Manifestations of polarity in plant cells which apparently are without centrosomes.

By

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With plate XIII.

The existing evidence seems to favor the view that the formation of the karyokinetic spindle occurs in quite a different fashion in the animals than in the higher plants. The most frequently noted and discussed difference in the two cases is the presence of the so-called centrosome in the one and its at least apparent absence in the other. While the existence of the centrosome as a permanent cell organ in the animal cell is by no means universally admitted, the evidence for its occurrence as such in some cases at least seems unquestionable. The presence of the center, as Rabl and Flemming most clearly pointed out, involves a visible polar organization in the cell; even in the resting stages the cell shows a polar organization whose main axis is a straight line passing through the center and the nucleus. When the cell prepares to divide, the centrosome divides first, the two halves separate, and the spindle figure develops about them as centers; from the beginning it is a bipolar figure. At any moment then such cells present a polar, never an isotropic structure. The most thoroughly studied animal cells in general show such an organization as do also many of the lower plants. Amongst the Algae for example *Fucus*[1]) and *Dictyota*[2]) show at least during the processes of division distinct central bodies, and in the apical cells of *Stypocaulon*[3]) centre is also present in the resting stages.

1) Strasburger, E.: Kernteilung und Befruchtung bei *Fucus*. (Jahrb. f. wiss. Bot. Bd. XXX. 1897. p. 351.)

2) Mottier, D. M.: Das Centrosom bei *Dictyota*. (Ber. d. Deut. bot. Gesel. Bd. XVI. 1898. p. 123.) Nuclear and cell division in *Dictyota dichotoma*. (Ann. Bot. Vol. XIV. 1900. p. 163.)

3) Swingle, W. T.: Zur Kenntnis der Kern- und Zellteilung bei den Sphacelariaceen. (Jahrb. f. wiss. Bot. Bd. XXX. 1897. p. 297.)

Amongst the fungi there is a whole series in which centers have been found, and in some of them also the central body stands out plainly in the resting as well as in the dividing cells. Of these *Phyllactinia*[1]) especially deserves mention for in it not only can the central body be traced uninterruptedly from one cell generation to another but its relation to the chromatin can be clearly followed.

For a considerable period[2]) similar relations were described for the higher plants (Phanerogams and vascular cryptogams). However, the investigations concerned were largely led by conceptions which had their origin in the results obtained on the zoological side; they left much to be desired in regard to both perfection of technique and completeness of the observations. It was not until the appearance of the „Bonner Studien"[3]) in 1897 that an approximately complete account of the development of a karyokinetic figure in the higher plants was presented. According to these observations a so-called „resting" cell shows a strictly isotropic structure, neither within the nucleus nor in the cytoplasm is there an organ or even a region which could in any way be compared to a centrosome. As nuclear division approaches, fibers appear in the cytoplasm which are at first arranged radially about the nucleus but later bend over till they form a felt about the nuclear membrane so that it lies as it were in a nest of fibers. Then these fibers draw together more distinctly in cone shaped groups, but even at this time there is no indication as to where the definitive spindle poles are to lie. The bipolar spindle arises from this multipolar stage, as it is called, in that the various cones unite into two which are opposite each other and whose bases rest against each other. After nuclear and cell division is completed there apparently is no longer any trace of a polar organization in the cell, it again presents an isotropic structure.

Our knowledge of spindle formation was further extended by a large number of observers and to most widely separated species of the higher plants with the result that everywhere the process was found essentially the same as described by Osterhout and Mottier. While some of these papers present a carefully worked out, detailed account, of spindle-formation (see for example the work of Mottier on the embryosac of *Lilium*[4]) and that of allen on the

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[1]) Harper, R. A.: Sexual reproduction and the organization of the nucleus in certain mildews. (Carnegie Inst. Washington. 1905.)

[2]) First by Guignard, Sur l'existence des sphères attractives dans les cellules végétales. (Compt. Rend. T. CXII 1891. p. 539.)

[3]) Jahrb. f. wiss. Bot. Bd. XXX. 1897. Especially Osterhout. Über Entstehung der karyokinetischen Spindel bei Equisetum, and Mottier: Beiträge zur Kenntnis der Kernteilung in den Pollenmutterzellen einiger Dikotylen und Monokotylen.

[4]) Mottier, D. M.: Über das Verhalten der Kerne bei der Entwicklung des Embryosacks und die Vorgänge bei der Befruchtung. (Jahrb. f. wiss. Bot. Bd. 31. 1898 .p. 125.)

pollen-mother-cells of *Larix*[1]) it is to be noted that a large number of them come no nearer to presenting a developmental history of the spindle figure carefully worked out on technically adequate preparations than did the earlier centrosome studies on plant cells.

There have also appeared since the „Bonner Studien" a large number of papers in which granules or aggregations of granules are described as lying at the spindle poles in various species of higher plants and considered as of the nature of centrosomes.[2]) The same criticism applies to all of these latter investigations — the content themselves too much with the finding of a granule or granules at the spindle poles during one or several stages of mitosis without tracing the development of these granules and their relation to the formation of the spindle. That however the mere occasional finding of a granule at the poles of the completed spindle or in any other isolated stage of mitosis shows nothing as to the function or nature of that granule has been repeatedly shown.

From the standpoint of our knowledge of the so-called blepharoplast, the question of the existence of central bodies in the higher plants assumes a somewhat different aspect. It has been shown by Belajeff,[3]) Shaw,[4]) and others that blepharoplasts occur quite generally among the vascular cryptogams, and the work of Hirase,[5]) Ikeno,[6]) and Webber[7]) has given us full data as to their presence and structure in the lower Gymnosperms. They appear as well defined organs of the cell although present, as far as known, during but a limited number of cell generations. In their function as ciliaforming organs, the blepharoplasts show a behavior analogous to that of the central bodies of animal cells during the

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[1]) Allen, O. E.: The early stages of spindle-formation in the pollen-mother-celle of Larix. (Ann. Bot. Vol. XVII. 1903. p. 281.)

[2]) See for instance Bernard, Quelques remarques a propos des centres kinétiques. (Jour. de Bot. XIX. 1905. p. 80.) who also gives an extensive list of works belonging in this category.

[3]) Belajeff, W.: Über den Nebenkern in spermatogenen Zellen und die Spermatogenese bei den Farnkräutern. (Ber. d. Deut. bot. Ges. Bd. XV. p. 337.) Über die Spermatogenese bei den Schachtelhalmen. (Ibid. Bd. XVII. p. 399.) Über die Ähnlichkeit einiger Erscheinungen in der Spermatogenese bei Tieren und Pflanzen. (Ibid. p. 342.) Über die Cilienbildner in den spermatogenen Zellen. (Ibid. Bd. XVIII. p. 140.)

[4]) Shaw, W. R.: Über die Blepharoplasten bei Onoclea und Marsilia. (Ber. d. Deut. bot. Ges. Bd. XVI. 1898. p. 177.)

[5]) Hirase, S.: Notes on the attraction-spheres in the pollen-cells of Ginkgo biloba. (Bot. Mag. Tokyo. Vol. VIII. 1894. p. 359); Études sur la fécundation et l'embryogénie du Ginkgo biloba. Jour. Coll. Sci. Imp. Univ. Tokyo. Vol. VIII. Pt. II. 1895. p. 307); Études sur la fécondation et l'embryogénie du Ginkgo biloba. (Ibid. Vol. XII. Pt. II. 1898. p. 103.)

[6]) Ikeno, S.: Zur Kenntnis des sogenannten „centrosomähnlichen" Körpers im Pollenschlauch der Cycadeen. (Flora. Bd. 85. 1898. p. 15.) Untersuchungen über die Entwicklung der Geschlechtsorgane und den Vorgang der Befruchtung bei Cycas revoluta. (Jahrb. f. wiss. Bot. Bd. 32. 1898. p. 557.) Also Jour. Coll. Sci. Imp. Univ. Tokyo Vol. XII p. 152.

[7]) Webber, H. J.: Peculiar structures occuring in the pollen tube of Zamia. (Bot. Gaz. Vol. XXIII. 1897. p. 453.) Spermatogenesis and fecundation of Zamia. (Bur. Plant Ind. Bull. No. 2. 1901. U. S. Dept. Agri.)

metamorphosis of the spermatid. Whether they also play a role analogous to that of the central bodies during nuclear division is perhaps still an open question. Webber concluded[1]) that the blepharoplast takes no part in nuclear division and that it is not the homologue of the centrosome, a conclusion which Strasburger[2]) also reached after reviewing the evidence at hand at the time. Hirase[3]) and Ikeno[4]) on the other hand considered the blepharoplast the homologue of the centrosome although they present no very convincing evidence of its participation in nuclear division. On the other hand, Belajeff[5]) in his observations on the microgametophytes of *Marsilia* traces the blepharoplast through a number of cell generations preceding the antherozoids and finds that they divide preparatory to nuclear division and that the spindle figure develops about the separating halves just as about the central bodies in animal cells. This account of Belajeff's is the most complete we have describing a participation of the blepharoplast in nuclear division. It is to be hoped that further investigation will throw more light on the origin of the blepharoplast and its possible relations to the spindle, both in the vascular cryptogams and in the Cycads.

While the multipolar stage in spindle development has been found to be of wide spread occurrence it appears that its multipolar polyarch origin, to employ Strasburger's terminology, is restricted in general to sporogenous tissues; that in vegetative cells the spindle is diarch from the beginning. Rosen[6]) gave an essentially correct sketch of spindle development in vegatative cells, but it is especially due to Němec's work[7]) that attention has been called to the differences between the mitoses in vegetative and in sporogenous tissues. Strasburger[8]) has however pointed out a series of intermediate types which indicate that there is no such great disparity in the methods of spindle formation in sporogenous and vegetative tissues as Němec at first maintained.

As far as the central bodies are concerned they are no more in evidence in those cells in which the spindle is diarch from the beginning of its appearance than in the sporogenous cells where the spindle has a polyarch origin, so that at present the view is

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[1]) Webber, H. J.: Notes on the fecundation of Zamia and the pollen-tube apparatus of Ginkgo. (Bot. Gaz. Vol. XXIV. 1897. p. 232.) Spermatogenesis and fecundation of Zamia. l. c. p. 77.

[2]) Strasburger, E.: Hist. Beitr. VI p. 185, 1900.

[3]) Hirase: Jour. Coll. Sci. Imp. Univ. Vol. XII p. 103.

[4]) Ikeno: Jahrb. f. wiss. Bot. Bd. 32 p. 571.

[5]) Belajeff, W.: Über die Centrosome in den spermatogenen Zellen. (Ber. d. Deut. bot. Ges. Bd. XVIII. p. 199.)

[6]) Rosen, F.: Beiträge zur Kenntnis der Pflanzenzellen. (Cohn's Beitr. z. Biol. d. Pflanzen. Bd. VII. 1895. p. 225.)

[7]) Němec, B.: Über die Ausbildung der achromatischen Kernteilungsfigur im vegetativen und Fortpflanzungsgewebe der höheren Pflanzen. (Bot. Ctblt. LXXIV. 1898. p. 1.) Zur Physiologie der Kern- und Zellteilung. (Bot. Ctblt. LXXVII. 1899. p. 241.) Über die karyokinetische Kernteilung in der Wurzelspitze von Allium cepa. (Jahrb. f. wiss. Bot. Bd. XXXIII. 1899. p. 313.)

[8]) Strasburger, E.: Hist. Beitr. VI p. 118, 1900.

held quite generally amongst botanists that in the vascular cryptogams and in the Phanerogams the karyokinetic spindle develops without the participation of a centrosome.

It should be mentioned that Guignard,[1]) who more than anyone else has busied himself with the question of the existence of centrosomes in the higher plants, has sought to bring his earlier statements into harmony with the discovery of the multipolar stage in spindle-development by the assumption of multiple centers. In so doing, however, he dispensed with one of the most important characteristics of the centrosomes as they are known in the animal cells: that is, their constant bipartition and the immediate establishment of a bipolarity of the cell as soon as the daughter centers separate.

According to the present most fully developed conception concerning the processes of division in the higher plants, that is, according to the „kinoplasm" theory as advanced by Strasburger and his pupils, we have in the cytoplasmic fibers or rays units which in coordination with one another go through the various movements and transpositions necessary to produce the bipolar spindle. At no time during the whole process do structures appear which could be compared with centrosomes. Not only is nothing to be seen of a central body, but the assumption of its invisible presence is entirely superfluous. The fibers considered as co-ordinated units give just as satisfactory an account of the processes observed in spindle formation as can be given by assuming the presence of a minute granule which in some mysterious manner controls the activities of the fibers.

The question still remains as to how on the kinoplasm hypothesis the fibers so coordinate their activities as to constantly produce a bipolar organisation and consequent bipartition of the mother cell, and further, as to how the process of division with the presence of a central body, whatever its function, as found in the algae and fungi has passed over into the type of division without a central body as found in the higher plants. From this standpoint I have undertaken the studies on nuclear and cell division in *Isoetes* described below.

Leare a wider space between these two paragraphs. The material for this work was collected at Devils Lake, Wisconsin, where *Isoetes lacustris* grows abundantly, putting forth new leaves from the beginning of spring till late in the fall. Of the various fixing fluids in common use, Flemming's stronger chrom-osmium-acetic mixture gave the best results. The material was imbedded and sectioned in the usual way. The sections were stained for the greater part either with Flemming's triple stain or with Heidenhain's iron haematoxylin.

Leaves which have reached a length of about 5 mm show a large number of nuclear and cell divisions and even a hasty survey

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[1]) Guignard, L.: Les centrosomes chez les végétaux. (Compt. Rend. TCXXV. 1897. p. 1148.) Les centres cinétiques chez les végétaux. (Ann. d. Sci. Nat. Bot. 8e Sér. TVI. 1897. p. 177.)

of the preparations suffices to show that the cell contents here present relations which are of the greatest interest with reference to the question of the organisation of the cell. Usually the cells of these young leaves contain starch, frequently in considerable abundance, and one is at once struck by the fact that the starch grains are collected in masses of which there are apparently without exception either one or two in a cell. In preparations treated with the triple stain these starch masses on account of the deep blue of the starch grains present a striking appearance. Equally conspicuous is the fact that the starch masses bear in every case a definite relation to the nucleus and that in resting cells there is without exception only one starch mass present which lies closely pressed against the side of the nucleus, while in the later stages of division there are just as regularly two masses present which in this case are invariably placed at the poles of the spindle and later in polar depressions of the young daughter nuclei. All this is to be observed, as it were, at a glance.

More detailed study shows that the starch-grains lie in a clear space which is surrounded by a more or less distinct boundary. Frequently it appears as though a well defined membrane surrounded the mass of starch grains, a membrane of varying thickness, or, possibly, a membrane of uniform thickness which appears thicker in places because of the irregular deposition of fine particles upon its surface. In general the walls bounding the starch masses appear thicker than those of ordinary vacuoles, in other cases, however, it is almost impossible to distinguish a definite bounding layer. But even in these cases the starch containing area stands out from the rest of the cytoplasm owing to the abrupt ending of the lamellae or strands of the cytoplasm at the surface of this areas. Frequently, too, if much starch is present, the outlines of the enveloping layer are clearly indicated by the position of the outermost grains since the group of crowded starch grains closely follows the contours of the envelop which holds them together. (Fig. 2.)

If the contents of these starch bodies is examined more closely, it appears that, in many cases at least, it does not consist entirely of starch grains but that in addition to these there are strands (which stain blue-black with the triple stain) of varying thickness extending between the starch grains and between these and the periphery of the body. There also appear to be small granules taking the red stain scattered between the starch grains, they cannot, however, be distinguished with certainty from minute starch grains in preparations stained with the triple stain. The presence of small granules other than starch can, however, be easily demonstrated in iron-haematoxylin-preparations. In these the starch grains are almost colorless, but the other granules take a deep black stain so that they stand out sharply in the otherwise clear space.

These starch-containing bodies appear in varying forms and it is noticeable at once that their starch content fluctuates. This has its explanantion partly in the fact that different leaves vary in the amount of starch they carry, and furthermore, that the starch is not uniformly distributed throughout a whole leaf. Not rarely there are cells which contain no starch at all, but in them this peculiar body is none the less present. It is merely not so conspicuous as when it contains deeply staining starch grains. While these variations in the starch content of different leaves make difficult a final judgment as to whether the amount of starch in any particular cell varies with its condition of development, it seems none the less as if the starch content of the individual cells undergoes periodical changes parallel with the karyokinetic cycle.

More important than the variations in the amount of starch contained in these structures are the changes in shape and in position which they undergo and which upon closer examination are seen to be in intimate relation with the processes of nuclear division. As was stated above, there are invariably either one or two of these structures in a cell, and examination shows that whether one or two are present depends upon the condition of the cell with respect to division.

Immediately after cell division, each daughter cell invariably contains a single starch mass, and this condition continues also in the completely reconstructed and so-called „resting" cell. In such cells the starch-body lies close to one side of the nucleus, in most cases presses into it so that the nucleus is deeply indented. (Fig. 1.) The shape of the starch body varies considerably. Frequently it is fairly well rounded, but at other times is more elongated as in Fig. 1, here it is seen extending out from the polar depression in the nucleus, bending to one side and running along closely pressed to the nucleus for some distance. By focusing deeper down into the section it can be seen that the starch body also extends beyond the polar depression of the nucleus on its under side, spreading out somewhat into the cytoplasm. In this figure, as always, the polar indentation in the nucleus is on the side opposite the newly formed cell wall.

In the young leaves of *Isoetes* cell divisions follow rapidly one upon the other so that there is some difficulty in deciding whether a cell is in a perfectly „resting" condition or whether the prophases of the next division are already in progress. The most characteristic indication of an approaching cell division is that the single starch body of the „resting" cell divides into two. Stages showing the division of this body are sharply marked and not difficult to find. The body elongates and at the same time it constricts in its middle portion until finally it separates into two. While this is going on the starch body maintains its close contact with the nucleus. In fact it is so pressed into the nucleus that a furrow is formed in the surface of the latter through which the separating halves of the starch body draw apart, at the same time the nucleus is also elongated in the direction of the separation of the daughter starch bodies. (Fig. 2.) The chromatin at this stage is still finely distributed through the nuclear cavity, at the edges of the furrow, however, the granules are more closely crowded than elsewhere in the nucleus, a distribution such as would result from the local indentation of a plastic vesicle which contains uniformly distributed particles.

The starch body does not always press deeply into the resting nucleus, sometimes it merely lies closely against it without indenting it, and in these cases when the starch body divides the two separating halves merely move along the surface of the nucleus. These cases are rarer, however, than those in which the nucleus is furrowed by the separating daughter starch bodies.

After the starch body has completely divided into two, the halves withdraw from each other until they lie at opposite poles of the elongated nucleus, close against its membrane, at the same time they change their shape or their position so that their long axes now are at right angles to that of the nucleus (figs. 2 & 3). Frequently at this stage the cytoplasm between the starch bodies and the plasma membrane is more vacuolated and clearer than elsewhere in the cell. At about this time the first indications of an approaching division become apparent in the nucleus itself, the chromatin begins to collect into larger aggregations. Frequently a more or less diagonal furrow which was formed by the starch body pressing into the nucleus in the earlier stages is still to be seen at this stage. (Fig. 3.) Up to this time the chromatin appears in the finely divided condition characteristic of resting nuclei and it is plain that we have in these cells a long series of visible changes preparatory to cell division before the appearance of the chromatin gives any indication of the beginning of the prophases; neither are there any fibers or rays visible in the cytoplasm up to this time.

As the chromatin passes into the spirem stage[1]), the polar structures leave the nuclear membrane, moving further apart from each other in opposite directions until frequently they lie close to the plasma-membrane. As they do so they become still more flattened and irregular in outline. This irregularity of outline is worthy of note for it is a further indication that the polar structure is not a vacuole whose form is determined by surface tension. As the polar structures move away from the nucleus, the cytoplasm between them and the plasma membrane assumes a denser appearance; if the amount of cytoplasm here present is small it has this denser appearance throughout its extent. It almost looks as if the polar structures in moving away from the nucleus had pushed the cytoplasm ahead of them and thus crowded its elements

[1]) The formation of a continuous spirem has been questioned in recent years. See Grégoire and Wygaerts who worked on Trillium. La Cellule (T. XXI. p. 5, 1904, and J. Kowalski who worked on salamander larvae in Grégoire's laboratory. La Cellule (T. XXI. p. 349.) In the young leaf-cells of Isoetes a continuous spirem seems to be formed. I have not, however, devoted any special attention to determine whether possibly there are breaks in the apparently continuous spirem.

closer together. These conditions are still more clearly shown in cells in which a more considerable space remains between the polar structures and the plasma membrane as frequently happens in cells which are elongated more than usual. In these cases the cytoplasm next to the outer boundary of the polar structures appears as a dense almost finely granular mass, further outwards towards the plasma membrane its structure becomes looser. Not rarely this denser part of the cytoplasm is fairly sharply bounded, resting on the polar structure like a more or less crescent shaped mass. The strands or lamellae of the less dense adjacent cytoplasm extend out from this denser mass to the cell periphery in a more or less radial arrangement. The appearances just described are especially well brought out in preparations stained in iron-haematoxylin. The dense cytoplasmic mass stains more intensely than the surrounding cytoplasm and strongly suggests on archoplasmic region such as has been figured for various animal cells.[1])

When the polar structures have reached their definitive positions or even as they were moving away from the nucleus, the spindle fibers begin to appear, and it is to be observed that without exception they extend between the polar structures and the adjacent surface of the nucleus. In the earlier stages of spindle formation the fibers present the appearance of a tangled network. Whether the fibers actually branch and anastomose or whether the appearance is merely due to an intertwining of sinuous but unbranched fibers may be left undecided for the present. While the spindle-fibers are developing the nucleus changes its shape. It expands laterally and at the same time shortens in the direction of its former long axis so that finally its long axis is perpendicular to that of the cell. The nucleus no longer has the smooth, regular, ellipsoidal outline it possessed in the earlier stages but shows a shallow indentation at the places where the spindle fibers come in contact with it. The appearance strongly suggests a pressing in of the nuclear membrane on the part of the spindle fibers. Whether such is actually the case I have not determined.

Simultaneously with these changes in the shape of the nucleus its membrane begins to disappear. The stages in the disappearance of the membrane are not easily followed since the nuclear membrane is never very conspicuous in these cells. However, to all appearances it seems evident that the membrane first disappears at the points where the spindle fibers come in contact with it. (Fig. 4) The chromosomes at this time lie, more or less wound about each other, fairly uniformly distributed through the nuclear space.

The spindle fibers begin to stand out more sharply now, assume a straighter course, and approach each other more closely at their distal ends until finally an exceedingly sharp pointed spindle

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[1]) Hermann, F.: Beiträge zur Lehre von der Entstehung der karyokinetischen Spindel. (Arch. f. mikr. Anat. Bd. XXXVII p. 569. See figs. 3.5. etc. Pl. XXXI.)

results, the ends being drawn out as it were to needle points. The polar starch-bodies round up more and more so that by the time the chromosomes are arranged on the equatorial plate they have once more a more or less irregular spherical shape. The sharp spindle poles press into these irregularly spherical polar structures producing an appearance not unlike that obtained when a pointed body presses into a mass of stiff dough. (Fig. 5.) We are here dealing with an actual indentation of the surface of the rounded polar structure and not with an appearance such as is sometimes met with in the astrospheres of animal cells. Here namely, if the spindle-ends extend into the astrosphere a part of the sphere is replaced by them though a superficial observation might give the impression that here too the spindle indents the sphere. This is especially true of those cases in which there are differences in the staining reactions of sphere-rays and spindle fibers, or where the spindle fibers are closely packed and distinctly fibrous while the sphere has more of a granular appearance. It requires but a glance, however, to show that in these cases the appearance is due to the absence of a sector of the astral rays and their replacement by the spindle fibers, and not to an indentation of the surface of the sphere.

The completed spindle of the young leaf-cells of *Isoetes* is made up of numerous fibers. They are delicate, unusually clean cut, and closely packed; the result is a spindle of sharp outline which stands out in strong contrast to the pale cytoplasm surrounding it. In fact, the surrounding cytoplasam at this stage seems to consist of little but watery cell-sap; the strands or lamellae of its more solid constituents are delicate and widely separated. The relation between the spindle and the surrounding cytoplasm is only imperfectly represented in Fig. 5, the spindle should be considerably darker in comparison to the surrounding cytoplasm. It is to be noted that there is a definitely fixed relation between the position of the polar structures and the axis of the spindle. Without exception the spindle lies so that its ends press into approximately the middle of the polar structures. This invariable relation stands out with especial clearness when, as is not seldom the case, the polar structures lie at diagonally opposite corners of the cell. In these cases the spindle also lies diagonally so that here as always its ends indent the approximate centers of the polar structures. Occasionally it happens that the spindle figure lies far over at one side of an unusually broad cell with the spindle axis parallel to the side of the cell, and in these cases the polar structures also show the same degree of displacement towards the side of the cell. In this connection Strasburger's conception[1]) of the anchoring of the spindle figure in those cases in which its poles do not reach to the plasma membrane comes to mind. He assumes that the dense trophoplasmic layer which frequently surrounds the spindle figure in these cases and into which the spindle-poles extend

[1]) Hist. Beitr. VI p. 152.

represents a provision for the anchoring of the spindle. If such is the case, it is not impossible that the polar structures of *Isoetes* exercise a similar function in the later stages of nuclear division. But it is to be noted that the cells here for the most part are not especially long in proportion to the size of the spindle, so that in most cases the spindles might easily reach from one wall to the other.

The passage of the chromosomes from the equatorial plate to the poles seems to progress more slowly in the young leaf-cells of *Isoetes* than it does in many other cases so that a large number of intermediate stages between early and late anaphases are met with.

The spindle-poles persist till a late stage so that when the chromosomes are closely packed together in the diaster, and it seems as if the new daughter nuclear membrane were about to appear, the two spindle poles are still sharply defined. They are no longer composed of fine clean-cut fibers, but show a finely granular structure, and appear almost homogeneous. They also have changed in their staining qualities. At the equatorial plate stage the spindle stains blue (with the triple stain) now, howewer, its remaining ends take a yellow to a reddish color. The shape of the spindle ends has also changed as a comparison of figs. 5 and 6 shows.

Sharply defined spindle-poles in the later stages of nuclear division are not common in plant cells. Rosen[1]) figures one case for *Psilotum* and Osterhout[2]) does the same for *Equisetum;* in *Fuligo*[3]) they seem to be of regular occurrence. Also in the pollen-mother cells of *Nymphaea*, the spindle-poles remain intact for a longer time[4]) but instead of their sides including a wider and wider angle as division progresses as in the case in *Isoetes* the remaining spindle ends become thinner and thinner. In animal cells it is not so rare to find the spindle ends persisting through the later anaphases.

As at the time of the equatorial plate stage, the spindle-poles continue during the anaphases to press into the polar structures. These have changed little, possibly they have become slightly flattened in the direction perpendicular to the spindle axis. Occasionally a starch grain lies exactly at one or at both of the sharply pointed spindle-poles, this, however, to all appearances is not at all a constant occurrence. As the chromosomes go back to the poles well developed connecting fibers appear between the chromosome groups, later they are seen to extend also to the sides of the chromosomes, ending at the polar structures. They are delicate, sharply defined fibers which stain blue in the triple stain. In the equatorial region they are crowded together in groups and more or less wound about each other while nearer the chromosomes

[1]) Beiträge zur Kenntnis der Pflanzenzellen. (Cohn's Beitr. z. Biol. d. Pflanzen. Bd. VII. Taf. IV fig. 4.)

[2]) Jahrb. wiss. Bot. Bd. XXX, Taf. II, Fig. 18.

[3]) Harper, R. A.: Cell and nuclear division in Fuligo varians. (Bot. Gaz. Vol. XXX. 1900. p 217.)

[4]) See Strasburger: Hist. Beitr. VI. Pl. IV, figs. 170 and 171.

they are more evenly distributed, as a result the figure appears lighter in the middle than at the ends. The cell from which figure 6 is taken shows in addition to this a narrow light streak across the equatorial region of the connecting fibers which is not reproduced in the figure. Whether this light streak is a regular occurrence in connection with cell-plate-formation and what its relation is to Timberlake's[1]) „orange zone" I have not determined.

When the chromosomes have about reached the poles and are crowding closer and closer together they accupy a space which in section is approximately kidney shaped, the concave side facing the pole. Soon the new nuclear membrane appears, the daughter nuclei steadily increasing in size during this period. The chromatin for a time appears to be arranged in a more or less spiral fashion, the irregular contours of the strands indicate that it is in process of being distributed through the nuclear cavity. (Fig. 7.) The nucleoles also make their appearance at this stage. The first indication of their presence is the appearance of from one to several faintly staining masses lying between the material of the chromosomes. At first the boundaries of these masses are indistinct gradually passing over into the clear nuclear sap, later however, they become more and more definite, and at the same time the affinity of the material of the masses for stains increases until finally completely formed nucleoles are present.

During these changes in the daughter nuclei the polar structures have considerably increased in size. Frequently at this time the starch contained in them stains with especial intensity so that the whole cell with its violet chromatin, blue gray connecting fibers, and deep blue starch grains presents a striking appearance.

As was previously mentioned the content of any particular starch body seems to fluctuate with the stages of cell-division, but it is difficult to decide with certainty whether there is more starch in a cell at any particular stage of mitosis than at another because of the great individual variations between cells in regard to their starch content. The indications are, however, that the starch decreases in amount during the prophases and that that which is present comes to stain less intensely. This condition continues until into the telophases when the starch grains again appear to become more numerous, and also to take a deeper stain. That such a periodic fluctuation in the starch content of the polar structures actually exists is made probable by the fact that in examining a large number of preparations it is only here and there that a cell appears which shows abundant starch in the anaphases or in the late prophases at the same time one frequently finds cells in the telophases whose polar structures are closely packed with starch. This observation would be fairly convincing as to a consumption of starch during spindle formation, possibly also during the time that the chromosomes are passing to the poles, and its

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[1]) Timberlake, H. G: The development and function of the cell plate in higher plants. (Bot. Gaz. Vol. XXX. 1900. p. 97.)

accummulation in the intervals between successive divisions if it were not for the fact that here and there a cell is found which is well supplied with starch in the late prophases or anaphases. It is possible that such cells possessed a superabundance of starch so that although a part was used during mitosis a large amount still remained, but as far as my observations go at present nothing can be said about this with certainty.

It should be mentioned, too, that now and then one or several starch grains are found lying outside of the polar structures in the cytoplasm. These are isolated cases and seem to have no particular significance.

The kidney shape assumed by the daughter nuclei at the time of their formation persists for a considerable period. Frequently it is still present when the new nuclear membrane has been completely formed and the chromatin quite uniformly distributed through the nuclear space. As is well known, the nuclei of animal cells frequently show during the telophases and even later a marked indentation on their polar side; in this indentation or just opposite it the central body lies. This relation is duplicated in the cells of the young leaves of *Isoetes* only that in this case the position of the central bodies is occupied by the polar starch masses. Usually they lie closely pressed to the nucleus completely filling its indentation. The cell represented in figure 7 has the upper polar structure developed just as well as the lower only the greater part of it lies in the next section.

Cell division takes place according to the method characteristic of the higher plants. A well developed spindle is formed, which, as it spreads out more and more laterally, is distinctly fibrous only at the periphery, its interior already shows the structure which the cytoplasm of the so-called „resting“ cell presents. The polar structures which till now had the shape of a more or less flattened and irregular sphere become more elongated and frequently press deeply into the nucleus. The nucleus has been increasing in size all this time and the indentation in it may be due either to its growing up around the polar structure, or to an active pressing into it on the part of the polar structure. With this the cell has passed once more into the typical resting condition. Before entering into a discussion of the more general bearings of the observations just described upon the problems of nuclear and cell-division it will be well to see to what extent structures similar to the polar structures of *Isoetes* occur in other plants.

As early as 1839 Von Mohl[1]) described for the spore-mother-cells of *Anthoceros* a green colored, starch-containing body (chromatophore) lying at one side of the nucleus. This chromatophore divides in the middle, and then each half again divides so that there are four of them placed about the nucleus at about

[1]) Von Mohl, H: Über die Entwickelung der Sporen von Anthoceros laevis. (Verm. Schrftn. p. 84.)

equal distances from each other. Nägeli[1]) in 1844 confirmed these observations. Both Von Mohl and Nägeli show fibers in their figures which represent spindle or connecting-fibers, neither of them, howewer, recognized their significance for nuclear or cell division. Strasburger,[2]) in 1880, for the first time described nuclear division in the spore-mother-cells of *Anthoceros* and he ascertained that the first karyokinetic figure is so oriented that each of the spindle-poles lies midway between two chromatophores, nuclear division not commencing until the chromatophore has divided twice in succession and the four daughter chromatophores have taken their position about the nucleus. He does not directly describe the position of the spindles of the second division but judging from his statements regarding the positions of the four tertiary nuclei it is to be concluded that the poles of the second spindles are directed each upon one of the four chromatophores. As far as the chromatophores themselves are concerned Strasburger's observation confirm the earlier accounts of Von Mohl and Nägeli. Strasburger also describes the connecting fibers in the spore-mother-cells of *Anthoceros* as extending between the chloroplasts, not between the nuclei, and points out that this is an exceptional occurrence. Davis[3]) in 1899 published a paper on the spore-mother-cells of *Anthoceros* in which, however, he does not materially extend the observations of the earlier workers as far as the chromatophores are concerned, neither does he mention having observed any relation between the position of the spindle and the position of the chromatophores. Van Hook[4]) also made some observations on cell division in *Anthoceros* He gives an interesting figure[5]) of a cell-plate stage showing numerous connecting fibers passing around the relatively small nucleus on all sides and centered upon the chloroplasts. Van Hook does not enter into a description of spindle formation and nuclear division.

The macrospore mother cells of various *Isoetes* species show conditions which resemble those in the spore mother cells of *Anthoceros*. Tschistiakoff,[6]) and Strasburger[7]) were the first to call attention to the structure of these cells and they were later more fully investigated by Fitting.[8]) Fitting found a dark mass consisting of starch grains and coarsely granular plasm lying against one side of the still undivided nucleus of the macrospore-

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[1]) Nägeli, C.: Zellkerne, Zellbildung und Zellwachstum bei den Pflanzen. (Ztschft. f. wiss. Bot. Bd. I. 1844. p 49.)

[2]) Strasburger, E: Zellbildung und Zellteilung. 3. Aufl. Jena 1880.

[3]) Davis, B. M: The spore mother cell of Anthoceros. (Bot. Gaz. Vol. XXVIII. 1899. p. 89.)

[4]) Van Hook, J. M.: Notes on the division of the cell and nucleus in liverworts. (Bot. Gaz. Vol. XXX. 1900. p. 394.)

[5]) l. c. Pl. XXIII. Fig. 13.

[6]) Tschistiakoff, J.: Beiträge zur Physiologie der Pflanzenzelle. (Bot. Ztg. Bd. 33. 1875. p. 1.)

[7]) l. c.

[8]) Fitting, H.: Bau- und Entwickelungsgeschichte der Makrosporen von Isoetes und Selaginella und ihre Bedeutung usw. (Bot. Ztg. Bd. 58. 1900. p. 107.)

mother-cell. Rays which extend almost to the cell periphery center upon this mass which elongates and finally constricts in the middle. The daughter masses separate from each other until they lie almost at the foci of the ellipsoidal mother cell. Arrived here they once more elongate, this time in two planes at right angles to each other and to the plane of the first elongation. Constriction in the middle again follows this elongation so that the mother cell now contains four such masses arranged in the form of a tetrahedron about the nucleus and surrounded on all sides by protoplasmic rays. Now the nucleus begins to divide. Although Fitting's material did not suffice for a detailed study of mitosis, he made the important observation that the poles of the first spindle lie respectively in the middle between two of the starch masses, apparently between two sister masses. The spindles of the second division are perpendicular to each other, the poles lie close beside one each of the four starch masses but they never end directly in a starch mass, always lying a little to one side of them and entirely free in the cytoplasm. Fitting also briefly indicates the theoretical interest of the described observations without going further, however, than to point out that in certain cases even amongst the Archegoniates cell division may be initiated by a division of the cytoplasm and the included reserve materials; and that the conditons as they are found in the macrospore-mother-cells of *Isoetes* deserve to be taken into account by the students of cell-mechanics. Fitting worked with preference on living material, which has its undeniable advantage, at the same time it is to be hoped that microtome sections prepared after successful fixation (which here meets with considerable difficulties) will show an abundance of additional important details.

The behavior of the chloroplasts in *Coleochaete* is also of interest. According to Oltmanns[1]) there is a single chloroplast lying at the basal end of the unfertilized oosphere. After the entrance of the male cell (which does not contain a chloroplast) the chloroplast divides, the halves taking position at opposite sides of the fusing sex nuclei. The division of the chloroplast is twice repeated so that finally there are eight of them in the oospore. Now nuclear division begins; usually there are three successive divisions of the nucleus resulting in the formation of eight nuclei. Cell division follows, producing eight carpospores, each of which contains one nucleus and one chloroplast. Aside from the fact that cell division occurs so that finally each carpospore contains one chloroplast there are no statements as to the relations between the chloroplasts and the karyokinetic figures. In his figure 7 Oltmanns shows two cells, apparently belonging to an antheridial branch, whose nuclei are in the equatorial plate stage. A chloroplast lies opposite each spindle pole; however, from this fact alone it is not possible to conclude much regarding the relations

[1]) Oltmanns, Fr.: Die Entwickelung der Sexualorgane bei Coleochaete pulvinata. (Flora Bd. 85. 1898. p. 1.)

between the chloroplasts and spindles for the cells are long and narrow so that the chloroplasts must necessarily occupy this position if each daughter cell is to receive one of them.

The chloroplasts of the epidermal assimilatory cells of the foliage leaves of *Selaginella* investigated by Haberlandt[1]) are also of interest in this connection. These cells usually have a single, large, bowl-shaped chloroplast placed at one end of the cell with its concavity in which the nucleus lies turned towards the interior of the cell. Unfortunately there are no data concerning the behavior of this chloroplast during cell division.

An observation of Rosen's[2]) on the root cap cells of *Oleandra nodosa* should also be mentioned here. He found in these cells two more or less kidney shaped bodies lying close to the nucleus, at opposite sides of it. Rosen regarded these bodies as possibly tannin masses; he gives no information concerning their behavior during nuclear division.

As relatively large bodies in the cytoplasm which undergo definite changes of position during cell division, the polar structures of *Isoetes* call to mind some observations of Conklin's[3]) on the dividing eggs off various gasteropods and ascidians. Conklin finds a differentiated mass, the sphere substance, lying at one side of the nucleus distinguished in color and density from the remaining cytoplasm, and derived from the polar material of preceding divisions. For a few cleavages this mass divides into two and the halves migrate to the spindle poles so that they are distributed to the daughter cells. In later cleavages, however, these masses in the cytoplasm do not divide as the cell divides and some of the daughter cells receive the entire mass while others receive none. In this way a visible differentiation of the cells in the early cleavage stages is brought about, a differentiation which is associated with the later differentiation of tissues in the embryo. It seems that these masses of sphere substance are not definitely bounded cell organs, but merely aggregations of material of different composition than the remainder of the cytoplasm.

Finally, the elaioplasts should perhaps be mentioned. The elaïoplasts are cell-organs presumptively concerned with the production of oily substances and according to Zimmermann[4]) are of wide spread occurrence in the plant kingdom. Usually, although there are exceptions, there is a single elaioplast in a cell, lying

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[1]) Haberlandt: Die Chlorophyllkörper der Selaginellen. (Flora. 1888. p. 291.) Über die Plasmahaut der Chloroplasten in den Assimilationszellen von Selaginella Martensii Spring. (Ber. d. Deut. bot. Ges. Bd. XXIII. 1905. p. 441.)

[2]) Beiträge zur Kenntnis der Pflanzenzelle. (Cohn's Beitr. z. Biol. d. Pflanzen. Bd. VII. Pl. III. Fig. 18.)

[3]) Conklin, E.: Karyokinesis and cytokinesis in the maturation, fertilization, and cleavage of Crepidula and other gasteropoda. (Jour. Acad. Nat. Sci. of Phila. 2nd. Ser. Vol. 12. Pt. I. 1902.) The origin and cell-lineage of the Ascidian egg. (Ibid. Vol. 13. Pt. I. 1905.)

[4]) Zimmermann, A.: Über die Elaioplasten. (Beitr. z. Morph. u. Physiol. d. Pflanzenzelle. 1893. p. 185.) Elaioplasten, Elaiosphaeren und verwandte Körper. (Beihefte z. bot. Ztblt. Bd. 4. 1894. p. 165.)

closely against the nucleus, in some cases at one end of the somewhat elongated nucleus. Frequently the elaioplast lies so close to the nucleus as to deform it. There are few data relative to the behavior of the elaioplasts during nuclear division. According to Raciborski[1]) they play no part whatever in it; he also maintains that they are formed de novo in the cytoplasm.

I may note in this connection a polar organization during synapsis which I have observed in two other of the higher Pteridophytes and which requires further study. If spore-mother-cells of *Equisetum hyemale* are examined during the synapsis stages it is seen that a more or less sharply bounded mass lies in the cytoplasm at one side of the eccentrically placed nucleus. This mass consists of numerous closely packed granules, largely starch, of varying sizes which frequently show an affinity for orange if stained with the triple stain. Upon this mass well developed cytoplasmic rays are centered (fig. 8) so that the figure has a striking resemblance to an archoplasmic region with radiations proceeding from it, as known for various animal cells.[2]) The spore mother cells of *Equisetum* are usually distinctly elongated at the time of synapsis, the nucleus almost invariably lying at one end of the cell. The chromatin is aggregated at the side of the nucleus nearest the cell wall. Just as regularly the dense aggregation in the cytoplasm lies on the side of the nucleus opposite the synaptic mass. These relations stand out strikingly in cells which, after fixation in osmic acid mixtures, are teased out of the spore sacs and examined whole. The relation is just the reverse of that obtaining between synaptic mass and centrosome in animal cells. Here, as has been frequently figured,[3]) the chromatin is aggregated on the side of the nucleus next to the centrosome.

I have observed a similar definite orientation of the synaptic mass in the spore mother cells of *Marsilia quadrifolia.* These cells also show a more or less sharply defined dense mass in the cytoplasm at one side of the nucleus consisting largely of small starch grains, and here the chromatin is regularly aggregated on the side of the nucleus adjacent to the mass of starch grains. (Fig. 9.) I have not worked out in detail the further history of these accumulations in either *Equisetum* or *Marsilia* and am not in a position to say whether they are related to each other or what their relation is, if any, to the polar structures of *Isoetes.*

But it is not only in these more or less isolated cases that we have evidence of a polar organisation of the higher plant cells. All the higher plants during at least one stage of their life history show such an organisation of their cells before any

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[1]) Raciborski, M.: Über die Entwickelungsgeschichte der Elaioplasten bei Liliaceen. (Bull. inter. d. l'acad. d. Sci. de. Cracovie. 1893. p. 259.)

[2]) Hermann: Arch. mikr. Anat. Bd. XXXVII. Pl. XXXI.

[3]) See for example Farmer, J. B. and Moore, J. E. S. The maiotic phase in animals and plants. (Quart. Jour. Micros. Sci. Vol. 48. Pl. XL Fig. 72) and Schreiner, A. und K. E. Über die Entwickelung der männlichen Geschlechtszellen von Myxine glutinosa. (Arch. de. Biol. T. XXI. Figs. 52, 71, 170 etc.)

indications of spindle formation are present, that is, during synapsis. During synapsis the chromatin collects at one side of the nucleus and thus for the time being brings about a visible polar organization of the cell. In animal cells the chromatin aggregates on the side of the nucleus next to the central body; according to Harper the same is also true of *Phyllactinia*.[1]) For the higher plants no explanation has as yet been offered as to what determines at which side of the nucleus the chromatin shall aggregate. That the position of the synaptic mass is not determined by gravity is seen at once by examining any spore sac whose spore-mother-cells are in synapsis.

The fact that a conspicuous polar organization of the cells of higher plants appears at or about at the time of synapsis, at a time when the plant is returning to a one celled condition, suggests that this may be a striking back to an ancestral condition.

Further evidence must be had as to whether the bodies in the cytoplasm described in any of the cases just mentioned bear any such definite and constant relation to the formation and position of the spindle as do the polar structures in the vegetative cells of *Isoetes*. The conditions described for *Equisetum* and *Marsilia* during synapsis suggest the existence of a definite polar organization in the cells of these plants, and Strasburger's observations on *Anthoceros* and Fitting's on the macrospore-mother-cells of *Isoetes* point to a similar condition in these cases also. It is highly desirable that a re-investigation of the spore-mother cells as well as the vegetative cells of *Anthoceros* and likewise of the epidermal assimilatory cells of *Selaginella* be undertaken from this standpoint.

The large chromatophores which are usually found singly in the resting cells in the vascular cryptogams certainly require further investigation. Usually the chromatophore in the higher plants is a structure of relatively small dimensions; the leucoplast may become hugely distended by a storage starch grain but in so doing becomes so thin and so closely pressed against the starch that it is difficult to distinguish it at all. The suspicion arises that possibly the structures found in Selaginella and in the spore mother cells of *Anthoceros* are not chloroplasts at all but that the chloroplasts lie inside of these structures, close about the starch grains. This, however, can only be determined by further investigation. There are undoubtedly points of resemblence between the polar structures of *Isoetes* and the bodies-described as chromatophores in *Anthoceros*. Still, the polar structures of *Isoetes* bear a relation to the karyokinetic figure which has not been described for chromatophores, so that to include these structures under chromatophores would, for the present at least by entirely unmarranted. I have so far called them starch bodies, but this term is not without objections since these structures also appear entirely

[1]) Harper, R. A.: Sexual reproduction and the organization of the nucleus in certain mildews. (Carnegie Inst. Wash. 1905. Pl. IV figs. 43—45.)

free from starch. They may therefore for the present be designated simply as polar structures.

It is perhaps conceivable that these „structures" are nothing more than a mass of starch grains and dissolved starch transformation-products which without further changes are not easily miscible with the surrounding cytoplasm and hence is separated from it by a surface tension film. i. e. forms a vacuole. To be sure the shape of these structures is not that of an ordinary vacuole. This is especially true during the prophases when the polar structures are markedly flattened and irregular in outline. However, if solution processes take place with varying intensity at various parts of the surface of a vacuole the differences in surface tension brought about by this might result in corresponding changes in form of the vacuole. It is possible further that after the last starch grains had disappeared their immediate transformation products would still be present and accordingly the vacuole which owed its formation to the presence of starch grains could persist even after the last starch grain as such had disappeared. This hypothesis is, however, highly improbable for the polar structures of *Isoetes* also occur in tissues which to all appearances have for long periods contained no starch whatever as for example the vegetative points of young root-tips. Here the cells are entirely free from starch and apparently have not contained any for at least a large number of cell generations; nevertheless all the cells here possess these polar structures.

If an explanation of the bodies in question involved merely the matter of accounting for the accumulation of a number of free starch grains at the spindle-poles one might follow the lines of the kinoplasmic theory and conceive the visible changes taking place in the cell during division as the expression of the coordinated activities of a system of kinoplasmic fibers. It is further assumed that in addition to these more active constituents of the cell other parts are more passive, the so-called „metaplasm", aggregations of food material, etc. If these are freely movable the may tend to collect at the points of equilibrium, or regions of greater quietude, in the active system, just as for example the sand grains aggregate at the points of rest in a vibrating plate in the production of the familiar Chladni figures. If we assume that the spindle poles in a dividing cell are regions of relative quiet, then the accumulation of passive bodies about those points follows as a matter of course. There are numerous statements scattered through the literature reporting the aggregation of more or less passive bodies in the neighborhood of the spindle poles. Karsten[1]) for example found that in diatoms the chloroplasts tend to collect about the spindle poles; Van Hook[2]) made similar observations for *Marchantia*. The

[1]) Karsten: Die sogenannten „Mikrosporen" der Planktondiatomeen und ihrer weiteren Entwickelung, beobachtet an Corethron Valdiviae. n. sp. (Ber. d. deut. bot. Ges. Bd. XXII 1904. p. 544. See figs. 2 and 2a Pl. XXIII.)

[2]) Bot. Gaz. Vol. XXX. Pl. XXIII. Fig. 2.

statements regarding the finding of the nucleoles near the spindle poles during nuclear division also belong here. The observation has been made by numerous investigators that the nucleoles, if they persist long enough, frequently come to lie in the neighborhood of the spindle poles. This has sometimes led to a confusion of nucleoles with centrosomes. However, in not a single case known at present does the nucleole invariably take its position at the spindle poles, more or less frequently it lies elsewhere in the cell. This alone suffices to distinguish it from the centrosome. The fact of the frequent occurrence of the nucleoles at the spindle poles remains to be accounted for, however. Fischer[1]) made use of the observation that the nuclear membrane in some cases first disappears at the point nearest the spindle poles to account for the polar position of the nucleoles. He conceived that as the nuclear membrane disappears at the poles the liquid contents of the nucleus escape at these points carrying the nucleoles with them and as a result these come to lie near the spindle poles. While this explanation is sufficient to account for the approximately polar position of the nucleoles it is entirely inadequate to account for the exact and unvarying position of the central bodies at the poles of the spindle to which Fischer also applies this explanation. It is also still an open question whether it is a general rule that the nuclear membrane breaks down first in the polar regions. When the break does take place at these points it seems probable that a tendency to bring the nucleoles near the spindle poles will result, but it is entirely possible that even where there is no such outstreaming of nuclear contents towards the poles the nucleoles may nevertheless frequently lie near the spindle poles. The same factors which bring about the aggregation of chloroplasts, etc. in the neighborhood of the spindle poles may also be responsible for the position of the nucleoles.

Considerations like the preceding are, however, inadequate to account for the position and behavior of the polar structures of *Isoetes*. In them we are not dealing with the aggregation of free starch grains but with a body which at every stage occupies a specific position in the cell. It divides and its halves migrate along definite lines and come to lie exactly at the poles of the spindle, behaving in all these respects as does the centrosome. The so-called „dynamic" theory of nuclear and cell division also does not account for the behavior of the polar structures of *Isoetes*. According to the dynamic theory there are especial structures or, perhaps, only especial regions at the spindle poles from which an attraction proceeds, an attraction which at times at least extends even to relatively inert bodies in the cell. In this way the accumulation of various cell-constituents at the spindle poles is accounted for. Behind this view lies the conception of magnetic attraction, for this, or a force acting according to similar laws as

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[1]) Fischer, A.: Fixirung, Färbung und Bau des Protoplasmas. Jena 1899 p. 248.

this, is the only attractive force which can be conceived as producing such figures as the dividing cell presents. So far little headway has been made toward a theory of cell-mechanics by the application of our knowledge of magnetic forces. It is a common weakness of the works of Ziegler,[1]) Gallardo,[2]) and Hartog[3]) that they concern themselves almost solely with a single stage of karyokinetic activity i. e. with the completed amphiaster. Success in producing by means of experimental arrangements a model in ironfilings of the same configuration as that shown in some cases by the completed spindle would help little toward an understanding of the formation of such a spindle.

Neither the kinoplasmic nor the dynamic theory is in my opinion adequate to account for the behavior of the polar structures of *Isoetes*. They give no explanation of the perfectly regular division of these structures preparatory to cell division nor for their unvarying position exactly at the poles of the spindles. The facts which have been brought out as to the structure, position, and behavior of these bodies all point to them as selfperpetuating and perhaps permanent organs of the cell, organs which in their behavior during cell division at once suggest the central body as it is known for animals and lower plants. There is of course much difference of opinion regarding the nature of the centrosome, its very existence as a structure of morphological or physiological value is still denied by some.[4]) There is, however, a large amount of apparently unquestionable evidence[5]) which goes to show that in some cases at least the centrosome is an organ of the cell which passes continuously from one cell generation to another, reproducing itself by division. Likewise there is a general consensus of opinion that the centrosome is most intimately connected with spindle formation and cell division.

The polar structures of *Isoetes* are of sufficient size to be readily distinguished at any period in the existence of the cell;

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[1]) Ziegler: Untersuchungen über die Zellteilung. (Verh. d. Deutsch. Zool. Ges. 1895. p. 62.)

[2]) Gallardo, A.: Essai d'interprétation des figures karyokinétiques. (An. Mus. de Buenos Aires T. V. 1896. p. 10.) A propos. des figures karyokinétiques. (Compt. Rend. de Soc. Biol. T. LII. 1900. p. 732.) Les croisements des radiations polaires et l'interprétation dynamique des figures de karyokinèse. (Ibid. T. LIII. 1901. p. 454.)

[3]) Hartog, M.: The dual force of the dividing cell. (Proc. Royal Soc. B. Vol. 76. 1905. p. 549.)

[4]) For evidence against the hypothesis that the centrosomes are permanent cell organs see Morgan, T. H. The production of artificial astrosphaeres. (Arch. f. Entw.-Mech. Bd. III 1896. p. 339.) The action of salt-solutions on the unfertilized and fertilized eggs of Arbacia and of other animals. (Ibid. Bd. VIII. 1899. p. 448.) Wilson, E. B.: Experimental studies on cytology I. A cytological study of artificial parthenogenesis in sea-urchin eggs. (Arch. f. Entw.-Mech. Bd. XII. 1901. p. 529.)

[5]) See for example Boveri, Th. Zellenstudien. Heft 4. Über die Natur der Centrosomen. 1901.

there is no doubt that they are differentiated structures of the cell which multiply by successive bipartitions. Furthermore the relations of these bodies and their motions to the formation and orientation of the spindle fibers indicate that they are most intimately connected with spindle formation.

Isoetes is somewhere on the border line between pteridophytes and phanerogams and it might possibly be assumed that in *Isoetes* we have a transition from a cell structure with well defined central bodies as found in some algae and fungi to a cell structure apparently without central bodies or anything corresponding to them as found in the spermatophytes. Still, the data we have seem to show that in the pteridophytes aside from the universal presence of blepharoplasts a cell organization essentially similar to that of the spermatophytes prevales. It is to be noted of course that *Isoetes* is not among the direct ancestors of the spermatophytes but stands rather at the head of a more or less independent developmental series, so that the conditions found in its cells may quite possibly be the outcome of another line of development from the lower forms with central bodies than that followed in the development of the spermatophytes. It is always to be further remembered that physiologically and anatomically equivalent structures, apparently may arise independently in widely separated organisms, note for example the similarity in structure and formation of some plant and animal spermatozoids, similar requirements and environmental conditions calling forth similar structures.

In conclusion I wish to heartily thank professor R. A. Harper for the encouragement and aid he has given me in the preparation of this paper.

Explanation of Figures.

The figures were drawn with the aid of the camera lucida.

Figs. 1—7 are taken from cells of young leaves of *Isoetes lacustris* and are magnified 1250 diameters. Fig. 8 is from a spore-mother-cell of *Equisetum hyemale,* magnified 700 diameters. Fig. 9 from a spore-mother-cell of *Marsilia quadrifolia*, mag. 700 diameters.

Fig. 1. „Resting" cell, the nucleus lies close to the last formed cell wall. The starch containing polar structure extends out from the polar depression in the nucleus.

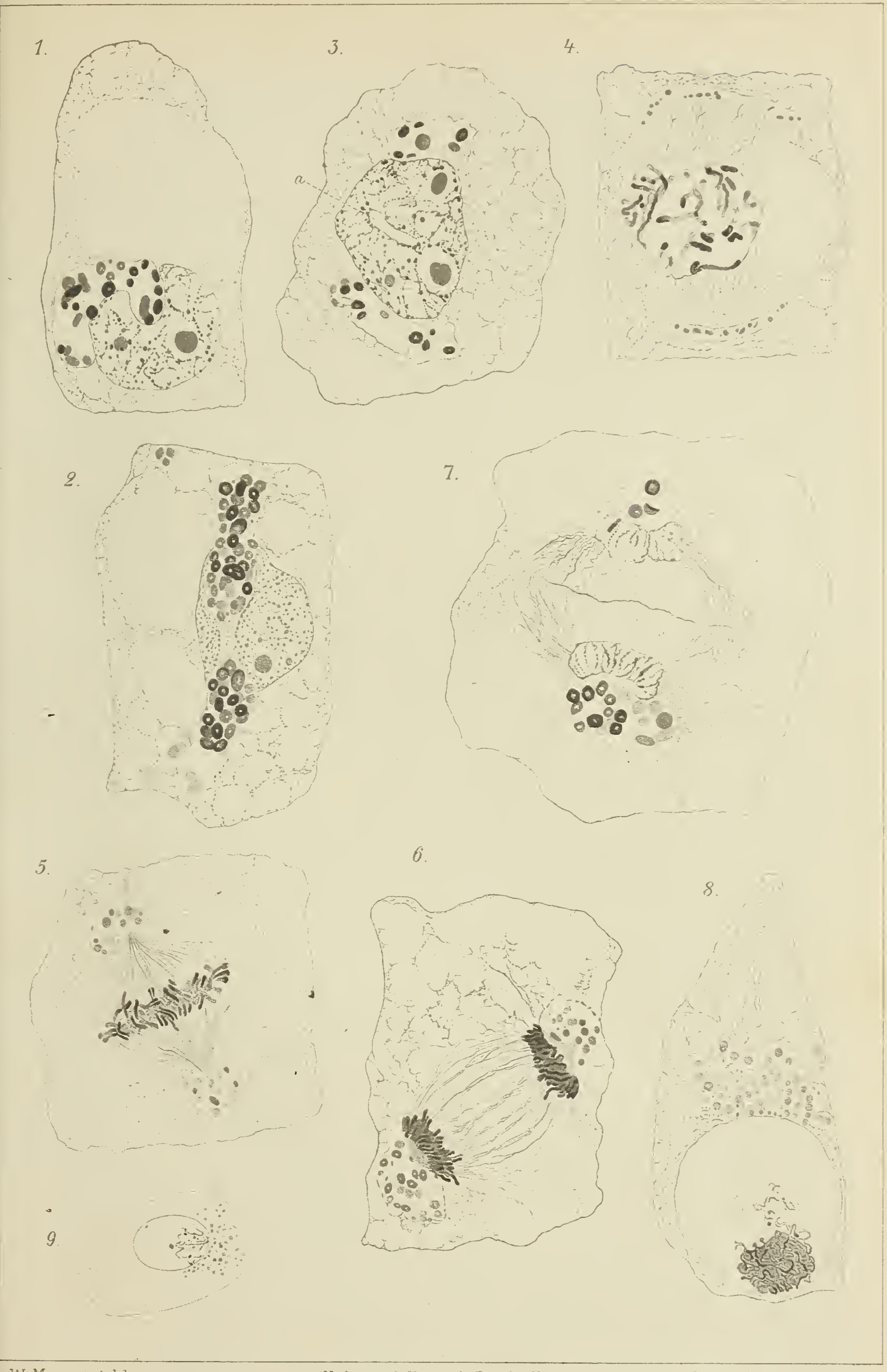
Fig. 2. Division of the polar structure, the separating halves drawing through a depression or furrow in the nucleus.

Fig. 3. The daughter polar structures at opposite sides of the nucleus, closely pressed against it. *a*, section of the still persisting furrow formed in the nucleus by the separating polar structures.

Fig. 4. Polar structures withdrawn from the nucleus, spindle fibers appearing, extending between the polar structures and the nucleus. The nuclear membrane disappearing.

Fig. 5. Completed spindle, equatorial plate stage.

Fig. 6. Late diaster showing well developed connecting fibers; the poles of the spindle still persisting.



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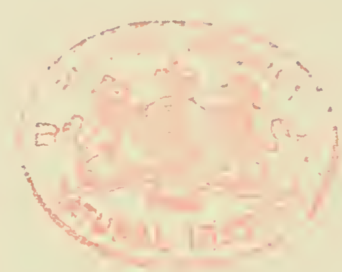

Fig. 7. Reconstructed daughter nuclei with polar depression, nucleoles beginning to appear. The polar structures contain abundant starch, the upper one is as well developed as the lower but most of it lies in the next section.

Fig. 8. Spore-mother-cell of *Equisetum hyemale* in synapsis. A dense aggregation in the cytoplasm lying against the nuclear membrane opposite the synaptic mass, the cytoplasmic fibers are centered upon this granular aggregation.

Fig. 9. Spore-mother-cell of *Marsilia quadrifolia* in synapsis. Here the granular aggregation (largely starch) in the cytoplasm lies against the nuclear membrane next to the synaptic mass.