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THE ACHROMATIC SPINDLE IN THE SPORE MOTHER CELLS OF OSMUNDA REGALIS.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY.
XXIII.

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(WITH PLATE XXII)

THE cytology of the vascular cryptogams has received scant attention at the hands of botanists, as a glance at the literature of the subject will suffice to show. Calkins (6) has described the divisions in the spore mother cells of *Pteris* and *Adiantum*, reaching the conclusion that in these plants there is a true qualitative reduction of the chromosomes, in Weismann's sense. Quite recently Calkins' view has been combated by Stevens (14), who studied the spore mother cells of *Scolopendrium*, *Cystopteris*, and *Pteris*. Osterhout's (11) article on *Equisetum* is memorable as the first complete account of the formation of a spindle uncontrolled by directive spheres or centrosomes. He found that the appearance of the spindle was heralded by a felt-work of threads about the nucleus, and that the threads later arranged themselves into a multipolar spindle, and afterwards into one characteristically bipolar. These three publications, together with that by Shaw (12) on fertilization in *Onoclea*, and those by Belajeff (2, 3, 4) and Shaw (13) which treat of the origin of the cilia of spermatozoids, make up almost the total of minute research into the cytology of the pteridophytes since the introduction of improved methods of technique.

Impelled by the meagerness of our knowledge, and by the want of accord among such papers as have been published on this subject, the writer two years ago began collecting and examining the sporangia of Lycopodium, Selaginella, and various ferns, to discover some of the forms most favorable for cytological investigation. None was found so good as *Osmunda*. All three of the species occurring in the northern United States were collected, but only *O. regalis* was found to be fixed and preserved in a condition suitable for the purpose, and it alone has received any considerable attention. For want of time a full examination of the reduction divisions was not possible, and therefore attention was centered only upon such features as it was hoped might be followed in detail in the time at disposal. Accordingly in this paper only the origin, structure, and fate of the achromatic spindle are to be considered, together with such comments upon the changes in the chromatin as seem necessary for clearness. A more extensive account of the chromatic elements of the spindle is reserved for a future occasion, after there has been opportunity for further study.

The methods of fixation, embedding, and staining require a brief mention. As fixing agents, chromo-acetic acid (1 per cent. chromic acid, 0.75 per cent. acetic acid), and Flemming's weaker solution were employed. Chloroform was used as the medium of transfer to paraffin. The sections were cut 5μ or 10μ in thickness. To differentiate the fibrillar structures most clearly, various combinations of the following stains were tried: nigrosin, Delafield's haematoxylin, erythrosin, iron-alum-haematoxylin, acid fuchsin, methyl-green, iodine-green, safranin, gentian-violet, orange G. Of these stains the two combinations which have yielded the best results are iodine-green and acid-fuchsin, which was found especially advantageous in dealing with the chromatic elements, and safranin and gentian-violet, which was most effective in bringing out the details of the achromatic figure.

The sporangia of *Osmunda* make their first appearance in the latter part of summer, and continue their growth in the autumn months. By mid autumn those of *O. cinnamomea* and

O. Claytoniana have reached the mother cell stage, in which condition they lie dormant during the winter. The division into spores takes place in the spring, usually in northern Indiana between April 15 and May 1. *O. regalis* does not reach so advanced a condition before winter as do the other species. Cell divisions in the sporogenous tissue occur in the spring, and it is not till after the middle of April that the mother cells are differentiated; the division into spores is effected about three weeks later.

A more exact statement will be instructive. April 6, material collected showed occasional karyokinetic figures in the sporangia. April 21, first collection in which no divisions were found in the sporogenous cells; the tapetal cells were in active multiplication. May 5, many nuclei were in synapsis, the first seen. May 10, most of the nuclei were in synapsis, but some in first division. May 13, in most cases both divisions were completed, and often no trace of the spindle remained.¹ This indicates that the resting and early spirem stages last for about two weeks, and the synapsis stage for three or four days; and that the two divisions are effected in quick succession within a period of two or three days. The difficulty of obtaining the various stages of the second division also affords evidence that the second division follows quickly after the first, and is completed in a much shorter time.

The resting mother cell (*fig. 1*) is angular in form, and presents a rather large nucleus. The cytoplasm is evenly distributed as a delicate network of stainable matter with irregular swellings at the points of intersection; it cannot be regarded as having the structure of foam. The nucleus has a structure similar to that of the cytoplasm, differing only in the greater coarseness of its meshes, the larger size of its granular thickenings, and its more intense reactions with stains. Two or three nucleoli are usually present. In the cytoplasm are numerous deeply staining granules of variable size, each usually lying in an unstained area, as if the granules had suffered contraction in

¹ It should be added that the collection of May 13 was not from the same locality as those of the earlier dates.

the process of fixation. Of the nature and function of these granules, which in staining properties resemble nucleoli, little was learned except that they are not starch. They are not peculiar to any phase of cell division, though rather more numerous in the resting mother cell, or the prophase. They cannot be extra-nuclear nucleoli, for there is no increase or diminution in their size and number contemporaneous with the dissolution or renewal of the nucleoli. Moreover, the nuclear membrane is yet unbroken, when, in the later prophase of division, the nucleoli disappear from view, and therefore, if the nucleolar substance passes out into the cytoplasm at this time, it must do so by osmosis, and any relation between it and the extra-nuclear granules must be conjectural. To the inquiry if they might not be the source, at least in part, of the material of the achromatic spindle, no satisfactory answer can be given; it may be so, but positive evidence is lacking.

The mother cells enlarge for about two weeks after they have reached their full number, gradually separating from one another and becoming more and more rounded in outline. The nuclei also grow larger and pass first into a spirem condition in which the chromatic ribbon is not a single thread but much branched and anastomosed, and later into synapsis. The details of these changes need not be considered in this paper.

Meanwhile, the cytoplasm has retained its reticulate structure. But towards the end of synapsis a change is discernible, and this change is the first indication of the future spindle. It begins as an aggregation of material immediately about the nucleus, causing this region to stain more deeply with gentian-violet. The structure of this accumulation of cytoplasm, when it is first recognizable, is so delicate as absolutely to baffle the powers of the microscope; nothing definite can be said with regard to it, except that it appears to be very finely granular, especially in preparations fixed in Flemming's solution. To a similar layer about the nucleus of the pollen mother cells of *Cobaea scandens*, Lawson (8) proposed to give the name perikaryoplasm, in virtue of its position. Since Strasburger's term

kinoplasm has the advantage of more general acceptance, it will be employed here to designate not only this differentiated layer of cytoplasm but also the spindle forming material in all its modifications.

As the kinoplasm increases in quantity, it begins to assume a somewhat definite outline and texture. It becomes distinctly granular, and the granules are often disposed in short rows which run nearly concentric with the periphery of the nucleus, but are intertangled more or less confusedly (*figs. 2, 4*). In cells which have been fixed in chromo-acetic acid a more distinct fibrous appearance is presented. In some cases the fibers appear to form a loose mat; in others they are so related as to resemble a delicate meshwork with the meshes flattened towards the nucleus. Though the appearance is such as to suggest that the fibers and meshes are only a modification of the cytoplasmic reticulum, the writer was unable to trace the steps of such a transformation. The kinoplasmic material, both in its earliest form and in that assumed after cell division before its final re-transformation into reticulate cytoplasm, could not be distinguished as either reticular or fibrillar, but was very indefinitely granular.

The reader will not fail to discover the similarity of these conditions to those described by Osterhout (**11**), as the preparatory steps of spindle formation in the spore mother cells of *Equisetum*, although in *Equisetum* no layer of granular matter was observed. But the similarity stops here. The spindle in *Osmunda* does not pass through a multipolar stage, nor is there at any time a zone of radiating fibers about the nucleus, such as were seen by Osterhout in *Equisetum*, and by Belajeff (**1**), Mot-tier (**9**), Lawson (**8**), and others in the pollen mother cells of various seed plants. Tripolar spindles, though occasionally met with (*fig. 10*), were of so rare occurrence that their beginning or fate could not be traced, and it is certain they are not normal stages in the development of the spindle.

The changes in the outline of the kinoplasmic mass are very simple and easy to follow. When it has become distinctly fibrillar in structure its fibers, all the time increasing in quantity at

the expense of the outer cytoplasm, begin to collect in greater abundance at opposite sides of the nucleus and to be pushed up into dome shaped prominences. This condition is very well shown in *fig. 3*. The threads of granules run nearly tangential to the nucleus, extending out some distance at the side, and it is obvious even at this time that the axis and the bipolarity of the spindle are already determined. It is rarely the case, however, that both poles are equally prominent from the beginning; commonly one develops considerably in advance of the other. A curious relation between the position of the chromatin in synapsis and the first formed pole of the spindle was observed so frequently that it cannot be regarded as accidental. In elongated cells in which the nucleus is situated excentrically, the first pole of the spindle is formed on that side of the nucleus where there is the greatest amount of cytoplasm, while in synapsis the chromatin was almost invariably observed to be gathered within the nucleus to the side farthest from the center of the cell; that is, the chromatin in synapsis and the kinoplasm are gathered towards opposite ends of the cell. Such a relation between chromatin and kinoplasm may be true of all the mother cells, but it can be traced only in those cells which have the distinction of a long and a short axis, since the polarity of the spindle is not apparent until some time subsequent to synapsis. These peculiarities will be referred to again when the second division is considered. Every care was taken to avoid the illusion into which an observer might be misled by the effects of irregularity of infiltration of the fixing or other reagents, and the conclusion was reached that these relations have a real existence and significance in the living cell.

The writer inadvertently had an excellent opportunity to examine a condition of the cell somewhat resembling synapsis. Finding that the young sporangia, which are ensheathed in a close covering of hairs, did not sink readily in the killing fluid, he first moistened some of them for a moment with alcohol. They sank immediately, but afterwards turned out to be quite unfit for study. It was perfectly easy to trace the path of the

invading alcohol. Both cytoplasm and nucleoplasm were pushed forward; but no one could mistake such a condition for synapsis if he had once become acquainted with the latter. Contrary to what might be looked for, it was the resting cell which suffered most by this treatment; the spindle fibers, the chromosomes, and the spirem thread were not seriously affected.

It will now be necessary to refer briefly to intranuclear changes. The chromatic material, emerging from synapsis, gradually unrolls and extends itself within the nuclear cavity into a much coiled spirem, apparently of one continuous thread, for no ends can be seen in uncut parts of the nucleus. The spirem shortens and thickens, and after a time is segmented into long irregular chromosomes, which continue the shortening and thickening process already begun in the spirem. It is easy to see that many of the chromosomes are split longitudinally into pairs, and the two parts of a pair are often twisted loosely about each other. They take up a peripheral position in the nucleus, being apparently in close contact with the nuclear membrane, The halves usually remain attached to each other for a time, giving rise to Xs and Ys, or loops (as in *fig. 5*), according to the mode of attachment. But while the shortening is still going on, some of the pairs fall apart; otherwise how can the number of chromosomes shown in *fig. 7* be accounted for, if Strasburger's (15) estimate of twelve² be correct? This number has been counted in a few cases in uncut nuclei of the age shown in *fig. 5*. and also in polar views of the late anaphase in which twelve daughter chromosomes have been seen symmetrically grouped about the pole. But in nuclei of the condition shown in *fig. 7* the number of separate chromatin masses is quite variable, fourteen, fifteen, or sixteen being most common, and even as many as twenty have been counted.³ If these numbers indicate a falling apart of some of the chromosome pairs, there must be a subsequent reunion before their arrangement into such an equatorial plate as that shown in *fig. 8*, in which of the twelve chromatic

² See note at the end of the paper.

³ Guignard counted as many as twenty-two.

masses eleven are clearly double. Whether in the case of the larger numbers all the groups represent pairs, or whether some of them are single chromosomes, has not been determined. It is by no means certain, however, that the number of chromosomes in the equatorial plate is so constant as theories of nuclear division which are accepted at the present time require us to assume.

There is a corresponding development of the spindle fibers coincident with the maturation of the chromosomes. The spindle acquires a more distinctly bipolar form, and its fibers, which become coarser and longer, run continuously from pole to pole. During the prophase they have the appearance of knotted cords or strings of loose beads. These features can be seen in *fig. 5*, and also in *fig. 6*, which represents a section of a cell cut sufficiently deep to remove one chromosome and part of the equatorial region of the spindle. But in the metaphase and anaphase the knotted appearance is no longer recognizable. The fibers then appear as stout uniform threads or rods (*figs. 9, 10*). The same statement can be made of the second division (*figs. 17, 18*); that is to say, the fibers at the time when they are functioning in the separation of the chromosomes are of uniform diameter and texture. They are rows of granules at all other times, either when they are disappearing or in process of formation.

It is evident that the achromatic spindle is wholly of cytoplasmic origin. If any nuclear material takes part in its formation, it can do so only after passing through the nuclear membrane. There can be no direct union of linin or other nuclear substance with the kinoplasm, for in nuclei, as far advanced in the prophase as those shown in *figs. 5* and *7*, the chromosomes are the only stainable constituent remaining; no fibers of any kind can be made out, nor any trace of the nucleolus.

A comparison of the shape of the spindle, as seen just before and after the dissolution of the nuclear membrane, suggests that this period is marked by a sudden change. While the nuclear membrane is still present, the spindle has its poles

rounded and ill defined, and its breadth relatively great in comparison with its length. But in all cells from which the nuclear membrane has disappeared, the spindle is seen to be considerably narrower, and at the same time longer and more sharply pointed. To explain these phenomena it seems necessary to suppose that a pressure, exerted by the spindle fibers upon the nucleus, is sustained by the nuclear membrane, and that when the membrane finally gives way, its collapse is attended by a sudden diminution of the diameter of the spindle (*figs. 7, 8*) together with a corresponding increase in length. At the same time, the pressure of the fibers crowds the chromosomes close together. If the fibers be conceived of as curved elastic rods, this action becomes intelligible; that they are rigid enough to exert pressure is probable from the fact that the second spindles frequently cause a widening of the ends of the mother cell, pushing out the wall so as to give it the form in section of a figure 8.

One effect of the sudden collapse of the nucleus is that the chromosomes, which were all necessarily on the inner side of the spindle fibers, are most of them forced to the outside. No distinction of central and mantle fibers was possible, though very great care was exercised at this point of the investigation. It is clear from *fig. 9* that the original fibers, which run meridionally, still remain so after the separation of the chromosomes. Repeated searching under a magnification of 2250 diameters failed to reveal any fibers other than those which run from pole to pole, or any thickening of the fibers towards the poles, as if some portion attached to the chromosome were undergoing contraction. How then are the chromosomes propelled? Inasmuch as there are no fibers discoverable by which they can be pulled, is it not possible that they have a power of motion in themselves?

This hypothesis is not absurd. It is quite as reasonable to assume an automobility of the chromosomes as a contractility of the spindle fibers. The initiatory separation of the chromosomes into pairs by longitudinal fission implies a power of movement

which is entirely independent of external tension; and the same inference is possible from the gradual shortening of the spirem to form the chromosomes, and from the re-expansion of the chromatin in the daughter nuclei.

Meanwhile, the outer region of the cytoplasm loses its reticulate structure (still evident in *figs. 3, 4*), and passing through a condition in which it appears to be a diffusely staining mass becomes converted into granules, as in *figs. 8* and *9*. This change, which is comparable to that preceding the organization of the ktoplasm into fibrillae, advances gradually from the spindle outwards towards the cell wall. Directly after the metaphase the granules of this outer zone arrange themselves into rows stretching from the poles out towards the equatorial region of the cell. This condition is shown in *fig. 9*, and again in the second division in *figs. 17* and *18*. These rows of granules, which soon become more threadlike, will be spoken of as *secondary* fibers, to distinguish them from the primary fibers that form the central framework of the spindle. They are not mantle fibers, for they do not enter into connection with the chromosomes, and are only just coming into existence when the latter begin to withdraw from the equatorial plate towards the poles. The secondary fibers multiply rapidly, and by the end of the anaphase fill all the outer part of the cell; in fact all the cytoplasm seems to have been exhausted in producing them.

Almost immediately there begins a breaking down of the spindle fibers, indicated in the first place by their reassuming the appearance of dotted threads. In all cases the primary spindle is the first to disappear; then the secondary fibers break down and all the stainable cytoplasm is of granular texture (*figs. 11, 13*). A cell plate is first formed, which however comes to nothing; it can be traced through *figs. 12-18, 21*.

Though the second division follows quickly after the first, there is a sufficient interval between to allow the formation of a nuclear membrane and the partial reorganization of the chromatin. The chromosomes after assuming a very symmetrical pattern about the poles unite into a chromatic mass which

takes the shape of a shallow cup with the concavity towards the center of the cell.

If there is any synapsis in the second division, it is represented by the case of *fig. 13*. It will be interesting to compare this with the conditions prevailing at synapsis of the mother cell. In *fig. 13*, as in the synapsis formerly described, the chromatin is bunched on the side of the cell most remote from the greater mass of kinoplasm. In this case it is easy to understand how these conditions have arisen from those preexisting. Whether the relation of the chromatin to the kinoplasm is determined in the mother cell in the same manner, by the position of the last preceding spindle, it is impossible to say, on account of the long rest which the mother cell passes through. If it is so determined, it argues strongly for the organic continuity of the kinoplasm, or at least of some specific substance which retains a definite position in the cell and reacts on the cytoplasm so as to cause it, at the proper time, to be transformed into a fibrillar texture and organize the spindle. It should be remembered, however, that the first visible signs of approaching division in the mother cell are intranuclear, hence so far as we can judge from appearances, if the stimulus to division originates from some special region or structure, and not from the activity of the cell as a unit, we must assume that the primary impetus proceeds from the chromatin.

Apparently the preparations within the nucleus for the second division, and those in the cytoplasm, do not always progress with equal rapidity relatively to each other, for one may meet with cases in which the second spindles are well formed, while the nuclear membrane is still unbroken, and others in which the nuclear membrane is gone and the chromosomes of the second division well organized, while the spindle is still very imperfect. Invariably, however, the development of the second spindle is preceded by an accumulation of material along those borders of the daughter nuclei which were in contact with the old spindle. The fibers first appear in this region also, and the spindle as seen in sections across this part of the cell is bipolar from the

beginning. The poles are not diametrically opposite each other, but both lie on one side of the nucleus, as shown in *fig. 15*¹. Invariably, too, the axes of the second spindle are at right angles to that of the first; that is, though they may be at any angle with reference to each other, they always lie in planes which are parallel to the first cell plate (*figs. 15-18*). In the preparations selected for drawing, the second spindles are either parallel (*figs. 14-17, 21*), or at right angles (*fig. 18*). It must not be understood, however, that these cases are typical in this respect; they were chosen because in them both the spindles are in view at once.

Undoubtedly the material used for the building up of the second spindle is obtained from the disintegration of the first, not as fibers, however, but as granules; and in this abundance of granular matter ready formed in the cell, we may perhaps see an explanation of the rapidity of the second division.

The phenomena attending the metaphase of the second divisions agree completely with those of the first division. There is the same absence of mantle fibers (*figs. 17, 18*), the same continuity of the fibers from pole to pole (*fig. 19*), the same excess of fibers over the number of chromosomes (*figs. 18, 19*). In the anaphase there is the same elongation of the chromosomes into crooked or lobulated rods, the same beaded appearance of the fibers as soon as the chromosomes have passed to the poles (*fig. 21*), and the same development of secondary fibers which run from the poles towards the equator.

The beginning of the secondary fibers is shown in *fig. 19*; they are more abundant and longer in *figs. 20* and *21*, in which it is seen that those put out from the poles of one spindle meet those from the other. The secondary fibers so meeting unite by their ends into continuous threads, which connect the four poles of the primary spindles. In this way four secondary spindles are formed; and thus the four daughter nuclei are now joined by six symmetrically placed spindles, of which two are primary and four secondary. Meantime, there has been a slight rotation of the primary spindles so that their planes, which were at

first parallel to one another, come to lie at an angle of 60° . This results in placing the four daughter nuclei equidistant from one another in the tetrahedral arrangement which is characteristic of fern spores. Whether the rotation is brought about by the action of the secondary fibers, or by some other influence acting on the nuclei, could not be determined. In *fig. 21* two primary spindles are shown, and some of the secondary fibers not yet united by their extremities. In *fig. 22* are one primary spindle (that on the left side) and two secondary; the axes of the other three spindles would make angles of 60° with these or with the plane of the paper.

The disappearance of the spindle has been followed with great care in order to discover, if possible, in what manner the kinoplasm is metamorphosed into ordinary cytoplasm. It was found that the middle fibers of all the spindles are the first to disintegrate, the material being used up in part to build the cell plate. After the spindles are no longer distinguishable, a considerable amount of granular or amorphous matter remains on the inner side of each nucleus (*fig. 24*); finally this disappears and the cytoplasm assumes a reticulate structure throughout (*fig. 25*). All attempts to identify the granules of kinoplasm with microsomata, or the fibrous matter of the spindle with the cytoplasmic reticulations, were unsuccessful. The fibers of the spindle are composed of modified cytoplasm, which in the transformation loses its characteristic structure and becomes first structureless in appearance, then granular, then fibrous, and in returning to its normal condition reverses these steps.

SUMMARY.

The achromatic spindle originates wholly from cytoplasmic material (kinoplasm) which accumulates about the nucleus in the synapsis or spirem stage in the form of an indefinitely granular mass of stainable matter.

The kinoplasm becomes distinctly granular; then the granules arrange themselves into short rows concentric with the nuclear membrane; finally the rows of granules are massed in

greatest abundance on opposite sides of the nucleus, foreshadowing the development of a bipolar spindle.

Usually one pole is formed considerably in advance of the other; and in cells cut parallel to their long axis, it can be seen that the first pole (the greatest accumulation of kinoplasm) is on the side of the nucleus remote from the chromatic mass of synapsis.

The spindle is bipolar from the beginning. Němec's (10) generalization, therefore, that sporogenous cells as compared with vegetative cells are characterized by their spindles passing through a multipolar phase, does not hold good of *Osmunda*.

The fully formed spindle shows no distinction of central and mantle fibers, and no bodies which can be interpreted as centrospheres; all the fibers run from pole to pole.

The dissolution of the nuclear membrane is attended by a sudden narrowing of the spindle and a corresponding increase in length.

During the anaphase new (secondary) fibers, not to be confounded with mantle fibers, are put forth about the poles and meet in the equatorial region of the cell.

In the late anaphase the primary fibers, and soon after them the secondary fibers, begin to disintegrate, taking the appearance of beaded threads, and then of granules; at this time all of the stainable cytoplasm of the cell appears granular in texture.

The spindles of the second division at first have their axes parallel to the first cell plate. They are constructed out of the granular products arising from the disintegration of the first spindle.

The phenomena of the second spindles exactly repeat those of the first, except that four secondary spindles are formed by the union of the secondary fibers put forth during the anaphase.

The primary spindles become rotated about each other so as to bring the four daughter nuclei into the tetrahedral arrangement.

Cell plates are formed across the six spindles (two primary and four secondary), and in connection with them the separating walls of the spores are laid down.

Such a relation between the fibrillae of the kinoplasm and the cytoplasmic reticulum as Blackman (5) reports in *Pinus*, and Lawson (8) in *Cobaea scandens*, could not be verified. Between well developed spindle and cytoplasm are the three stages, (1) dotted fibers, (2) granules, (3) amorphous kinoplasm (structure too delicate for the microscope to reveal). The same phases in reverse order were traced in the first formation of the spindle.

This investigation was conducted in the Hull Botanical Laboratory of the University of Chicago during the spring and summer of 1899. The writer, while assuming full responsibility for the views expressed, takes pleasure in acknowledging his indebtedness to the members of the Botanical Staff for their courtesy and encouragement, and especially to Dr. Bradley M. Davis, under whose more immediate direction the work was undertaken.

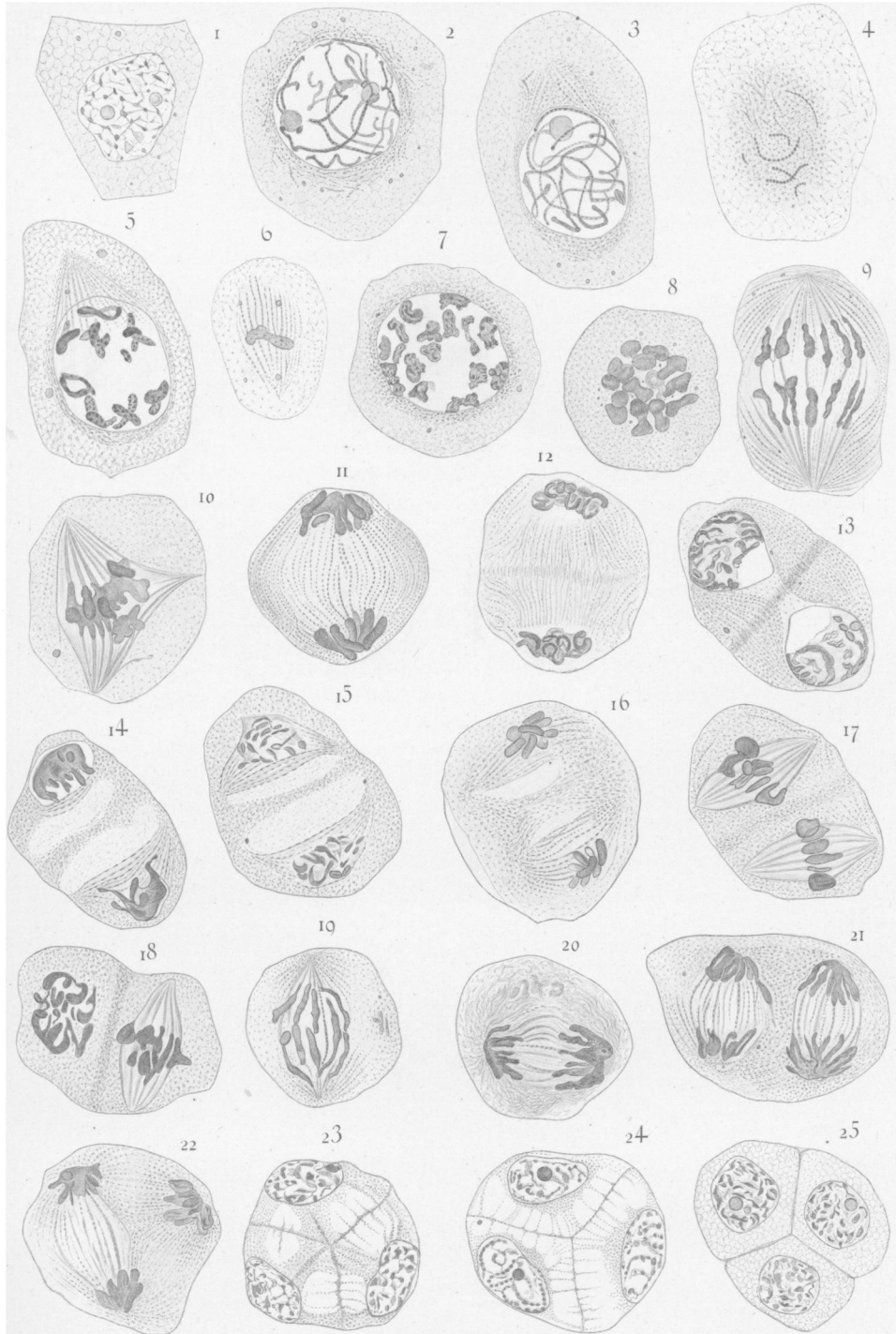
*** Since the foregoing account was written (in February 1900), a comprehensive work on karyokinetic problems (15a) has been issued by Strasburger, in which, among other topics, he discusses the divisions of the spore mother cells of *Osmunda*, and the general formation of achromatic spindles in plants. He distinguishes two types of spindles, those possessing centrosomes and those without such controlling centers. The latter, which are characteristic of higher plants, are again subdivided into multipolar polyarch spindles, such as those of the spore mother cells of *Equisetum*, and multipolar diarch spindles, such as are common in various spermatophyte root-tips; and he seeks to minimize the divergence between these two extreme types of multipolar spindles by instancing numerous intergradations between them. My observations on *Osmunda* accord well with his view; for though I have described the spindle as bipolar from the beginning, its appearance soon after its inception is such that it may very properly be termed multipolar diarch. Strasburger recognizes in the spindle two kinds of fibers, which, having the same origin and reaction to stains, he prefers to call not central and mantle fibers, but supporting and attracting fibers (*Stützfasern* and *Zugfasern*). He pronounces against a power of movement in the chromosomes themselves, and attributes their withdrawal from the equatorial plate to the action of the attraction fibers. This conclusion is quite at variance with that expressed in the preceding pages. To the view that the nucleolus is used up to help complete the achromatic spindle, my observations, though not contradictory, are not altogether favorable; the spindle fibers are too

nearly complete before the disappearance of the nucleolus. In his renewed observations on *Osmunda*, Strasburger has noted the large and varying number (20–22) of chromatic groups in the spore mother cell. Each such group he regards as a chromosome pair. He finds the number of chromosomes in the prothallial cells is not so constantly twelve as he formerly (15) stated; it may reach sixteen or more. It is therefore certain that the current view as to the constancy of chromosome numbers cannot be maintained, at least as regards *Osmunda*.

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EXPLANATION OF PLATE XXII.

The figures were outlined by the aid of an Abbé camera under the magnification given by a Zeiss apochromatic oil immersion lens 2^{mm} aper. 1.30, in combination with a Zeiss compensation ocular no. 8. In all cases the preparations were studied with the higher oculars, 12 and 18.

FIG. 1. Mother cell with resting nucleus.

FIG. 2. Mother cell with nucleus in spirem stage, and the kinoplasm distinctly granular and fibrillar.

FIG. 3. The same, somewhat more advanced, showing the bipolarity of the kinoplasmic mass.

FIG. 4. A section which cuts off only a portion of the nucleus.

FIG. 5. Chromosome stage.

FIG. 6. Portion of a cell, showing part of the equatorial region of the spindle.

FIG. 7. Later chromosome stage.

FIG. 8. Chromosomes in equatorial plate after the disappearance of the nuclear membrane.

FIG. 9. Anaphase, showing continuity of the primary fibers from pole to pole, and secondary fibers extending out from the poles.

FIG. 10. A tripolar spindle.

FIG. 11. Late anaphase.

FIG. 12. Late anaphase.

FIG. 13. Telophase; the primary spindle has quite disappeared.

FIGS. 14-16. Early stages of second division.

FIGS. 17-18. Metaphase of second division.

FIG. 19. Anaphase; secondary fibers beginning to form.

FIGS. 20-21. Late anaphase.

FIG. 22. Late anaphase; one of the primary spindles on the left side; the two other spindles shown have been formed out of the secondary fibers.

FIGS. 23-25. Successive telophases.