Studies in Spore Development.

III. The Premeiotic and Meiotic Nuclear Divisions of *Equisetum arvense*.

BY

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With Plates LI-LIII.

IN 1909 I published (1) a short account of the spore development of *Equisetum* with the principal object of recording the result of my observations upon the origin of the much disputed 'middle coat' of the spore-wall. In contradiction to those who had written on this subject before, I found that this layer of the spore-membrane was derived from the secretory activity of the tapetal plasmodium which surrounds the developing spores.

It had been my intention to publish a more detailed account of the spore development of this plant, but the appearance of a careful and thorough study of this subject by E. Hannig in 1911 (3) has rendered this unnecessary. Hannig, in his much more extensive memoir, has reached similar conclusions regarding the 'middle coat' (as well as in several other respects) to those to which I gave expression in my short note of 1909.

The nuclear divisions associated with the spore development of *Equisetum* were, however, not dealt with by Hannig, and I thought that it might be of interest to describe these at greater length in the present communication. This seemed the more desirable since a fresh study of many new preparations has caused me to modify some of the views which I expressed upon this subject in my previous note.

The material of *Equisetum arvense* (which was the only species used in this research) was collected in the autumn of 1908. A collection made on September 3 yielded sporangia, showing the various phases of the arche-sporial divisions. On September 8 and 9 the majority of the sporangia contained spore mother-cells in all stages of meiosis. On September 13 the meiotic divisions had been completed in almost all the sporangia and were only occasionally met with, but the strobili showed all stages of spore development from the naked, membraneless spore to the spores of 38μ diameter furnished with elaters. Later gatherings were made to complete

the series of spore development. The different sporangia upon a strobilus are usually found to differ considerably from one another with regard to the stage of development which they have reached. In a single transverse section of a cone it is quite usual to find a sporangium containing mother-cells with nuclei in diakinesis (of the first division) lying side by side with another sporangium enclosing the spore-tetrads already formed. Besides this difference between the sporangia of a strobilus a good deal of variety can also, not infrequently, be seen amongst the spore mother-cells of one and the same sporangium. For example, a sporangium containing in one part tetrads in which the young, membraneless spores were just commencing to separate from one another, enclosed at another spot spore mother-cells still in the metaphase of the homotype division.

Hofmeister (4) found a very similar variation to exist among the mother-cells of the sporangia of *E. palustre*. He wrote (pp. 283-4): 'In the same sporangium of *Eq. palustre* there may be found mother-cells with the primary nucleus in the act of dissolution, others with two flattened nuclei, and others with four globular daughter nuclei; there may also be found sets of four tetrahedral cells, individual daughter cells of a globular form, and lastly, others which already exhibit the transparent halo slightly developed.'

My material was fixed in absolute alcohol, alcohol and acetic acid mixture, and in Flemming's solutions. Of these the stronger solution of Flemming gave the best results and was chiefly employed for this study of the nuclear phenomena. For staining the sections I used Heidenhain's iron-alum-haematoxylin, either alone or in conjunction with Bismarck brown; Flemming's triple stain; and gentian violet and orange G without safranin. As I obtained excellent results with the gentian violet and orange G without safranin, I used either this combination or the Heidenhain's haematoxylin method for the majority of my preparations.

PREMEIOTIC DIVISIONS.

The resting nuclei of the archesporial cells of *Equisetum arvense* enclose a chromatic reticulum which varies somewhat in the degree of its fineness. In what are probably nuclei in a condition of complete rest this reticulum is very delicate and shows comparatively few and small chromatic aggregates. Pl. LI, Fig. 1 gives a good idea of a nucleus in this condition. In other archesporial nuclei the reticulum is coarser and contains here and there somewhat more obvious chromatic aggregates. These small collections of chromatin most often occur singly upon the reticulum, but occasionally two such lumps may lie side by side. This paired arrangement of the chromatin bodies, where such occurs, is without doubt a chance phenomenon depending upon natural or artificial tensions in the nucleus which stretch

the meshes of the network, here and there, so as to bring the two sides of the mesh closer together. When these two sides of the stretched mesh happen to contain chromatic thickenings upon them these will naturally form, in such cases, a parallel pair.

The degree of coarseness of the nuclear reticulum is also, no doubt, to some extent influenced by the penetration and action of the fixative.

At the same time, in the majority of cases, the coarser reticulum evidently denotes a nucleus which has not passed into such a complete condition of rest as the one enclosing the finer and more even network. It is possible that the coarser reticulum may always become more evenly distributed before the next division is entered upon, but it appears more probable that the nuclear condition in rest depends upon the rapidity with which the divisions are following one another.

The nucleoli are numerous in these resting nuclei. Three to six—or even more—nucleoli occur in each nucleus, and several of these can often be seen to be elongated, vermiform, or hour-glass shaped (Pl. LII, Fig. 16). The reticulum is usually contracted away from the nucleoli so as to leave a clear space round each of them. The nucleoli do not stain deeply, but colour as plasmosomes.

When a resting nucleus, such as that described above, is about to enter upon a mitotic division we find that certain of the arms and anastomoses of the network are withdrawn so that the meshes of the reticulum become larger and more open. This withdrawal of the cross connexions takes place along certain lines, so that there is a tendency manifested for lengths of filament, uninterrupted by anastomotic communications, to differentiate out of the network. At the same time the filaments of this looser and more open reticulum become thicker and more deeply stained with dyes. The nucleoli still continue to be large, numerous, and plasmatic in their standing reactions (Fig. 2).

The continued retraction of the branches and anastomotic connexions gradually leads to the formation of a spireme in which the filaments are smooth and stain fairly deeply in haematoxylin, gentian violet, &c. Quite early in its differentiation the spireme often shows a distinction of more darkly stained (chromatin) granules embedded in a lighter (linin) thread, but in my preparations of older stages I have failed to see this appearance.

The filaments which have arisen from a stage such as I have drawn in Fig. 2 by the further withdrawal of the branches of the reticulum, are still comparatively straight and show a certain amount of polarization (Fig. 3). This polarization of the filaments as they become differentiated out of the reticulum recalls the arrangement of the chromosomes during the telophase of the preceding division. It is not improbable, therefore, that the chromosomes which emerge at the prophase of a division may roughly occupy the

same relative positions as they did during the telophase of the previous division, when their outlines became lost to view owing to the development of numerous branches and cross connexions with one another.

It is especially to be noted that the spireme which develops from the reticulum forms at no time a continuous thread. From the beginning it is segmented into separate lengths which at first are long and slender, but which become thicker, shorter, more deeply staining, and more curved as mitosis advances. These facts are shown in Figs. 3, 4, and 5. The last of these figures shows that the definite polarization of the chromosomes has eventually become entirely lost, and that the curved filaments composing the segmented spireme coil in every direction through the nuclear cavity. Several conspicuous plasmosomes still remain enclosed within the coils of the spireme.

It will be seen that in the development of this spireme there is no concentration of the filaments by the approximation of longitudinal halves of previously alveolized chromosomes such as has been described in a number of other plants by several writers.

Indeed, the spireme, both when fully formed and during its development, appears in *Equisetum* to be quite unsplit longitudinally. Each chromosome appears in this plant to develop during the prophase solely by the drawing in of the anastomoses which had been formed at the close of the preceding division and by the concentration of the long, slender, but homogeneous filament, which first appears, into a shorter and thicker structure.

The nuclear membrane then disappears and the coil of chromosomes, together with the nucleoli, lies free in the cytoplasm. The chromosomes next become drawn upon the equator of the spindle which has developed in the usual manner. The chromosomes appear at this time as rather long and not very thick bodies which are bent into a longer arm directed towards the pole of the spindle and a shorter arm arranged upon the equator of this structure.

Soon after the chromosomes have become regularly arranged upon the spindle-equator they can be seen to be longitudinally divided (Fig. 6). As the longitudinal halves begin to separate from one another they not infrequently remain adherent or closer together at their distal ends whilst they are already quite widely apart at their proximal extremities. In these cases loop-like structures are formed such as I have represented in Fig. 7. The nucleoli can for some time be seen lying in the cytoplasm in the neighbourhood of the chromosomes, either among the spindle fibres or free from the mitotic figure, but as the daughter chromosomes begin to move apart the nucleoli are lost sight of.

In Fig. 8 I have represented a stage of the anaphase. It will be seen from this that the premeiotic spindle forms a blunt¹ structure, which differs

¹ It is of course possible that the blunt spindle may be due to the removal of the actual spindle

markedly in appearance from the sharply defined heterotype spindle, with its acute polar extremities.

The daughter chromosomes continue to move apart until they reach the ends of the spindle, where they become closely massed and intertangled in two groups corresponding to the two daughter nuclei (Fig. 9). After a time the tangle of chromosomes begins to open a little in each daughter nucleus, and a new nuclear wall is developed. At this stage of the telophase the chromosomes are still seen to be arranged for the most part with their long axes parallel with the longitudinal axis of the spindle. That is to say, they still roughly occupy the positions in which they reached the spindle extremities at the conclusion of the metaphase. No vacuolation, such as occurs in some other plants, is to be seen in the chromosomes of *Equisetum*. Their substance remains adherent at certain spots where they had come into contact when closely massed together at the conclusion of the metaphase, and these points of junction become drawn out into connecting threads when the chromosomes again separate from one another during the telophase. The chromosome substance becomes distributed along these anastomotic junctions and new lines of connexion appear to develop between the chromosomes, so that gradually the outlines of the chromosomes become obscured and the young nucleus is seen to contain a chromatic reticulum (Figs. 10, 11). This condition endures until the prophase of the succeeding division, when the anastomoses are again withdrawn and the chromosomes once more become recognizable as distinct entities, occupying, roughly, the same relative positions which they held at the conclusion of the telophase.

The activities performed by the so-called 'resting' nucleus can evidently be most satisfactorily performed when the chromatic contents are diffused more or less evenly through the nuclear cavity, whilst for the mitotic division concentration of this material is essential. It appears to be of relatively small importance how the diffusion of the chromatin is effected. In some cases it is attained chiefly through the vacuolization of the chromosome bodies; in others the chromosomes break up into shorter lengths and eventually into granules, whilst in such cases as *Equisetum* the development of anastomotic connexions between the chromosomes and the distribution of their substance along these branches are the most important factors in obtaining a diffusion of the chromatin through the nucleus. In any case, however this diffusion of the chromosome substance is brought about in the telophase, the same steps usually appear to be retraced during the prophase of the succeeding division.

In *Equisetum*, therefore, the telophase finally leads to the development of a typical resting reticulum such as the one with which we started this

apex by the microtome knife, but the occurrence of obtuse ends was so constant and invariable a phenomenon in my preparations that I felt obliged to reject this interpretation.

account. It depends upon the degree to which the chromosome substance becomes diffused whether a coarse or a fine network results.

Soon after the new nuclear membranes have developed round the daughter nuclei, and therefore at an early stage of the telophase, one or more nucleoli can be observed lying among the chromatin contents (Figs. 10, 11).

FIRST MEIOTIC DIVISION.

At the conclusion of the last premeiotic division the nucleus passes into a complete condition of rest indistinguishable from that separating one premeiotic division from another. Such a nucleus I have already illustrated in Fig. 1. At the commencement of meiosis there is a small but distinct growth in size of the nuclei, the average nuclear diameter of the resting cell being 14μ , whilst that of the nuclei shortly before synapsis is 17μ . During this growth many of the anastomoses of the chromatic reticulum become withdrawn, so that the close network of the resting nucleus is converted into a much looser one with wider meshes (Fig. 12). The nucleoli still remain in about the same number (usually from three to eight), and with the same appearance, as in the resting nuclei.

The next step in the progress of meiosis is the contraction of the chromatin network. In Fig. 13 we see that this contraction has commenced on one side of the nucleus, whilst in Fig. 15 the synaptic contraction has been completed. The nucleoli, which not infrequently appear vacuolated at this period, are partly enclosed among the contracted meshes of the chromatin reticulum, whilst others may lie free from the tangle.

During synapsis the chromatin network becomes finally transformed into an unbranched spireme free from all anastomotic connexions. This is already clear at the stage represented in Fig. 17 *a*, in which the coil of chromatin is becoming loosened and redistributed through the nuclear cavity. Another fact is apparent in the same drawing, and this is the clear demonstration of the existence of a longitudinal split in the filaments which are uncoiling. The short length of spireme depicted in Fig. 17 *b* shows this particularly well.

Gradually the spireme, free from all cross connexions or anastomoses, unfolds entirely and winds freely through the nuclear cavity (Figs. 18, 19). The longitudinal division of the threads, which could be clearly seen as the synaptic tangle loosened, is usually again lost sight of when the spireme has completely developed. As a rule the filaments are smooth, slender, and homogeneous in appearance. Occasionally, when the differentiation of the stain has been brought to a particular point, the appearance of lighter and darker coloured areas, corresponding to a differentiation of linin and chromatin, can be seen, but usually the thread is stained uniformly. There is a good deal of difference between the arrangement of the coils of the

spireme to be seen according as we examine a median or a peripheral section of the nucleus.

In Fig. 18 I have represented a median section of a nucleus, where it will be seen that the filaments appear to be arranged without order. In Fig. 19 is shown a peripheral section through a neighbouring nucleus at precisely the same stage. Here it will be observed that there is a very definite order in which the threads are grouped, and that they exhibit a certain amount of parallelism with one another.

Another feature of some importance is very evident in the spireme of this plant, and that is the discontinuity of the thread. It is only necessary to glance at any of the figures which I have given (Figs. 18, 19, 20, 27) to see at once that free ends of the filaments are of frequent occurrence, and that the spireme is segmented up into comparatively short lengths. Up to this time the cells of the sporogenous tissue have remained united as a single block of tissue surrounded by the tapetum. Each spore mother-cell is bounded by a thin wall, which gives the reactions characteristic of pectic bodies.

During the stage of the spireme, however, the membranes separating the spore mother-cells become split and the cells begin to separate from one another. Each mother-cell is at this time still covered by a delicate film of membrane which is the remains of the septum which formerly separated one cell from another.

Each spore mother-cell rounds itself off as it becomes isolated from its neighbours. In the meanwhile the tapetal cells have also lost the membranes which separated them from one another, and have flowed together to form a richly nucleated plasmodium. This tapetal plasmodium now begins to penetrate into the interior of the sporangium and to surround the sporogenous cells as these become isolated. As I pointed out in my previous note, and as Hannig (3) has confirmed, the tapetal cytoplasm first makes its way between the sporogenous cells, and the tapetal nuclei follow suit slightly later.

In somewhat older spore mother-cells the nuclear spireme has increased a little in thickness and become more retentive of chromatic dyes. In such nuclei the longitudinal division of the spireme can usually be again seen (Fig. 20). This stage immediately precedes a period during which the nuclear contents lose their regularity of outline and become drawn together. During this phase, which may be called that of the 'second contraction' (although there is not such a profound massing of the nuclear contents as is the case in some other plants),¹ it often becomes very difficult to correctly interpret the changes which are taking place within the nucleus. It is only when the almost fully developed bivalent chromosomes emerge from the confusion that it again becomes easy to follow the course of events.

¹ Cf. Miss Digby's figure of *Primula verticillata*, for example, (2) Plate XLII, Fig. 46.

One is at first tempted to conclude that each half of a longitudinally divided spireme segment condenses to form one of the two members of a bivalent chromosome, and therefore to consider that the heterotype chromosomes develop by a process of parasynapsis.

This was the conclusion to which I first came and to which I gave expression in my former note. Since that article was written, however, I have had the opportunity of subjecting the phase of 'second contraction' to a much closer scrutiny, and I have been successful in finding cases in which the entire course of events could be uninterruptedly followed from the stage of the longitudinally divided spireme segments (previous to the second contraction) up to the appearance of the fully developed heterotype chromosomes.

The study of these unusually clear examples has shown that my former conclusions were erroneous, and that, instead of having an example of the parasynaptic origin of the heterotype chromosomes in *Equisetum*, this plant really furnishes a rather striking instance of their origin end to end.

Before dealing further with the second contraction, it is first necessary to consider the appearance of the nucleoli in these nuclei during the preceding stages of meiosis, as the peculiar behaviour of these bodies during the contraction adds no little to the confusion of that stage. In the resting nucleus, following the telophase of the last premeiotic division, a large number of medium-sized plasmatic nucleoli occur. It is not uncommon to find as many as eight nucleoli in a single such nucleus. Many of the nucleoli are spherical, whilst others are elongated, vermiform, or hour-glass shaped. Several forms are represented in Fig. 16. Some of these constricted nucleoli may possibly be undergoing division, but, in the light of what occurs a little later in their history, I believe that the majority of these hour-glass-shaped bodies are instances of nucleolar fusion rather than of their division. The outlines of these nucleoli are usually perfectly smooth, and it is only very rarely that any budding of material from their surface can be seen at this period. In older nuclei which are just about to pass into the synaptic contraction it can often be seen that the nucleoli, six to eight in number, approach one another and unmistakably fuse into a few larger bodies.

In Fig. 14 it will be seen that on the left five nucleoli are grouped together but have not yet fused, whilst on the right of the nucleus three nucleoli have merged together into a single vermiform structure which still clearly shows the points of fusion. Fig. 27 shows further nucleolar fusions at this stage. During synapsis the fusion of nucleoli is completed, and by the time the spireme has unfolded the nucleoli have been reduced to one or two comparatively large bodies (about 3 or 3.5 μ in diameter). That the single (or few) large nucleolus of the spireme is derived from the fusion and not from the partial absorption of the numerous nucleoli of the pre-

ceding stages is clearly shown by the comparison of the amount of nucleolar matter which occurs in the two cases.

Up to the time of the establishment of the spireme the nucleoli are smooth in outline and show no evidence of budding. During the stage of the spireme, and especially a little later when the second contraction is taking place, we find the nucleolus actively extruding material from its surface in the form of droplets or buds (Figs. 20, 21).

It is noteworthy that this active extrusion of nucleolar material into the nuclear cavity is synchronous with the commencement of a period during which there is growth in thickness of the spireme segments and a great increase in their staining power.

Droplets of nucleolar material, varying from very minute globules to quite large bodies, are rapidly given off from the surface of the nucleolus. These pass into the nuclear cavity and become distributed along the spireme threads, to which they adhere (Fig. 21). At the same time the spireme segments are drawn more closely together, and often appear to be centred in the neighbourhood of the nucleolus (Fig. 22).

This tendency for the spireme filaments to become drawn together at one or more points, together with the presence of deeply staining nucleolar matter on and between them, leads to the obscuration of detail which marks this stage of meiosis. We may probably conclude that during this period chromatic material, elaborated by the nucleolus, is leaving that body and passing into the spireme segments, which in consequence grow in size and exhibit a greatly heightened affinity for dyes. At first the droplets of nucleolar substance merely adhere to the surface of the developing chromosomes and give these an irregular outline (sp. Figs. 21, 22, 23), but gradually the material becomes absorbed into the interior of these bodies.

It has already been mentioned that the spireme is segmented into a number of parts from a very early period in its history, and it now remains to be seen what relation these segments bear to the bivalent chromosomes of the heterotype division. Even in the early spireme many of these segments are more or less curved or bent. During the later stages, and especially during the second contraction, this curvature of some of the spireme segments becomes more marked, and in many instances leads to the formation of definite loops (Fig. 22). In the case of some other segments the curvatures may be only very slight or they may remain almost straight. Many of these loops and bent or straight spireme segments still show, at places, the existence of the longitudinal split, which was very obvious just before the commencement of the second contraction (Figs. 22, 24). Some of the loops are seen to be breaking across transversely at the point of curvature (see the loop on the left of Fig. 22), and by this means two completely separate, longitudinally divided elements, derived from the bending over of a single spireme segment, come to lie side

by side in the nucleus. Other segments maintain their integrity as unbroken loops or more or less bent filaments for some time longer.

In Fig. 24 it can be seen that there has been a great increase in the thickness and staining power of the segments, partly due to the condensation of their substance and partly owing to the absorption of chromatic material derived from the nucleolus.

In Fig. 25 the condensation of the segments has proceeded further, and we now see that these structures form unquestionable bivalent chromosomes in diakinesis. The passage of one structure to the other has, however, been so gradual and continuous that it is impossible to mistake the true relation existing between them. Many of the bivalent chromosomes at diakinesis are still in the form of unbroken loops, like the longer and more slender spireme segments which preceded them; others have separated across the loop, so that their two constituent elements are free from one another and lie side by side or in the shape of a cross or in some other similar position. No one with an unbiased mind who closely studies the series of steps which intervene between the first unfolding of the segmented spireme from the synaptic coil and the appearance of the fully formed heterotype chromosomes in diakinesis can be in doubt regarding the manner of origin of these chromosomes.

The facts show in an unmistakable manner that each segment of the spireme is composed of two elements (somatic chromosomes) joined end to end. These somatic chromosomes are at first long and slender, but they gradually become shorter and very much thicker, partly by the condensation of their substance and partly by the addition of fresh chromatic material derived from the nucleolus. At the same time each pair of somatic chromosomes usually becomes more or less sharply bent across into the form of a loop, so that the two chromosomes come to lie side by side or, at any rate (in the majority of cases), to approach one another laterally. Sooner or later the loops become ruptured at the bend so that the two members of each heterotype chromosome are finally independent of one another. For a long time during their development these chromosomes can be seen to be longitudinally divided, but when their condensation has been completed this split again becomes lost to sight (Fig. 25).

In Fig. 26 I have represented a few of the typical forms which these heterotype chromosomes may assume in *Equisetum*. In *a* we have a short, thick loop formed by the two constituents of the bivalent chromosome; in *g* and *e* the form is also that of a loop—but of a much more shallow character. In *b* the two somatic chromosomes have developed end to end in a straight line without any signs of curvature. In *f* the pair of chromosomes are arranged in the form of a cross; in *c* they lie side by side, but diverge from one another at one extremity; in *d* the two curved chromosomes also lie side by side, but owing to their curvature both ends

diverge from one another. This last form of chromosome when viewed under unfavourable optical conditions can easily be mistaken for a tetrad of spherical bodies lying close together. This fact no doubt explains the 'tetrads' described and figured by Osterhout (see Osterhout (5), p. 160 and Tafel I, Fig. 1).

Ring-shaped chromosomes (Fig. 26, *h*) are also frequently met with in these nuclei. During diakinesis the chromosomes are mostly arranged peripherally just below the nuclear membrane, but a few chromosomes usually remain closely grouped round the nucleolus as though the concentration of the nuclear contents which is seen at this point during the second contraction had not completely passed away (Fig. 25).

The surface of the chromosomes is, at this time, often somewhat irregular owing to the presence of minute, thorn-like processes upon it. At these points very fine filaments can be seen to be attached, and these join each bivalent chromosome to other neighbouring ones (Fig. 25).

Besides these delicate filaments some flocculent achromatic material occurs in the nuclear cavity between the chromosomes (Fig. 25). Each nucleus contains either one large nucleolus alone or one large one and several smaller ones.

When the spindle is developed and the nuclear membrane has disappeared one or more nucleolar bodies can usually be seen to have been extruded into the cytoplasm. These bodies occupy various positions, sometimes lying free in the cytoplasm and sometimes caught up among the spindle fibres. They usually exhibit no definite relation to the spindle poles, but occasionally one may lie in the neighbourhood of one of the poles of the multipolar spindle.

These nucleolar bodies are still quite distinctly visible during the late anaphase and the early stages of the telophase (Pl. LIII, Fig. 33).

In a few of my preparations of diakinesis in *Equisetum* some of the nucleoli appear to be disintegrating within the nuclear cavity. These cases suggest that at least some of the flocculent achromatic material in the nucleus may be derived from the nucleoli.

Osterhout (5) has already investigated the development of the spindle in great detail, and my observations, so far as they go on this point, entirely confirm his work. A multipolar polyarch spindle is developed in the cytoplasm immediately surrounding the nucleus; the nuclear membrane disappears and spindle fibres develop within the former nuclear cavity. These intranuclear fibres become attached to the chromosomes and place these in connexion with the extranuclear spindle fibres (Pl. LII, Fig. 28). The dissolution of the nuclear membrane is gradual, and it can often be observed that it has disappeared completely at one spot whilst it is still clearly visible at another.

The multipolar polyarch spindle is later converted into a multipolar

diarch structure (Fig. 29), and this subsequently develops into a bipolar structure (Fig. 31). The bipolar spindle is remarkably well defined and is characterized by its very acute and attenuated pole ends. No centrosomes are to be observed at this or any other stage of the division. The chromosomes become arranged upon the equator of this spindle. They are at this time smaller and denser bodies with a more even outline than they previously possessed. At this stage an estimate of their number can be more satisfactorily made than at any other time. Although I have made repeated counts I have found it impossible to do more than arrive at an approximate estimate of the chromosome number in *Equisetum*. Where the chromosomes are so numerous and closely crowded together it is almost inevitable that some should become hidden beneath their neighbours and thus be missed when the count is made.

Moreover, when an equatorial plate of chromosomes appears in more than one section, as is usually the case, the same chromosome may be cut so as to be represented in two (or more) sections, and it then becomes extremely difficult to avoid counting it twice over. In those cases in which the clearest view of the chromosomes could be obtained by counts varied from 94 to 136. Probably the mean of these numbers, viz. 115, is not very far removed from the true number of chromosomes in *Equisetum*.

The form of the chromosomes when arranged upon the equatorial plate of the spindle is shown in Fig. 30. The spindle fibres, which are attached to the chromosomes, are each composed of a bundle of fine filaments, as can be seen from the drawing (Fig. 30). During the metaphase the spindle poles show no trace of polar radiations, but during the anaphase when the chromosomes are approaching the poles radiations extending into the cytoplasm become apparent (Pl. LIII, Figs. 32, 33).

The distribution of the univalent chromosomes to the two poles takes place very irregularly. From Fig. 32, which represents a typical case, it will be seen that whilst some of the chromosomes are already far on their way to the spindle ends, others are as yet only commencing their journey. The majority of the chromosomes become more or less elongated in form during the earlier stages of the anaphase as though stretched longitudinally by the spindle fibres. On reaching the poles of the spindle the chromosomes again become short, dense bodies which are small in comparison to their size at an earlier period (Fig. 33). They become so closely massed together at each pole that it is often difficult to distinguish the outline of the separate chromosomes. Nucleolar bodies, extruded into the cytoplasm, can frequently be still recognized at this stage.

Soon the mass of chromosomes begins to loosen again, and a new nuclear membrane develops round each group. As the chromosomes move apart their soft mucilaginous substance remains adherent at certain spots which become drawn out into connecting branches between the separate

chromosomes (Fig. 36). The chromosome bodies become, to a certain extent, vacuolated (Fig. 36), but this is not so conspicuous a phenomenon as is the case in some other plants. Besides this vacuolation the substance of the chromosomes is distributed along the anastomotic connexions which join them together; new anastomotic junctions develop and the chromosome substance becomes diffused along these in turn. By these means the material composing the chromosomes becomes gradually more or less evenly distributed through the nuclear cavity to form a typical resting nucleus in which the bodies of the chromosomes are only faintly indicated (Fig. 35) and eventually altogether lost to view (Fig. 37). One or more small nucleoli make their appearance in each of the daughter nuclei.

A feature of some interest in the transformation of the chromosomes during the telophase is the gradual elongation of these short, dense bodies and the dispersal of the greater part of their material along certain definite lines. In Fig. 34 the elongation of the chromosome bodies, which are already united with one another by anastomotic junctions, is clearly seen, whilst in Fig. 35, which represents a later stage, the substance of the chromosomes has become much more diffused, but the definite lines along which the chromatic material has principally spread, and which represent the cores (so to speak) of the chromosome bodies, are perfectly distinct.

A little later, when the diffusion of the chromatic contents has proceeded still further and new cross connexions have been developed, the nucleus attains a condition of complete 'rest' in which the elongated remains of the chromosome bodies can no longer be traced (Fig. 37). During the prophase of the succeeding homotype division, however, the chromatic contents of the nuclei again become concentrated along certain definite lines which by further concentration develop into the elongated, slender chromosomes characteristic of the homotype division in this plant (Fig. 38). It would seem, from these facts, to be quite probable that the transformation of the short, thick heterotype chromosomes into the comparatively long, slender chromosomes of the succeeding division is already prepared for during the telophase of the heterotype division. The first manifestation of the homotype division is seen, as I have already mentioned, in the appearance of certain definite lines of concentration into which the strands and cross connexions of the nuclear reticulum become withdrawn (Fig. 38). These lines of concentration map out the bodies of the future chromosomes. There are usually several medium-sized nucleoli present at this stage. As concentration proceeds the chromosomes become more distinctly recognizable, but they are for some time irregular in outline and remain united together by anastomotic junctions. Fig. 39 shows this stage, and it will also be noticed from the figure that the chromosome rudiments have increased considerably in staining power. The nuclear membrane still remains intact, but a mantle of spindle fibres has developed

round the nucleus. In Fig. 39 the nucleus has shrunk slightly, so that the relation between nuclear membrane and extranuclear spindle fibres is seen unusually well.

Very soon, however, the nuclear membrane disappears and the rather confused tangle of chromosomes lies among the spindle fibres, which are now both intranuclear and extranuclear in origin. At first the chromosomes do not appear to be quite free from one another, but soon they free themselves and arrange their long axes parallel with the long axis of the spindle. They are now long, thin, smooth structures (Fig. 40). The spindle in many cases is multipolar diarch in origin (Fig. 40), but in other instances it is clearly multipolar polyarch (Fig. 41). In either case, however, it always becomes bipolar later on (Fig. 43). The nucleoli may remain entangled among the chromosomes or they may pass out at once into the surrounding cytoplasm.

Owing to their close arrangement during the prophase the individual chromosomes are usually rather difficult to study satisfactorily at this stage, but in a few favourable cases it was observed that some at least of the chromosomes were longitudinally divided (Fig. 42). Soon, however, the chromosomes arrange themselves upon the equator of the spindle, and then it is easily possible to repeatedly observe the longitudinal split in their substance (Fig. 43). The daughter chromosomes separate from one another during the anaphase, and in Fig. 44 these chromosomes are seen to have reached the poles of the spindle and are there closely grouped together with their free ends directed towards the equatorial region. A nuclear membrane develops round each group of daughter chromosomes, but certain of the longer chromosomes still protrude for a time beyond the general surface of the nuclei (Fig. 45). A little later the protruding chromosome-extremities are retracted, and the nucleus presents a smooth outline (Fig. 46). The substance of the chromosomes becomes dispersed along the anastomotic connexions which develop between them, so that the outlines of these bodies are gradually lost to view. No distinct vacuolation of the substance of the chromosomes could be seen, but the slender character of these bodies makes observation of this point very difficult.

One or two nucleoli make their appearance in each nucleus at this time. The nuclear division is followed by cell division, and four spores are produced which are at first completely unprotected by a distinct membrane. The nucleus of the young spore soon after cell division has been completed contains one or two distinct nucleoli and a reticulum in which the outlines of the chromosomes have been quite lost to view.

A little later, when the spores of a tetrad are just becoming separated from one another by the intrusion of tapetal cytoplasm, the nuclear reticulum has become slightly coarser and more chromatic than before, but the most striking difference between the nuclei at this stage and those at a somewhat

earlier phase lies in the considerable increase of nucleolar matter which they exhibit. Fig. 47 represents a typical example of a spore at this stage, and it will be noticed that not only has the number of nucleoli increased within the nuclear cavity, but other bodies with all the appearances and staining powers of nucleoli also occur in the cytoplasm.

I have not succeeded in tracing an actual extrusion of nucleolar material from the nucleus, but it appears not unlikely that this does take place and thus accounts for the origin of the nucleolus-like bodies lying in the cytoplasm.

Very probably the increase in nucleolar matter is associated with the active metabolic processes which are taking place within the spore at this time in connexion with the elaboration of an exospore.

SUMMARY.

A. *Premeiotic divisions.*

1. The resting nucleus of the archesporial cells contains a reticulum which varies in the degree of its fineness. It may be very delicate and even or it may be coarser with small chromatic aggregates upon it. These chromatic aggregates, when they occur, show no constancy either in their arrangement or in their number.

2. A spireme develops from the nuclear reticulum by the gradual withdrawal of branches and anastomotic connexions, so that first a close reticulum is converted into an open one, and then certain lines of concentration become apparent.

3. The spireme is discontinuous from the first.

4. By further concentration the segments of the spireme shorten into the chromosomes, which become arranged upon the equator of the spindle which has in the meanwhile developed. The chromosomes are now seen to be longitudinally divided.

5. The daughter chromosomes next move to the poles of the spindle, where they become closely massed together. Subsequently they open out, and their substance becomes distributed along numerous branches and anastomotic connexions which develop between them. It appears to be entirely by these means, and without any indications of internal vacuolation, that the reticulum of the resting nucleus develops from the chromosomes in *Equisetum*. A nuclear membrane is formed round each daughter nucleus.

6. A period of complete 'rest' intervenes between the last premeiotic division and the heterotype division.

B. *Meiotic divisions.*

7. Meiosis commences with the gradual withdrawal of many of the branches and cross connexions of the reticulum.

8. Synaptic contraction then occurs, during which the final conversion

of the reticulum into the spireme takes place. This spireme is seen to be longitudinally divided, and segmented into a number of separate pieces.

9. A period of great nucleolar activity and of partial contraction of the spireme elements ('second contraction') then occurs. During this period the nucleoli are seen to be actively extruding droplets (or 'buds') of material which pass into the nuclear cavity, where they adhere to the spireme filaments. A portion of this nucleolar material appears to be absorbed by the spireme segments and to contribute to their growth and staining power.

10. The spireme segments, which are often bent into the form of loops, become gradually shorter, thicker, and more chromatic, until they can be recognized as unmistakable heterotype chromosomes. The whole process of the transformation of the spireme segments into the heterotype chromosomes can be followed so continuously that there can be but little doubt regarding the relation of one structure to the other.

Each spireme segment consists of two univalent chromosomes arranged end to end, and each such pair develops, by concentration, into one of the bivalent chromosomes of the heterotype division.

11. The number of chromosomes is about 115.

12. By vacuolation and the development of anastomotic connexions the material of the chromosomes becomes dispersed through the nuclear cavity during the telophase and a 'resting' nucleus results during interkinesis.

13. The spindle of the homotype division shows some variation in the mode of its origin. In some cases it is multipolar polyarch during the earlier stages of its development, in others it is multipolar diarch. Eventually it always becomes a bipolar structure.

14. The daughter chromosomes which reach the poles of the homotype spindle develop numerous branches and cross connexions, along which their substance passes, but no distinct vacuolation of their bodies could be seen during the telophase of the division.

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EXPLANATION OF PLATES LI-LIII.

Illustrating Mr. Beer's paper on Spore Development in *Equisetum*.

All the figures were drawn with the aid of a camera lucida. Zeiss's apochromatic objective 2 mm. (apert. 1.40) and compensating ocular 18 were used for making the drawings. The magnification in all cases is about 2,700 diameters.

PLATE LI.

- Fig. 1. Archespore cell with 'resting' nucleus.
- Fig. 2. Archespore cell ; prophase of division.
- Figs. 3, 4. Archespore cells ; later stages of prophase of premeiotic division.
- Fig. 5. Spore of premeiotic division.
- Fig. 6. Metaphase showing the longitudinal division of the chromosomes.
- Fig. 7. Metaphase showing the separation of the longitudinal halves of the chromosomes.
- Fig. 8. Anaphase of premeiotic division.
- Fig. 9. Daughter chromosomes massed at the two poles of the spindle.
- Figs. 10, 11. Telophase of premeiotic division.
- Fig. 12. Commencement of prophase of first meiotic division. Open reticulum.
- Fig. 13. Beginning of synapsis.
- Fig. 14. Nucleus shortly before synapsis, showing fusion of nucleoli.
- Fig. 15. Synapsis.

PLATE LII.

- Fig. 16. Nucleoli from 'resting' nuclei of archespore cells.
- Fig. 17, *a, b, c*. Spore emerging from synapsis. The longitudinal split in the spore is shown.
- Figs. 18, 19. Spore.
- Fig. 20. Spore segments with longitudinal division.
- Figs. 21, 22. Second contraction.
- Figs. 23, 24. Stages in the development of the heterotype chromosomes.
- Fig. 25. Heterotype chromosomes in diakinesis.
- Fig. 26, *a-h*. Various forms of heterotype chromosomes.
- Fig. 27. Fusion of nucleoli previous to synapsis.
- Fig. 28. Multipolar polyarch spindle of first meiotic division.
- Fig. 29. Multipolar diarch spindle developed from the multipolar polyarch structure.
- Fig. 30. Chromosomes arranged on the spindle during the metaphase of first meiotic division.

PLATE LIII.

- Fig. 31. Fully developed bipolar spindle.
- Fig. 32. Anaphase of first meiotic division.
- Fig. 33. Conclusion of anaphase.
- Figs. 34, 35. Telophase of first meiotic division.
- Fig. 36. Details of chromosomes during telophase, showing vacuolation and branching of their substance.
- Fig. 37. Nucleus during interkinesis.
- Figs. 38, 39. Prophase of second meiotic division.
- Figs. 40, 41. Two modes of development of spindle during second meiotic division.
- Fig. 42. Prophase of second meiotic division, showing chromosomes longitudinally divided.
- Fig. 43. Metaphase of second meiotic division.
- Fig. 44. Anaphase of second meiotic division.
- Figs. 45, 46. Telophase of second meiotic division.
- Fig. 47. Spore of *Equisetum* at early stages of its development.

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