Studies in Nuclear Division of *Preissia commutata*.¹

BY

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With Plates LIV and LV.

IN the gorges and smaller ravines in the vicinity of Ithaca there are many places where the water, dripping almost constantly over the rocks, affords favourable environment for the development of various species of Liverworts. While studying the morphology of the Bryophytes, my interest in the Liverworts that grew here first began. In an effort to learn more about one of them which is very abundant near Ithaca, this piece of work was undertaken.

Material for the study of the vegetative cells was collected from several localities. During July, 1910, plants were sent twice a week from Coy Glen, near Ithaca, and kept in good growing condition on the bank of a brook in Mount Vernon, New York. Other material was collected during July and August, 1911, in Buttermilk Gorge and Enfield Gorge, in the vicinity of Ithaca, and the material fixed in the laboratory. The plants sent to Mount Vernon and those collected in Buttermilk Gorge were equally satisfactory for the study of somatic division.

I. MITOSIS IN THE VEGETATIVE CELLS.

(Plate LIV.)

Cells on or near the dorsal surface of the thallus of *Preissia commutata*² contain so many metaplasmic bodies, such as granules, plastids, starch, &c., that it is difficult to make a careful examination of the cytoplasm and nucleus for the presence of certain structures, especially centrospheres and centrosomes, which have been found in some Liverworts. A region almost free from the above-mentioned bodies was found on the ventral side of the thallus, just behind the turn made by the upward growth of the gametophore.

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² Portions of thalli were killed in Flemming's fluids, the stronger, medium, and weaker solutions; in Merkel's fluid, and in chromacetic solution. The Flemming's fluid, weaker solution, gave the best results. Air was pumped out of all tissues after being placed in the killing fluids. Sections were cut 5μ in thickness.

The cytoplasm in the resting cell is finely granular, and in addition there are coarse granules scattered through it which stain more deeply. Several large vacuoles are also present in the cytoplasm. The resting nucleus is rounded and contains a small number of delicate linin strands. These strands stretch across the nuclear cavity, occasionally anastomosing. At low magnification they seem to radiate from the nucleolus. Some of the chromatin granules are distributed on the linin network, either at points where the linin strands meet or here and there on long portions of a strand free from anastomosing ones. Possibly all the linin strands are not differentiated by the stain used. The nucleolus in the resting cell is a large irregularly rounded body lying in the centre of the nucleus. In addition to this large nucleolus, a smaller one of the same general form frequently accompanies it (Fig. 1). Conspicuous nucleoli and a delicate network of linin threads were described in the vegetative cells by Van Hook ('00), who studied cell-division in the stalk of the archegoniophore of *Marchantia polymorpha*.

The first indication of prophase is the elongation of the nucleus, usually in the direction of the long axis of the cell. All of the nuclear contents become more conspicuous. The linin increases in quantity, not only in the number, but also in the increased width of the strands, thus making a more complex and dense reticulum than the open, delicate reticulum of the resting cell. Large irregular chromatin masses appear in this elongated nucleus, either on the linin or lying apparently free in the nuclear cavity. These masses are formed partly by the union of the chromatin granules, partly from another source; for, considering their size and number, it is probable that the union of the granules seen in the nucleus of the resting cell is not the only source through which their bulk has been increased. The size, staining reaction, and surface appearance of the nucleolus remain the same as in the resting nucleus. Therefore it cannot be said to contribute any appreciable amount of chromatic substance to the chromatin masses (Fig. 2). An account of the behaviour of the nucleolus in the vegetative cells of *Preissia commutata* will be given later.

The cytoplasm at this phase of the nucleus shows a differentiation into areas that are finely granular and coarsely granular. The coarseness of the latter areas is due to a grouping of the granules that were scattered throughout the cytoplasm of the resting cell. This condition is shown in Figs. 2, 6, and 7.

Certain of the strands of linin forming the complex network described above thicken and form a more or less continuous spireme, while other strands anastomosing with this remain very delicate. These delicate strands finally disappear, fragments of them remaining for a time as lateral projections from the forming spireme, which thus adds to the irregular outline of the surface of the spireme (Figs. 3, 4, 5). The masses of chromatin

gradually lose their irregular outline and become distributed on the linin in the form of chromomeres. Grégoire and Wygaerts ('03) believe that there is no distinction between linin and chromatin, that it is but one substance. They look upon the smaller dense bodies in the nucleus as chromatin and the network as a thinner part of the dense bodies, that lies between them. The nuclei in *Preissia commutata* are so very small that it is impossible to say whether the reaction of the dense bodies is unlike the reaction of the filaments when triple stain is used. The nuclei in the Liverworts are so small that they are not favourable objects for the study of this question. When the spireme is first formed it is narrow, and the chromomeres, which are very conspicuous at this stage, appear on it at unequal distances (Figs. 3, 4, 5). Later, when the spireme thickens, the chromomeres are not distinct (Figs. 9, 10, 13, 14). This account of the formation of the spireme in *Preissia commutata* agrees, in general, with the account of the formation of the spireme in higher plants, as described by Farmer and Shove ('05) in the vegetative cells of *Tradescantia virginica*, and by Wager ('04) in cells in the root-tip of *Phaseolus* and others. Coincident with its formation, there is a general contraction of the spireme from the periphery of the nucleus towards the nucleolus (Figs. 4, 5). The spireme finally contracts so closely about the nucleolus as to almost hide it (Fig. 6). Finer linin threads extending from the contracted spireme to the periphery of the nuclear membrane may also be seen in this figure. This contraction is a conspicuous stage in the mitosis of the vegetative cells of *Preissia commutata*, and has been observed in the germinating spores of *Pellia epiphylla* by Davis ('01), who calls it synapsis, and in *Carex* by Stout ('11). Later the spireme moves away from the nucleolus. At this time it is very uneven, being slender in some places and thick and jagged in others (Figs. 7, 8). These uneven regions disappear, and a spireme of uniform consistency comparable to that found in the higher plants by many students lies loosely coiled in the nuclear cavity. This spireme appears to be continuous. Chromomeres, so conspicuous in the early stages of the spireme, cannot now be distinguished readily. An unsegmented spireme in the vegetative division of the Liverworts has been mentioned several times, but up to the present time has not been fully described nor illustrated. This may be due to the fact that little work on the cytology of these plants has been done. Van Hook ('00), who studied the vegetative cells of *Marchantia polymorpha* in the stalk of the archegoniophore, says that he did not observe a spireme. Davis ('01) says that the linin and chromatin in the germinating spores of *Pellia epiphylla* form a broad spireme. Chamberlain ('03), who also studied the germinating spores of *Pellia epiphylla*, states that the nuclei elongate after the spireme is formed. Neither of the last two investigators figured the spireme.

That there is a contraction of the spireme may be objected to on the

ground that it is an artifact, due to faulty fixing. Cells in all other phases of division lie near those in which the spireme is contracting about the nucleolus, and no evidence of faulty fixing can be observed. Cells showing a recovery of the spireme from the contracting state lie in the growing region, and cannot be explained on the assumption that this contraction is an artifact.

At early prophase, when the nucleus is elongated, the cytoplasm becomes massed at the ends of the nucleus. These masses are dense and contain numerous granules. They are closely applied to the membrane of the nucleus at its ends (Figs. 2, 3). These granular masses may be found applied to the nuclear membrane at the ends of all nuclei in early prophase (Figs. 4, 5, 6 are not cut parallel with the long axis of the nucleus). Between the granular cap and the nuclear membrane there now appears a cone-shaped hyaline cap that rests on the ends of the nucleus. The boundary of the hyaline polar cap presents a sharp outline which may be pointed or blunt at its apex (Figs. 11, 12). This boundary appears slightly granular in cross-section. The granular areas are not so prominent after the appearance of the hyaline caps (Figs. 11, 12). Whether part of the granular area of cytoplasm is transformed into a hyaline substance that forms the hyaline caps, or the caps are composed of a hyaline fluid that is extruded from the nucleus, was not determined. In cells in the root of *Hyacinthus orientalis*, Rosen ('95) found a layer of hyaline kinoplasm surrounding the nucleus. Later the kinoplasm does not envelop the nucleus, but appears on opposite sides of the nucleus at the elongated ends as polar caps. Their appearance in Rosen's material and in *Preissia commutata* is similar at this later stage. Rosen ('95) believes that the substance of the cap is derived from the cytoplasm. Němec ('99) describes hyaline caps in the vegetative cells of *Vicia Faba* and several other Seed Plants. He also described an enveloping layer of hyaline kinoplasm around the nucleus that later takes the form of polar caps. He believes that this substance surrounding the nucleus, and later forming the caps, is identical in chemical peculiarities with the nuclear sap. Němec's ('99) experiments show that the form of the hyaline polar caps is due to a force pulling them in the direction of the long axis of the nucleus. By chloroforming or plasmolysing the living tissue, the hyaline polar caps return to the form of a hollow sphere surrounding the nucleus. In *Preissia commutata* the spindle fibres are developed in the hyaline polar caps. These fibres have a finely granular appearance. When the spireme is segmenting to form chromosomes, spindle fibres are being formed in the hyaline polar caps (Fig. 13). These fibres converge at the apex of the cap (Fig. 14). The form of the polar cap after the development of the fibres is unchanged. Sections showing the development of the fibres, in which the outline of the cap of granular fibres presents a blunt appearance, such as Rosen ('95) illustrates in his Fig. 3 c, were not

found. Rosen ('95) believes that they grow from the nuclear membrane towards a point some distance from it.

Polar caps such as are described above have been observed by many investigators. Many have figured them in the Algae (*Spirogyra*). Hof ('98) describes them in a Fern, a Gymnosperm, and an Angiosperm, and Chamberlain ('03) in the germinating spores of *Pellia epiphylla*.

The nucleus in *Preissia commutata* elongates, as has been said, in the direction of the long axis of the cell, and the polar caps and spindle fibres appear against the nuclear membrane at these elongated ends. These fibres, therefore, appear as portions of the nuclear membrane, against which there is a greater mass of cytoplasm than there is on the sides of the elongated nucleus. The entire cells from which Figs. 13 and 14 are drawn are not represented; some portion at each end is omitted. The long axis of both these cells is parallel with the long axis of the nucleus. These fibres, therefore, appear on portions of the nucleus against which there is the greater amount of cytoplasm. The behaviour of these bipolar caps accords with Němec's ('99) theory, namely, that they appear in vegetative cells on the sides of the nuclei against which the mass of cytoplasm is the greater.

While the spireme is segmenting and while the nuclear membrane is still intact, a considerable number of spindle fibres have developed in the polar caps. Part of the spindle, therefore, is derived from the cytoplasm.

Diligent search was made for a centrosome at the apex of the converging fibres, but none was found; neither were there any cytoplasmic radiations. Many granules are in the cytoplasm at this stage, some of which may happen to lie at the apex of the cone of fibres, as shown in Fig. 13. The poles of the spindle at metaphase, as is shown in Figs. 16, 18, and 19, are sharply pointed, and, arising as they do from bipolar caps, it is at these poles that a centrosphere or centrosome might be expected, if it existed. No structures which could be justly interpreted as centrospheres or centrosomes were observed prior to or during the formation of the spindle. In one instance a multipolar diarch spindle (Fig. 18) was observed. The formation of a spindle of this type was not followed.

At the time the cone of fibres is fully developed at the ends of the nucleus, the spireme begins to break up into segments. Fig. 13 shows the spireme partly segmented. At this stage the segments are very little broader than the continuous spireme. Soon, however, they become broader and the ends of each chromosome curve (Figs. 14, 15). This curving makes it difficult to determine their exact form at metaphase. In longitudinal section of the spindle at metaphase the chromosomes appear as oval bodies. After segmentation of the spireme appears to have been completed, the segments are large and there are probably not more than eight (Fig. 14). In Fig. 15 the segments have shortened somewhat and there are probably not more than eight. In longitudinal section of the spindle at metaphase,

and before any division of the chromosomes has taken place (Figs. 16, 17), not more than eight chromosomes can be found. Fig. 20 probably represents the stage just after the division of the chromosomes. A cross-section at metaphase corresponding to the grouping of the chromosomes seen in Fig. 20 represents sixteen chromosomes in polar view (Fig. 21). Fig. 22 is a polar view of metaphase before division of the chromosomes in which eight are represented. This section is cut in a slightly oblique direction. In the first division of the spore mother-cell in a longitudinal section of the spindle at anaphase, there are seen eight chromosomes. At this division the chromosomes are very much longer than in the somatic division. This number was counted so often that it is probably the correct one for the haploid number. The chromosomes in the vegetative cells are very small bodies. Fig. 19 is a longitudinal section through the mitotic figure. Here the chromosomes appear larger than those in any other section. By far the greater number of sections at metaphase appear as in Figs. 18 and 20.

During anaphase the chromosomes elongate. When they reach the poles (Fig. 23) they are elliptical, but are still distinct from one another. Later, while still grouped at the poles, they become so crowded that it is impossible to distinguish individual chromosomes (Fig. 24). The elements of this crowded mass elongate considerably (Fig. 25) and appear as stout threads with clear spaces between them. Some of these thick threads are curved (Fig. 26). It is probable that all the curved threads represented in Figs. 25 and 26 are chromosomes since their number agrees with the number in late telophase illustrated in Figs. 23 and 24. Clear spaces between the stout threads are enlarged probably by the increase of nuclear sap (Figs. 25, 26).

A nuclear membrane cannot be distinguished until the chromosomes have become more tenuous, longer, and as individual bodies have become lost in their union to form the reticulum of the nucleus. With the appearance of the nuclear membrane a nucleolus appears which is at first very small (Fig. 27). At this time also the chromatin is distributed on the linin reticulum in the form of granules, as has been described above in the resting cell.

The nucleolus in the resting cell is, as has been stated, very large. In the resting condition and during the early stages of mitosis it may be spherical, irregularly rounded, or angular (Figs. 2, 3, 4, 5, 6). When using Flemming's triple stain it stains deeply with the aniline-saffranin during all the early phases of mitosis. In all preparations which I have examined, up to the time of the segmentation of the spireme, the nucleolus was present, and in no case has it presented the appearance of being vacuolate. I have found no evidence that the nucleolus contributes any portion of its material for the formation of the spireme and chromosomes, although during the resting stage of the nucleus and in the early prophase of division strands

of the reticulum are at least in contact with the nucleolus. The actual connexion of the nucleolus with the reticulum and with the chromosomes has often been observed by other students, some of whom believe that it furnishes a portion of the substance¹ for the chromosomes. Wager ('04) says that in the vegetative cells of *Phaseolus* the nucleolus contributes a large part of its substance to the formation of the spireme and the chromosomes. During this process the nucleolus undergoes changes of form, becoming amoeboid, then vacuolate, and finally spongy. After the chromosomes were fully formed, the remainder of the nucleolus divided into unequal portions which moved towards the poles of the spindle. In *Preissia commutata* I have not observed the nucleolus after the dissolution of the nuclear membrane. At metaphase, of which there are many sections, all the chromatin is readily identified as chromosomes. A dividing nucleolus, such as Wager ('04) found in *Phaseolus*, and a dividing nucleolus and fragmented portions of the nucleolus lying in the cytoplasm surrounding the spindle at metaphase, such as Thos. Martins Mano ('04) found in *Phaseolus* and *Solanum*, and as has been described by others, were not observed by me in the vegetative cells of *Preissia commutata*. When the spireme has segmented and the chromosomes begin to shorten and thicken (Figs. 13, 14) the nucleolus is of the same size as it is in the earliest prophase. Nor has its surface appearance and staining capacity changed so far as I could observe. These facts lead me to believe that the nucleolus does not give up its substance to the formation of the chromosomes, or, at any rate, not to any appreciable extent. But the sudden disappearance of the nucleolus coincident with the formation of the central spindle suggests that its substance may be transformed into spindle fibres. That the substance of the nucleolus is concerned with the formation of the spindle fibres is a view held by a number of students. Němec ('99) studied mitosis in the Seed Plants and came to the conclusion that the nucleolus takes part in the formation of the spindle. He describes the reverse process, that is, the mantle fibres become granular and by consolidating form the nucleoli of the daughter nuclei. Strasburger ('05), who worked at both Monocotyledons and Dicotyledons, was convinced that the nucleolus contributes some substance for the formation of spindle fibres. Miyake ('05) is in entire accord with Strasburger's view. A number of Monocotyledons were investigated by Miyake, and he describes and figures fully formed chromosomes while the nucleolus still persists. Many others hold the same view. Chamberlain ('03), who studied the germinating spores of *Pellia epiphylla*, suggests that the substance of the nucleolus is used partly in the formation of the

¹ In the 11th edition of *Lehrbuch der Botanik* (by Strasburger, Jost, Schenk, and Karsten), 1911, in the section 'Morphology', p. 73, Strasburger says that the nucleolus appears to represent reserve stuff which serves for the nourishment of the chromosomes and also furnishes some substance for the spindle threads.

chromosomes and partly in the formation of the central spindle fibres. It is generally accepted that in many Thallophytes the nucleolus often contributes a portion of its substance to the formation of the chromosomes, and it would not be surprising if in some of the Liverworts the nucleolus also contained some of the chromatin.

Indications of a cell plate appear in the earliest telophase observed (Fig. 23). In later telophase the cell plate, in section, appears as a line of granules. These increase in number until a distinct plate is formed (Figs. 24, 25, 26). At first the cell plate lies only between the two daughter nuclei, finally extending its circumference to the periphery of the cell. The formation of the cell plate in *Preissia commutata* agrees with the formation of the cell plate in the vegetative cells of *Pellia epiphylla* described by Farmer and Reeves ('94), and in the vegetative cells of the archegoniophore of *Marchantia polymorpha* described by Van Hook ('00).

DISCUSSION.

Although a number of papers on mitosis in the gametophyte of the Liverworts have appeared, many of them were concerned primarily with the centrosphere or centrosome problem. Farmer and Reeves ('94) describe centrospheres in the germinating spores of *Pellia epiphylla*; Farmer ('95) also describes them in the germinating spores of *Fegatella conica* (*Conocephalum conicum*). Van Hook ('00) reports them associated with centrosomes in cells in the archegoniophore of *Marchantia polymorpha*. Chamberlain ('03) describes the presence of asters in the prophase division in the germinating spores of *Pellia epiphylla*, their disappearance at metaphase, reappearance at telophase, and second disappearance with (see p. 33) the formation of the nuclear membrane. Usually they have nearly disappeared at the time of the third division. Davis ('01) describes prominent asters in the first three mitoses of the germinating spores of *Pellia epiphylla*, but they are less plainly shown in the second and third division. He does not find them in the stalk of the sporophyte. There is, therefore, agreement between these investigators as to the presence of centrospheres or asters in cell-division during certain stages of development of the vegetative cells of some Liverworts. The fact that asters are prominent in the first division of the germinating spore of *Pellia epiphylla*, that they become less prominent in the second and third division, finally disappearing, makes it reasonable to expect that they may not be formed during cell-division in the growing thallus of some Liverworts. The same may be said with reference to the presence of centrosomes. Both are surely absent from all phases of mitoses in the cells of the thallus of *Preissia commutata*, as far as my observations are concerned. So many cell generations lie between the mitoses in the germinating spore and the fully formed thallus that, if they are present in

the first mitosis (a division that has not been studied) they disappear entirely by the time the thallus is formed. Farmer and Reeves ('94), in their study of the germinating spores of *Pellia epiphylla*, believe that the elongation of the nucleus in the early prophase is due to the influence of the centrospheres. If there is some controlling force at the poles of the nucleus which causes it to elongate, it is certainly not to be ascribed in all cases to demonstrable centrospheres, for they are not present in the somatic cells of *Preissia commutata*, where the elongation of the nucleus is marked. Van Hook ('00) does not attempt to explain the elongation of the nucleus in cells in the stalk of the archegoniophore of *Marchantia polymorpha*; nor does Chamberlain ('03), who found that the nucleus in *Pellia epiphylla* elongated after the spireme had become segmented into chromosomes. Němec ('99) believes that some force acts on the nuclei of cells in Seed Plants which tends to pull the nuclear wall in the direction of the force. By chloroforming or plasmolysing the living tissue the nuclei become spherical.

The formation of the spindle fibres in *Preissia commutata* follows the method of their formation in many other forms, and shows that the process in this plant is similar to that in the seed plants. Upon the elongation of the nucleus the granular masses of cytoplasm at the ends of the nuclei are conspicuous. When the triple stain is used, the granules take the aniline-saffranin stain. The appearance of the kinoplasmic caps are described in the vegetative cells of a Liverwort (*Pellia epiphylla*) by Davis ('01), and his illustrations show that this cap is composed of granular cytoplasm. Farmer and Reeves ('94) state that the achromatic spindle develops from the cytoplasmic radiations of the centrospheres in the germinating spores of *Pellia epiphylla*. In *Marchantia polymorpha*, according to Van Hook ('00), the radiations from the centrospheres meet to form a spindle, while Chamberlain ('03) believes that the spindle fibres in the germinating spores of *Pellia epiphylla* are formed from hyaline caps that become granular and then fibrous.

The earliest prophase in *Preissia commutata*, in which the nucleus elongates and the chromatin substance increases materially, is similar to the same stage in the higher plants. This stage in higher plants has been described many times by many students. In a Liverwort it has been described by Van Hook ('00), and the results of my investigation agree with his, except that the centrospheres he finds at this stage are absent from the plant now under consideration.

The results of this work show that from the earliest prophase to the reconstruction of the daughter nuclei, mitosis in the vegetative cells of the Liverworts agrees with that in the higher plants, as described by many investigators.

SUMMARY.

1. In earliest prophase the nucleus elongates. The linin becomes conspicuous and large chromatin masses appear.

2. Strands of linin thicken and form a spireme comparable to the spireme in many seed plants. The masses of chromatin lose their irregular outline and become distributed on the linin in the form of chromomeres.

3. As the spireme is forming, it contracts about the nucleolus. Fine linin threads extend from the contracted spireme to the nuclear membrane.

4. As the spireme recovers from the contraction and moves away from the nucleolus, it is very uneven. Later its width is even.

5. The spireme now segments to form chromosomes, while the nuclear membrane is intact and the nucleolus still persists. The haploid number of chromosomes is probably eight.

6. During late telophase the chromosomes elongate. They then become crowded so closely together that it is difficult to distinguish the individual ones, after which they elongate into slender threads which finally lose their individuality as they unite to form the reticulum of the daughter nuclei.

7. The nucleolus is very large. It persists for some time after the segmentation of the spireme. Its disappearance is very sudden and coincident with the formation of the central spindle fibres. Neither vacuolization nor fragmentation of the nucleolus was observed at any time. This suggests that it may contribute some of its substance to the formation of the central spindle fibres.

8. Granular masses of cytoplasm are applied to the elongated ends of the nucleus at early prophase. Between the granular cap and the nuclear membrane a cone-shaped cap rests on the ends of the nucleus. Fibres appear in these hyaline caps when the spireme is segmenting and the nuclear membrane is intact. The development of fibres in the hyaline cap in *Preissia commutata* is like their development in other groups of plants.

9. Neither centrospheres nor centrosomes were found.

II. SPOROGENESIS.

(Plate LV.)

The material for the study of the development of the spores of *Preissia commutata* was collected every day, beginning with April 18, until May 17, 1912. In December the young sporogonium is fully formed. The stalk is short and lifts the receptacle but very little above the dorsal surface of the thallus. At this time the cells in the sporogonium form a solid tissue, and they remain in this condition until the following spring. Division in the sporogenous tissue begins early in April, but there is no differentiation of the tissue until May 1. On May 6, 1912, the sporogenous tissue was

differentiated into spore mother-cells and elaters. All the sporogonia are not in the same stage of development, though they are all near the same stage.

When the differentiation in the sporogonia takes place, the spore mother-cells are embedded in a gelatinous substance. There are plastids in all the cells in the sporogonia and those in the elaters develop chlorophyll. There is also chlorophyll in the wall of the sporogonium. When the wall of a sporogonium is cut and its contents are forced out on a slide the chlorophyll bodies in the elaters are conspicuous. The gelatinous substance in which the spore mother-cells and elaters are embedded is colourless, viscid, and holds the contents of the sporogonium together on the slide.

In preparation for fixing, the sporogonia were separated from the gametophore and the pseudoperianth was cut away before they were placed in the killing fluid. Hermann's fluid, Carnoy's fluid, and Juel's fluid were used. Material killed in Hermann's fluid was best. Through experience during the two preceding springs, it was found that any killing solution containing chromic acid tended to harden the mucilaginous substance in which the spore mother-cells are embedded, and the cells were plasmolysed. Sections were cut 4, 5, 6, 7, and 9 μ . They were stained with Flemming's triple stain, Heidenhain's iron-alum-haematoxylin, and the Flemming's triple stain, substituting pyoktanin for gentian violet.

The spore mother-cell in the resting condition is broadly elliptical. The cytoplasm presents a coarse open or foamy appearance. It has the same appearance in the living condition. Lying in the cytoplasm between the nucleus and the cell wall are groups of plastids. In the centre of the cell there is a spherical nucleus. The reticulum in the resting nucleus is made up of a number of fine anastomosing threads. The chromatin bodies are small, very numerous, and are situated at the angles of the reticulum (Fig. 1). One or more vacuoles appear in the reticulum at this stage. There may be as many as four large nucleoli in the nucleus of the spore mother-cell.

The spherical nucleus of the resting cell enlarges. The fine reticulum changes into a coarse network of threads (Fig. 2). Some of the threads making up the network can be traced for some distance, and it is evident that at this early prophase a long slender thread is being formed. Near the nucleoli in the section represented by Fig. 2 are fine linin threads. The reticulum is of unequal thickness at this phase; the strands near the nucleoli remain in the condition of the linin of the resting nucleus the longest time. The chromatin at this early prophase is more conspicuous than in the resting nucleus, the individual bodies having increased in size. Here, as in the nucleus of the resting cell, the chromatin bodies lie at the angles of the reticulum.

The growth of the nucleus continues. The condition of the reticulum

in which it is possible to trace a continuous thread for some distance (Fig. 2) is replaced by a slender thread, the whole reticulum having been converted into a slender thread (Fig. 3) which is called leptonema by some authors (Winiwarter ('01), Grégoire ('07), and others). From the frequency of its occurrence in sections at this stage of prophase, it is evident that the leptonema spireme is formed a considerable period before synapsis. In this figure many threads may be seen lying side by side. This suggests a pairing of the threads. The threads of many of these pairs are more slender than others which lie singly. It would be interesting to know if these stouter single threads are formed by the side-by-side union of a pair of the slender ones, or by such a close approximation that the line of division between them cannot be seen. The pairing of these threads suggests the condition of the spireme in *Osmunda regalis*, *Allium fistulosum*, &c., described and figured by Grégoire ('07) as 'noyaux zygotènes'. Grégoire ('07) does not find a continuous spireme—the chromosomes or gemini arise as long slender threads; while in *Preissia commutata* my preparations in early prophase have the appearance of the spireme being a continuous thread. Because of the very small size of the nuclei and the great length of the thread it is difficult to speak with certainty. There was no evidence, however, that prochromosomes such as are described by Strasburger ('05) in *Galtonia candicans*, by Miyake ('05) in the same plant, and by Overton ('09) in *Thalictrum purpurascens*, and by others, were present in the spore mother-cell of *Preissia commutata*. The appearance is that of a spireme that persists for a considerable time.

The spireme next enters into synapsis (Fig. 4). In the synaptic knot there is evidence of the persistency of the spireme, as the appearance of the knot in Fig. 4 plainly shows, but it is so tightly drawn together that it cannot be followed throughout its length. In the synaptic knot there are many chromatin bodies of equal size and shape. These bodies are extremely prominent and are always present at this stage of the prophase, but they are arranged in rows or in the form of a spireme. These prominent chromatin bodies of equal size and shape in the synaptic knot are very suggestive of the condition found by Strasburger ('05), Miyake ('05), and Overton ('09) in seed plants. At this phase, according to these investigators, the prochromosomes, or gamosomes, pair. In *Preissia commutata* there is no evidence in the synaptic knot that these chromatin bodies come in contact in pairs. It may be, however, that during synapsis the pairs of threads described above may be brought into closer approximation and actually fuse, thus producing this stout spireme with the chromatin masses situated at quite regular intervals. The nucleolus may be included in the synaptic knot or lie by its side.

As the spireme emerges from synapsis, there is a general loosening of the entire structure (Fig. 5). At this time it is quite slender and the chro-

matin bodies on it are very distinct. The loosening of the synaptic knot and the emergence of the spireme continue until the spireme is uniformly distributed throughout the nuclear cavity (Fig. 6). The spireme is at this stage still quite slender and its appearance is very different from the leptonema spireme. The chromomeres are of the same size and form and are placed at almost equal distances from one another. They become more prominent as the spireme thickens (Fig. 7). A post-synaptic spireme in Liverworts has been described by Davis ('01) in the spore mother-cell of *Pellia epiphylla*, where it appears as a delicate, closely wound spireme. The spireme shown in his illustration is evidently in the same stage as that in Fig. 6 of the present work. The nucleolus in *Preissia commutata*, however, is not vacuolated at this stage, while in *Pellia epiphylla* Davis ('01) found the nucleolus vacuolated.

There is a gradual shortening and thickening of the spireme (Figs. 8, 9) which results in the formation of what is sometimes called the 'pachynema' spireme. At this stage the width of the spireme has increased very much (compare Figs. 6 and 9).

From this time there is another contraction of the spireme. It contracts away from two opposite sides of the nucleus, so that it occupies a rather broad plane, extending entirely across the nucleus. The loops of the spireme do not have any definite arrangement (Figs. 8, 9), but the entire spireme is drawn rather closely together across the diameter of the nucleus. Some of the loops touch the nuclear membrane (Figs. 8, 9). This stage is the second contraction described by Farmer and Moore ('05). However, in *Preissia commutata* it occupies, as has been said, a broad plane extending entirely across the nuclear cavity and does not extend in all directions in the nuclear cavity with large spaces between the contracted portions, as is the case during the second contraction in *Lilium candidum* as described by Farmer and Moore ('05) and in *Lilium speciosum* by Grégoire ('07). Many sections with spiremes in the same general position as those shown in Figs. 8 and 9 were obtained, which indicates that this stage lasts for some time in *Preissia*.

When the spireme is entering upon the second contraction, it begins to split longitudinally throughout its entire length. Some portions of the spireme are so situated that the split is plainly seen (Figs. 8, 9). The chromomeres that are so prominent in the earlier prophases (Figs. 4, 5, 6, 7) are now not recognizable (Figs. 8, 9). Fine short threads project from the margin of the spireme, giving it a somewhat jagged appearance.

The spireme is always contracted about or near the nucleolus. At this stage the centre of the nucleolus stains less deeply than the periphery. This may be due to the method of staining or to the chemical changes that have taken place in the nucleus. It is certain, however, that at this stage the nucleolus is not vacuolate, and its size and shape remain the same as in

the earlier prophases. After the segmentation of the spireme, the nucleolus disappears, but the manner of its disappearance was not determined. In sections showing the chromosomes in process of formation no fragments of the nucleolus were found. Fragmented nucleoli in some of the Liverworts have been observed. Farmer ('95) describes a fragmented nucleolus while the chromosomes were being formed in the spore mother-cell of *Fossombronia Dumortieri*. He also describes a fragmented nucleolus at the time when the spireme splits longitudinally in *Fegatella conica* (*Conocephalum conicum*). Davis ('99) also found that the nucleolus in *Anthoceros* fragmented after synapsis. In *Pellia epiphylla* vacuolization and fragmentation of the nucleolus take place, according to Farmer ('95), during the formation of the chromosomes, and he thinks that it furnishes some material for the formation of the spireme and chromosomes. The fact that the central portion of the nucleolus stains less deeply in *Preissia commutata* prior to and during the segmentation of the chromosomes possibly indicates that some of its substance goes to the formation of the latter. But, as the greater part of it disappears after the formation of the chromosomes, it would seem that it contributes also to the formation of the spindle, if we accept the view of Strasburger ('05), Miyake ('05), Chamberlain ('03), and others on this point. It is to be regretted, however, that no direct evidence was obtained on this point in *Preissia commutata*, and that the way in which the nucleolus disappears was not observed. It apparently disappears quickly.

After the second contraction, the spireme segments (Fig. 10). The line of the longitudinal splitting observed in sections illustrated by Figs. 8 and 9 persists in the segments of the spireme (Fig. 10). The segments curve somewhat, sometimes throughout their entire length, sometimes at one end, in very much the same manner as was noted for the chromosomes in the somatic cells. The nucleolus shows no further change in its appearance than was noted at the second contraction.

The segments of the spireme next shorten and thicken (Fig. 11). At this stage the chromosomes stain very deeply, and the split shown so clearly in Fig. 10 is now more difficult to see. It may sometimes be seen throughout the length of the segment, but the free ends show it plainly. As the chromosomes shorten and thicken they bend and twist in various directions (Fig. 11). The nucleolus has disappeared completely and the nuclear membrane is still intact. At a late stage of this prophase the split may be seen only at the free ends of the chromosomes.

The achromatic spindle appears so quickly, its mode of formation was not determined. The results of efforts made to observe its formation will be given later.

At metaphase the chromosomes are arranged on the equator of the spindle (Fig. 13), but their method of splitting was not clearly made out.

The spindle lies in a space almost free from cytoplasm, plastids, &c. The poles of the spindle are broad and blunt, though there may be a multipolar diarch (Fig. 14).

As the chromosomes draw towards the poles (Figs. 14, 16) some are often in advance of the others. At this stage the chromosomes are plainly two-pointed (Fig. 17), a condition described and figured by Overton ('09) and others. As the chromosomes reach the poles (Fig. 18) they are grouped together, but do not, at least at the early telophase, lose their individual outline. In cross-section the cell plate appears as a line across the equator of the spindle at the time the chromosomes have reached the poles (Fig. 18).

The reconstruction of the daughter nuclei of the heterotypic division takes place very quickly. The wall separating the two daughter nuclei of the first division is scarcely formed, when they begin to show signs of early prophase preparatory to the formation of the four spores. No sections showing the steps between the grouped chromosomes at telophase and a daughter nucleus with a reticular content, a nucleolus, and a nuclear membrane (Fig. 19) were found. The reconstruction of the daughter nuclei is evidently passed over very quickly. After a short time the nuclei enlarge (Figs. 20, 21) and the chromatin strands thicken. The nucleolus also enlarges and chromatin bodies appear (Figs. 20, 21).

In the second mitosis of the spore mother-cell the daughter nuclei divide simultaneously. In Fig. 23 three newly formed nuclei are represented; the other one and the first wall laid down in the spore mother-cell are in another section.

Spindle fibres, so difficult to obtain in the first division of the spore mother-cell, are most conspicuous in the early prophase of the daughter nuclei. Whether they are preceded by a cap of hyaline cytoplasm, as is the case in the somatic cells of *Preissia*, was not determined. At the earliest prophases of the spore mother nuclei in the heterotypic division hyaline caps are closely applied to the nuclear membrane (Figs. 22, 24, 25). In an effort to understand the origin and function of these caps, sections were cut 5μ thick. This is too thin a section to study the nuclear phenomena, but Figs. 22 and 24 are probably both in the reticulum stage. The form of the hyaline cap varies, as a comparison of Figs. 22 and 25 shows. Their position against the nuclear membrane also varies; they may lie on opposite sides of the nucleus, or there may be a greater distance between them on one side of the nucleus than on the other side (Fig. 22). Coarse strands of cytoplasm may extend over the hyaline caps from a group of plastids to the nuclear membrane. Whether the outer spindle fibres are developed from these hyaline caps, as is the case in the somatic cells, or whether the spindle fibres are developed from a zone of fibres circling about the nucleus, as is the case in Seed Plants, the present investigation does not prove.

It has been stated that groups of plastids lie in the cytoplasm around the nucleus (Figs. 1, 7). In many sections four groups were well defined, and it was at first thought that these groups might bear some relation to the number of spores formed; that is, that each group constituted the plastids found in a mature spore. More than four groups were, however, found in sections obtained later. From metaphase to telophase the plastids are almost always divided into two large groups that lie near the poles of the spindle, few or none lying in the cytoplasm near the equator of the spindle (Figs. 13, 14, 15, 18). The coarse strands of cytoplasm extending from the groups of plastids to the nuclear membrane are very prominent (Fig. 26), but whether there is any relation between these strands and the spindle fibres was not determined.

During the earliest prophase a most careful search was made for centrospheres and centrosomes. The membrane of many nuclei in all stages of prophase was closely searched for a body exerting a pull on the membrane. None was found in *Preissia commutata*. Centrospheres with a centrosome have been described by Farmer ('95) in the earliest prophase of the spore mother-cells of *Fossombronia Dumortieri*. He also observed that the nuclear membrane of this plant was pulled out through the influence of the centrospheres, and that the quadripolar spindle is at first a 'nuclear distortion'. Farmer ('95) found that the spindle in *Pellia epiphylla* was also formed through a pull exerted on the nuclear membrane as described above for *Fossombronia Dumortieri*. Not having found spindle fibres while the nuclear membrane was still intact, nor that the nuclear membrane was pulled out, I am unable to say whether *Preissia commutata* agrees with *Fossombronia Dumortieri* and *Pellia epiphylla* or not. In view of the presence of the hyaline kinoplasmic caps described above in *Preissia commutata*, and the work of Němec ('99), Rosen ('95), and others, in which it is shown that spindle fibres are developed in the hyaline kinoplasmic caps, it is probable that at least the outer spindle fibres in the plant now being considered are formed from these caps. Heretofore these hyaline kinoplasmic caps have been reported only in somatic or vegetative cells, and their occurrence in the heterotypic division of *Preissia* is therefore interesting.

SUMMARY.

1. In early prophase the nucleus of the mother-cell enlarges. The fine reticulum of the resting cell is changed into a coarse network of threads, some of which can be traced for some distance. The individual chromatin bodies increase in size and number.

2. The nucleus continues to grow. The reticulum is replaced by a leptoneuma spireme. Many strands lie side by side.

3. The spireme enters synapsis. In the knot there is evidence of the persistency of the spireme. The chromatin bodies are prominent in the knot.

4. As the spireme emerges from synapsis it is quite slender, and the chromomeres are distinct on it.

5. The spireme shortens and thickens. It contracts away from opposite sides of the nucleus, occupying a broad plane, extending entirely across the nucleus. Its loops do not have any definite arrangement. At the time of the second contraction the spireme splits. The nucleolus at this time is not vacuolate nor does it fragment. It, however, does not stain as deeply as in earlier stages. It disappears suddenly when the chromosomes are being formed, and while the nuclear membrane still persists.

6. After the second contraction the spireme segments. At this time the splitting is plainly seen. The segments shorten and thicken.

7. Prominent hyaline caps in polar position are applied against the nuclear membrane in the heterotypic division. Whether spindle fibres are developed in these caps or not was not determined.

8. The poles of the spindle are broad and blunt. The method of the splitting of the chromosomes at metaphase was not observed. At anaphase they are plainly two-parted. When the chromosomes reach the poles, the cell plate appears as a line in cross-section.

9. The reconstruction of the daughter nuclei takes place very quickly. The nuclear contents have a slight reticular appearance and there is a small nucleolus. The nuclei soon elongate preparatory to the second division. Spindle fibres are conspicuous at the elongated ends.

10. Groups of plastids lie in the cytoplasm. There may be four or more of these groups. Coarse strands of cytoplasm extend from the groups to the nuclear membrane.

11. No centrospheres or centrosomes were found.

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LITERATURE CITED.

- CHAMBERLAIN, CHARLES J. : Mitosis in *Pellia*. Bot. Gaz., xxxvi, 1903, pp. 28-51, Pls. XII-XIV.
DAVIS, BRADLEY MOORE : The Spore Mother-cell of *Anthoceros*. Bot. Gaz., xxviii, 1899, pp. 89-109, Pls. IX, X.
——— : Nuclear Studies on *Pellia*. Ann. Bot., xv, 1901, pp. 147-80, Pls. X, XI.
FARMER, J. BRETLAND : On Spore Formation and Nuclear Division in the Hepaticae. Ann. Bot., ix, 1895, pp. 469-523, Pls. XVI-XVIII.
——— and MOORE : On the Meiotic Phase (Reduction Divisions) in Animals and Plants. Quar. Jour. Micr. Sci., xlviii, 1905, pp. 489-557, Pls. XXXIV-XLI.

- FARMER and REEVES: On the Occurrence of Centrospheres in *Pellia epiphylla*. Ann. Bot., viii, 1894, pp. 219-24, Pl. XIV.
- and SHOVE: On the Structure and Development of the Somatic and Heterotypic Chromosomes of *Tradescantia virginica*. Quart. Jour. Micr. Sci., xlviii, 1905, pp. 559-69, Pls. XLII, XLIII.
- GRÉGOIRE, VICTOR: La formation des gemini hétérotypiques dans les végétaux. La Cellule, xxiv, 1907, pp. 369-420.
- and WYGAERTS: La reconstitution du noyau et la formation des chromosomes dans les cinèses somatiques. Beih. Bot. Centralbl., xiv, 1903, pp. 11-19.
- HOF, A. C.: Histologische Studien und Vegetationspunkt. Bot. Centralbl., lxxvi, 1898, pp. 65-69, 113-18, 166-71, 221-5, Pls. III, IV.
- MANO, THOMAS MARTINS: Nucléole et chromosomes dans le méristème radiculaire de *Solanum tuberosum* et *Phaseolus vulgaris*, La Cellule, xxii, 1904, pp. 57-77, 4 Pls.
- MIYAKE, KÜCHI: Ueber Reductionsteilung in den Pollenmutterzellen einiger Monokotylen. Pringsheim's Jahrb. wiss. Bot., xlii, 1905, pp. 83-120, Pls. III-V.
- NĚMEC, BOHUMIL: Zur Physiologie der Kern- und Zelltheilung. Bot. Centralbl., lxxvii-lxxviii, 1899, pp. 241-51, Text-figs. 1-7.
- OVERTON, JAMES BERTRAM: On the Organization of the Nuclei in the Pollen Mother-cells of Certain Plants, with especial reference to the Permanence of the Chromosomes. Ann. Bot., xxiii, 1909, pp. 19-61, Pls. I-III.
- ROSEN, F. VON: Beiträge zur Kenntnis der Pflanzenzellen. Beitr. Biol. Pflanz., vii, 1895, pp. 225-308, Text-fig. 3, Pls. II, III, IV.
- STRASBURGER, EDUARD: Typische und allotypische Kernteilung. Pringsheim's Jahrb. wiss. Bot., xlii, 1905, pp. 1-70, Pl. I.
- STOUT, A. B.: The Organization of the Chromosomes in *Carex*. Science, N.S., xxxiii, 1911, p. 264.
- VAN HOOK, J. M.: Notes on the Division of the Cell and Nucleus in Liverworts. Bot. Gaz., xxx, 1900, pp. 394-9, Pl. XXIII.
- WAGER, HAROLD: The Nucleolus and Nuclear Division in the Root-apex of *Phaseolus*. Ann. Bot., xviii, 1904, pp. 29-55, Pl. V.
- WINIWARTER, H. VON: Recherches sur l'ovogenèse et l'organogenèse de l'ovaire des Mammifères (Lapin et Homme). Arch. de Biol., xvii, 1900, pp. 33-199.

EXPLANATION OF PLATES LIV AND LV.

All figures were drawn with the aid of a Zeiss camera lucida.

PLATE LIV. Mitosis in Vegetative Cells.

(All the figures, except Figs. 1 and 22, were drawn to the same scale, using a Zeiss microscope, compensating ocular 12, and a 2 mm. homogeneous immersion objective, numerical aperture 1.30, magnification 2,040. Fig. 1 was drawn using the same objective but an 18 Zeiss compensating ocular, magnification 3,250. The same objective was also used for Fig. 22, but an 8 Zeiss compensating ocular, magnification 1,430. The images were all drawn at a plane even with the base of the microscope. The figures are reproduced from the drawings without any reduction.)

Fig. 1. A resting cell. The reticulum in the nucleus is delicate and open. Chromatin knots lie on it. Two large nucleoli are in the nucleus. Several vacuoles lie in the cytoplasm. Cell highly magnified.

Fig. 2. Elongated nucleus. Linin strands have thickened and their number increased. There is also an increase in the number and size of the chromatin bodies.

Figs. 3, 4, 5, 6. The chromatin bodies are becoming distributed on the linin in the form of chromomeres. A spireme is being formed, and it is contracting about the nucleolus.

Figs. 7, 8. The spireme moving away from the nucleolus. It is then very uneven in thickness.

- Figs. 9, 10. Later ; the spireme is of even thickness.
 Figs. 11, 12. Early prophase, showing hyaline kinoplasmic caps at elongated ends of nucleus.
 Figs. 13, 14. Segmented spireme. Nuclear membrane intact. Nucleolus shows no sign of vacuolization. Spindle fibres have appeared in the hyaline caps. A granule lies at the apex of the fibres in Fig. 13.
 Fig. 15. Segments of the spireme are shortening and thickening.
 Figs. 16, 17. Metaphase with the undivided chromosomes.
 Figs. 18, 19, 20. Early anaphase. The oval chromosomes moving towards the poles.
 Fig. 21. Polar view of early anaphase ; at same stage as shown in Fig. 20.
 Fig. 22. Polar view at metaphase, cut slightly obliquely. Eight chromosomes are seen.
 Fig. 23. The chromosomes at the poles elongate. The first indication of a cell plate.
 Fig. 24. The chromosomes at the poles are crowded. The cell plate is more distinct.
 Figs. 25, 26. The chromosomes elongate and their identity is lost in the formation of the reticulum.
 Fig. 27. The daughter nuclei in which there is a fine reticulum. Chromatin granules lying on the linin or free from it. There is a well-defined nucleolus and a definite nuclear membrane.

PLATE LV. Sporogenesis.

Fig. 23 was drawn using a Zeiss oil-immersion, numerical aperture 1.30, and a 6 Zeiss compensating ocular. Figs. 1, 7, 14, 15, 18, 19, and 25 were drawn using the same objective and an 8 compensating ocular, magnification 1,430. Figs. 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 16, 17, 20, 21, 22, 24, and 26 were also drawn using the same objective but a 12 Zeiss compensating ocular, magnification 2,040.

- Fig. 1. A mature spore mother-cell. The resting spherical nucleus has a close reticulum with chromatin knots at its angles. Groups of plastids lie in the cytoplasm.
 Fig. 2. Enlarged nucleus. The masses of chromatin becoming more conspicuous and the reticulum thickening. Parts of the latter may be traced for some distance.
 Fig. 3. Leptonema spireme, some portions of which lie parallel to each other. The chromatin bodies larger than in stage represented in Fig. 2.
 Fig. 4. Synapsis. The chromatin bodies are of equal size and are very conspicuous in the synaptic knot.
 Fig. 5. Recovery from synapsis. The spireme as it emerges from synapsis is very slender.
 Fig. 6. The spireme is distributed throughout the nuclear cavity, and the chromomeres lie at equal distances on it.
 Fig. 7. The spireme somewhat thickened. The chromomeres present a bead-like appearance.
 Figs. 8, 9. The spireme has thickened and is contracted across a broad plane of the nuclear cavity. At this time it shows a split.
 Fig. 10. A segmented spireme showing the split formed at the stage represented in Figs. 8, 9.
 Fig. 11. The segments of the spireme are shortening and thickening. The split is still evident. The nucleolus has disappeared and the nuclear membrane is intact.
 Fig. 12. The chromosomes have become short and thick.
 Fig. 13. Metaphase showing broad, blunt spindle.
 Figs. 14, 15, 16. Anaphase.
 Fig. 17. Late anaphase, showing two parted chromosomes.
 Fig. 18. The chromosomes at the poles are as yet distinct.
 Fig. 19. Reconstruction of daughter nuclei, showing a slight reticular appearance. The nucleolus formed and the chromatin granules present. Indication of the first wall.
 Figs. 20, 21. Daughter nuclei in prophase. Polar caps of spindle fibres very prominent.
 Figs. 22, 24, 25. Hyaline polar caps on the spore mother nucleus.
 Fig. 23. Section showing three of the spore nuclei ; the fourth nucleus and the first wall laid down is in another section.
 Fig. 26. Coarse strands of cytoplasm extend from groups of plastids across the hyaline cap to the nuclear membrane.

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Graham, Margaret. 1913. "Studies in nuclear division of *Preissia commutata*." *Annals of botany* 27, 661–679.

<https://doi.org/10.1093/oxfordjournals.aob.a089482>.

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