Fig. 53). The latter disappears, the fibres enter the nuclear cavity, and the chromosomes become arranged in an equatorial plate (Fig. 53 A). During this process the body at each pole of the spindle persists; at the same time other bodies may be scattered through the cytoplasm or in the region of the spindle (Fig. 53 A). By late anaphase the polar bodies have disappeared and the cytoplasm may or may not contain granular bodies (Fig. 54). As in *Marchantia* there is no evidence that the bodies which occupied the poles of the spindle during metakinesis persist as individuals in the sperm cells.

DEVELOPMENT OF THE SPERM.

The nuclear network of the sperm cell of *Porella* soon after the last division shows no nucleolus. The nucleus is smaller than in earlier stages of the antheridium, and the amount of cytoplasm is less (compare Fig. 12 with 1). The cytoplasm is granular and shows a denser aggregation in one end of each cell. Around the nucleus it is lighter, but in the opposite ends of the cells there are also denser areas (Fig. 12). The densely stained body in each sperm cell, as shown in Fig. 13, corresponds exactly in position to the smaller dense collection of cytoplasmic granules in Fig. 12. These figures give strong evidence that the body shown in Fig. 13, from which the blepharoplast develops, is of cytoplasmic origin. Fig. 14 shows one of the pair of sperm cells viewed from the side, if we speak of the view in Figs. 12 and 13 as edgewise. The chromatin lumps of Figs. 12 and 13 seem to be drawn out into longer and irregular pieces. Otherwise the cell has changed but little. The densely staining body now begins to develop directly into the form of the mature blepharoplast. It lengthens as a cord, following quite closely the plasma membrane (Fig. 15), until, as shown in Fig. 17, it may extend half-way or more around the periphery of the cell. Fig. 18 shows a pair of the sperm cells with the blepharoplast extending along the edge past the nucleus. The forward end of the blepharoplast, or the one from which the development has proceeded, remains slightly larger for a short distance backward than the remaining posterior portion. The

cytoplasm in the peripheral region of the cell through which the blepharoplast grows is denser than in the interior (Figs. 15, 16, and 17).

Frequently a very dense band of cytoplasm extends somewhat beyond the posterior end of the developing blepharoplast (Figs. 17 and 18), as if possibly contributing to the growth of the latter. The blepharoplast throughout seems to be composed of similar material; at least, no differentiation could be brought out in the staining. The conditions observed in the development of the blepharoplast indicate that the body shown in Fig. 13 represents cytoplasmic constituents; that this body begins to grow into a cord, and enough material is added from the cytoplasm to produce the mature threadlike blepharoplast. The position of the blepharoplast is quite similar (Figs. 17-20) to that of *Chara*, as described by Mottier ('04), but the form and development differ. The blepharoplast of *Chara* arises as a differentiation of the plasma membrane with the posterior end the thicker, while in *Porella* it grows from a spherical body, and the forward end is the larger.

The chromatin granules gradually lose their identity, forming a homogeneous mass which begins to draw out along the blepharoplast into a curved or crescent-shaped band (Figs. 19, 20, and 21). The nuclear material now becomes so closely applied to the blepharoplast that the exact extent of the latter cannot be determined. The nuclear band continues to lengthen, describing eventually a little more than one turn within the cell-wall. The blepharoplast, or as much of it as can be seen extending from the nucleus, becomes thinner, but the forward end from which the cilia spring remains slightly thicker than the rest (Figs. 22 and 23). Fig. 22, which is still enclosed within the cell membrane, shows the cilia coming off from the rear of this thickened forward end of the blepharoplast and extending around the body of the sperm, and at certain points in contact with the cell membrane. Fig. 23 shows more distinctly the point of insertion of the cilia. The cytoplasm collects in coarse granules within the concave side of the sperm, mainly within the posterior or nuclear part (Figs. 21, 22, and 23). Mature sperms (Fig. 24) were secured by dissecting out antheridia from the antheridial branches, and allowing the sperms to escape in water on a slide. In about thirty minutes after escaping, they were killed with 2% osmic acid and stained with anilin safranin and gentian violet. The blepharoplast is readily distinguished, extending forward from the nuclear portion and bearing close behind its tip a pair of cilia, which are somewhat longer than the entire body of the sperm. The blepharoplast is nearly straight, but the nuclear part is curved and forms a little more than one complete turn. The blepharoplast cannot be traced back along the side of the nuclear portion, but a distinct line extends beyond, which, together with the posterior end of the nucleus, encloses a small vesicle of apparently homogeneous cytoplasm. On comparing Fig. 24 with Figs. 17 to 20, we can readily see that this line

around the vesicle may be the posterior end of the blepharoplast; however, this is only a conjecture and cannot be proved from the material thus far observed. The mature sperm, then, consists of a nuclear portion which makes up the main body, a blepharoplast bearing two cilia, and a small cytoplasmic vesicle. The evidence so far brought to light on *Porella* favours the view that the blepharoplast and cilia represent specialized cytoplasmic material, which is first distinguishable in the origin of the blepharoplast, as a spherical granule or body, in the cytoplasm of the sperm cell; that it represents, as suggested by Mottier ('04), individualized parts of the kinoplasm which arise *de novo* in the spermogenous cells.

As already stated, the sperm cell of *Marchantia* does not contain a centrosome-like body which has persisted throughout the last division of the spermogenous tissue. However, it is possible at almost any stage occasionally to locate granules or bodies within the cytoplasm. But it is only after the last division of the spermogenous cells that any body or granular collection can be shown to persist as an individual throughout the subsequent metamorphosis of the resulting sperm cell, and to become a part of the mature sperm. This has already been described for *Porella*, which, on account of the slow development of the sperm, gives better opportunity for the observance of a complete series of stages.

Soon after the last division in *Marchantia* a globular or slightly elongated body appears in one point of the sperm cell (Figs. 38, 39, and 40). This body, as in *Porella*, is the beginning of the blepharoplast, and in appearance and subsequent behaviour is practically the same. As it proceeds around the side of the cell, denser areas, or aggregations, of cytoplasm evidently contribute to its growth (Fig. 43). The direction which the blepharoplast takes around the cell is shown by comparing Figs. 41, 42, and 43. In Fig. 41 it comes around next to the observer, while in Fig. 42 it goes behind the nucleus. When the pairs of sperm cells appear as shown in Fig. 41, the blepharoplast does not usually extend around the edge, but more or less next to, or away from, the observer. The mature sperm is quite similar to that of *Porella*, except that it is relatively thicker and shorter and not so much coiled. The cytoplasmic vesicle attached to the posterior end is more distinct. The greater rapidity with which the sperm of *Marchantia* develops would probably account for all of these differences.

Just after the oblique division in *Fegatella*, we find the daughter nuclei lying in a more or less granular cytoplasm, which does not immediately divide (Figs. 55 and 56). The latter figure shows a number of granules in the cytoplasm, but it would be entirely arbitrary to select any particular one as the future blepharoplast, or as the body which occupied the pole of the last spindle. A little later, however, when the two sperm cells have separated, a dense body similar to the one found in *Marchantia* or *Porella*, at first spherical then slightly elongated, appears in one angle of the cell

(Fig. 57). Fig. 58, which is a side view, shows this body developing as a cord along one edge of the cell. So far as stages were observed, the blepharoplast developed and the nucleus changed in shape and structure as in *Porella* and *Marchantia*. Fig. 59 A compares very favourably with Figs. 18, 19, and 20 of *Porella*, although the blepharoplast cannot be traced throughout its entire course. The rapidity of development, however, compares with that of *Marchantia*. The mature sperm (Fig. 60) is somewhat larger than that of *Marchantia* (Fig. 44), otherwise they appear quite similar. The cilia arise from nearer the tip of the blepharoplast in *Marchantia* and *Fegatella* than in *Porella* (compare Figs. 44 and 60 with Fig. 24). The cytoplasmic vesicles in the two former differ slightly from the latter, and all three differ in size ; but the general form and structure is the same in all three plants.

CONCLUSIONS.

The evidence furnished by these studies seems to warrant certain conclusions. In the first place there is no evidence that centrosomes occur in the spermogenous tissue of *Porella* and *Asterella*, or in *Marchantia* and *Fegatella*, as has been claimed by certain investigators. When occasional bodies are found in the cytoplasm or the region of the spindle they do not present the behaviour or appearance of centrosomes. Miss Black found the same conditions to prevail in *Riccia Frostii*, as did also Escoyez in *Marchantia*.

During the last division of the spermogenous tissue of *Marchantia* and *Fegatella*, which results in the formation of the sperm cells, a darkly staining body, as a rule, occupies each pole of the spindle. There is no evidence leading one to believe that this body is of other than cytoplasmic (kino-plasmic) origin. There are no indications that it arises in the nucleus. No radiations except the spindle fibres extend from this body. Van Hook ('00) found centrosomes with profuse radiations extending into the cytoplasm in dividing vegetative cells of *Marchantia*. I compared my slides with those of Prof. Van Hook, but found that my preparations of the spermogenous tissue showed no figures similar to his of the vegetative tissue. I also compared my slides with those of Prof. Mottier showing centrosomes in *Dictyota*, but found in mine no similar structures. No figures similar to those found by Farmer ('95) in the germinating spores of *Fegatella* and *Pellia*, and by Farmer and Reeves ('94) in *Pellia*, were found.

Furthermore, there is no conclusive evidence that this body, which occupies the poles of the diagonal spindle, persists as an individual in the resulting sperm cell. No genetic continuity can be traced throughout even two cell generations. Whatever may be the nature of this body, its appearance and behaviour do not agree with that of a true centrosome in those places where this latter organ is found.

The fact that a body does sometimes occupy the pole of the spindle does not necessarily imply anything more than a probable concentration of cytoplasmic or kinoplasmic materials.

The development of the blepharoplast in all these plants proceeds from a dense granular mass or spherical body, located usually in the most distant angle of the sperm cell. Evidence so far obtained indicates its origin as a condensation or aggregation of cytoplasmic material. The blepharoplast becomes a cord, growing in close contact with the plasma membrane and entirely past the nucleus. The latter then draws out along the posterior portion of the blepharoplast and may extend beyond it, while from near the forward and slightly enlarged end two cilia develop. My observations lead me to believe that the 'Cytoplasmatischer Fortsatz' of Ikeno is merely a part of the blepharoplast.

No body corresponding in size and appearance to the 'Nebenkörper' of Ikeno was found.

According to some authors, the mature sperm represents three metamorphosed elements of the cell, each of morphological rank, namely, the nucleus, the cytoplasm, and the blepharoplast or centrosome; the centrosome and blepharoplast being held by some to be homologous. In the light of my observations I believe that no centrosome exists in *Porella*, *Marchantia*, or *Fegatella*, and that the blepharoplast arises *de novo* in the cell in which it is to function as the cilia-bearer. Escoyez takes the same view, save that he describes the blepharoplast as originating in the spermatogenous tissue just previous to the last division, and occupying the pole of the diagonal spindle. These considerations tend to confirm the view expressed by Mottier ('04), that the fundamental substance known as kinoplasm possesses genetic continuity, and that the blepharoplast represents individualized parts of the kinoplasm arising *de novo* in certain spermatogenous cells. We cannot at all times, by present cytological methods, differentiate the kinoplasmic part of the cytoplasm.

We may thus consider the mature sperm to represent the two constant cell elements, nucleus and cytoplasm; the main body, or the nuclear portion, representing the nucleus, the blepharoplast and cilia representing specialized parts of the cytoplasm, and the remainder of the latter being found in the cytoplasmic vesicle.

These studies were carried out under the direction of Professor D. M. Mottier, to whom I wish to express my thanks for his kindly assistance and for his valuable and timely criticisms.

INDIANA UNIVERSITY,
August 31, 1910.

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EXPLANATION OF FIGURES IN PLATE XXV.

Illustrating Mr. Woodburn's paper on Spermatogenesis in Hepaticae.

All figures were drawn with the aid of a camera lucida and with a Leitz apochromatic 2 mm. objective, 1.30 apert. with compensating ocular 18. Magnification 3000 at table level.

Figs. 1-24. *Porella*. (Figs. 1-11 previous to the last division of the spermogenous tissue.)

- Fig. 1. Cytoplasm and nucleus in young antheridium. Nucleus in resting stage.
- Fig. 2. Nucleus in resting stage.
- Fig. 3. Cell in very young antheridium. Nucleus in early prophase.
- Figs. 4-6. Nuclei in various stages of prophase.
- Fig. 7. Cell showing number of chromosomes.
- Fig. 8. Metakinesis in young antheridium.
- Fig. 9. Anaphase.
- Fig. 10. Telophase.
- Fig. 11. Two cells shortly before entering upon last division.
- Fig. 12. A pair of sperm cells without the enclosing cell-wall.
- Fig. 13. A pair of sperm cells enclosed in cell-wall. Seen from the edge.
- Fig. 14. One sperm cell, seen from the side, showing blepharoplast primordium.
- Fig. 15. Sperm cell. Blepharoplast beginning to develop in length.
- Fig. 16. Blepharoplast slightly longer than in Fig. 15.
- Fig. 17. Blepharoplast half-way around the sperm cell.
- Fig. 18. A pair of developing sperms, showing blepharoplast.
- Figs. 19 and 20. Showing nucleus becoming more homogeneous than in Fig. 18.
- Fig. 21. Nucleus beginning to draw out along the blepharoplast.
- Figs. 22 and 23. Later stages of the sperm with the cilia partly developed.
- Fig. 24. Mature sperm escaped from the antheridium.

Figs. 25-44. *Marchantia*. (Figs. 25 A to 28 A are drawn from spermatogenous tissue.)

Fig. 25 A. Spermatogenous cell in resting condition.

Fig. 25. Chromatin in a central mass. Nuclear cavity very finely granular or filled with a homogenous substance.

Fig. 26. Nuclear cavity as in Fig. 25. Chromatin in two or three lumps.

Fig. 27. Nuclear cavity a little more coarsely granular. Chromatin nearly ready for the formation of chromosomes. Cytoplasm unevenly granular.

Figs. 28 and 28 A. Spindle stages.

Fig. 29. Just previous to the last division. Cytoplasm containing two dense bodies.

Fig. 30. The same stage as 29, but more bodies in the cytoplasm.

Figs. 31 and 32. Formation of the oblique spindle with the cytoplasmic bodies occupying the poles of the spindle.

Figs. 33 and 34. Spindle stages. Chromosomes in equatorial plate.

Fig. 35. Anaphase. Bodies at poles disappearing.

Figs. 36 and 37. Anaphase and telophase, bodies at poles having disappeared or nearly so.

Fig. 38. A pair of sperm cells. One showing blepharoplast primordium.

Figs. 39 and 40. Single sperm cells. Blepharoplast beginning to lengthen.

Fig. 41. Two antheridial cells containing respectively a pair of sperm cells seen from the edge.

Figs. 42 and 43. Single sperm cells showing the course of the blepharoplast. The stage is about the same as in Fig. 41, but from a different view.

Fig. 44. Mature sperm after escaping from the antheridium.

Figs. 45-60. *Fegatella*. (Figs. 45-52. Before the diagonal division.)

Fig. 45. Nucleus of a cell in resting condition.

Fig. 46. Cell with nucleus in early prophase.

Fig. 47. Later prophase. Chromatin forming an irregular spireme of a double nature.

Fig. 48. Spireme about as in Fig. 47. Cytoplasm showing indications of spindle formation.

Fig. 49. Showing number of chromosomes.

Figs. 50 and 51. Spindle stages. Chromosomes in equatorial plate.

Fig. 52. Telophase.

Fig. 53. Formation of spindle in oblique division.

Fig. 53 A. Oblique division. Equatorial plate and bodies at poles.

Fig. 54. Anaphase. No definite bodies persisting in region of poles.

Fig. 55. Pair of sperm cells. Blepharoplast cannot be identified.

Fig. 56. Pair of sperm cells. Numerous granules scattered throughout cytoplasm.

Fig. 57. Blepharoplast primordia. Sperm cells seen from the edge.

Fig. 58. One sperm cell seen from the side. Blepharoplast beginning to lengthen.

Fig. 59. A pair of sperm cells. Blepharoplast much elongated and nuclei drawing out along the rear portion of the blepharoplast.

Fig. 59 A. Somewhat later than Fig. 59. The blepharoplast disappears behind the nucleus.

Fig. 60. A mature sperm after escaping from the antheridium.

Figs. 61 and 62. Two spindles from spermatogenous tissue of *Asterella*.

W.L. Woodburn, del.

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