On Spore-Formation and Nuclear Division in the Hepaticae.

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With Plates XVI, XVII, and XVIII.

THE present paper is primarily intended to give some account of the behaviour of the nucleus during the formation of the spores in a few genera of Liverworts, but I have not scrupled to add other matters where they seemed to possess interest. Thus the process of germination of the spores of *Fegatella*¹ offers certain points of contrast as well as of similarity, with the corresponding occurrence in *Pellia* which has already been described in this journal². And again the mode of separation of the maturing spores seemed to deserve a few remarks.

As regards the methods employed in fixing the cell-contents, absolute alcohol, alone or in conjunction with formic or acetic acids, gave fair results, but on the whole Hermann's solution proved perhaps the most satisfactory reagent. The

¹ Now often called *Conocephalus*.

² Farmer and Reeves, On the occurrence of centrospheres in *Pellia epiphylla*. *Annals of Botany*, Vol. viii.

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protoplasmic structures at the critical period of spore-formation are unfortunately exceedingly difficult to fix properly, although this observation does not apply to the nuclei of any of the other cells, whether of gametophyte or sporophyte. For staining, I chiefly used Heidenhain's iron-haematoxylin, followed by a ground stain of Orange G. In this way it was possible to trace the developmental history of the chromosomes, though the results obtained were checked by the use of many other dyes, used both singly and in combination. In all cases the microtome was used for cutting the sporogonia, which had been previously embedded in paraffin, but observations were also made on uninjured spore-mother-cells which had been teased out of the sporogonium.

Fossombronia.

I examined three species of this genus, *F. Dumortieri*, *F. longiseta*¹, and *F. angulosa*. I found the first-named species on the whole the best for my purpose, as I had a large quantity kindly sent me by Mrs. Tindall; nuclear divisions were not so frequent in the material of the other two species.

The archesporial cells are fairly large, and contain very prominent nuclei. The nuclei seldom include more than one nucleolus, which is of a relatively large size; the linin consists of a freely anastomosing fine threadwork, which is very delicate in the resting nucleus. The anastomosing spots, and also intercrossing threads, give to the nucleus a granular appearance at first sight. The nuclear wall is very obvious. The protoplasm outside it is of a granular texture, and in the resting cells I was unable to determine the presence of either centrospheres or centrosomes. If the centrosomes exist as such, they would be very difficult to distinguish from the other granules of the cytoplasm.

When the nucleus is about to divide, however, two centrospheres appear, one at either end of the nucleus (Figs. 14, 15). They consist of a somewhat dense mass of protoplasm (archo-

¹ I am indebted to the kindness of Professor D. H. Campbell for a supply of this material.

plasm or kinoplasm), from which extends a beautiful system of radiations, some passing towards the cell-periphery, whilst others curve over the surface of the nucleus. A single central body could in some cases be easily distinguished, although I failed to find it in others. Shortly after the appearance of these structures, the nuclear elements begin to alter in character. The linin becomes more chromatic, and disposed more regularly just beneath the nuclear wall. The nucleolus, so far as I could determine, does not begin to change until *after* the linin has begun to do so; but in the stages which rapidly follow, it is seen to alter its form. It then becomes angular, and, in extreme cases, almost star-shaped, whilst at the same time the linin begins to exhibit a very striking increase in the amount of chromatin which it contains. This substance is not evenly distributed, but is aggregated along certain tracts of the filament, and eventually the intervening parts give way and thus set free the chromosomes. But this is only accomplished when a considerable interval of time has elapsed after the chromatic tracts have been well mapped out. The young chromosomes then appear as rods, which are often bent in an elbow-sort of fashion, and they lie much more irregularly in the nucleus than in both previous and subsequent stages. The nucleolus, which now is very much distorted in form, is often connected with several of the chromosomes, and it soon afterwards disappears. The centrospheres at this time become very prominent, and indeed they are the finest I have seen in any plant-tissues. The chromosomes split longitudinally, and almost immediately afterwards the achromatic spindle is completely differentiated, and the chromosomes, or rather the still closely approximated respective halves, become regularly arranged equatorially around its periphery. I could not follow out with certainty the stages in the formation of the spindle, owing to the suddenness with which it appears in its fully formed condition. Whether it is in this case largely of nuclear origin, as seems not improbable, or whether it is of cytoplasmic nature, must remain for the present undecided. The spindle when fully formed still exhibits radiations at its poles, though

these become very faint for a time, a circumstance often met with in spindles when at this stage in their development. Presently, however, the radiations again become clear, and the daughter-chromosomes move off to their respective poles. There is a sheaf of fibres commonly attached to each daughter-chromosome, and the whole process of the separation of the daughter-chromosomes is such as to suggest a passive 'roping-up' to the poles, rather than any spontaneous movement on the part of the chromosomes themselves.

It is easy to distinguish the number of the chromosomes in the archesporial nuclei. They are, in all the nuclei in which I could count them, sixteen in number. I estimated them both at the equatorial plate-stage and also, in other instances, while the daughter-chromosomes were travelling polewards. The membrane very early appears around the daughter-nuclei, but the connecting spindle-fibres persist for a considerable time, and across them the cell-plate is formed in the normal manner. I was unable to distinguish the centrospheres of the two daughter-nuclei after their walls had been formed. I saw, occasionally, vesicular bodies which occupied the place where the centrosphere might have been expected to be found, but they were very irregular in character. Sometimes they were altogether absent, and at others I saw as many as three lying together. The protoplasm is somewhat vacuolated at this stage, and I believe that the structures in question were nothing more than small vacuoles which happened to lie upon the surface of the nuclei. The nucleoli reappear early within the nuclei, first as two or three small bodies which finally fuse to one large one, and the linin concomitantly loses its chromatin constituent, and passes quickly into the ordinary resting condition. I was unable, however, to distinguish any difference in the staining capacity of either the nuclear sap or of the cytoplasm, such as would indicate that the stainable substance (chromatin) had become diffused out of the linin. The only structures which do change in this respect are the growing nucleoli. This does not necessarily imply that the chromatin passes, as such, into the nucleolus. Indeed, all the

more recent results obtained from microchemical examination of the cell, so far as they afford any evidence at all, are against it. But it may very well be that some constituents of chromatin find their way ultimately into this body, since both chromatin and the nucleolus readily yield albumen on appropriate treatment¹.

THE DEVELOPMENT OF THE SPORES OF *Fossombronina*
Dumortieri.

After the last archesporial divisions have been completed, the spore-mother-cells loosen their union with each other, and grow for a time before undergoing any further change. The nuclei are at first rather small, and contain a very prominent nucleolus. The linin constituent is very poor in staining substances, and is rather difficult to trace. As the cells approach their full size they become somewhat four-lobed, each protuberance corresponding to a future spore. But the lobed appearance is not nearly so striking here as in most other genera of the *Jungermannia*-series of *Hepaticae*. When the cell reaches its full size the nucleus alters in structure. The linin becomes very clear as a delicate threadwork within the nucleus. It seems always to pass through a peculiar phase about this time, being much massed up together, and this aggregation occurs almost always in the vicinity of the nucleolus. Afterwards it again is visible as an anastomosing mass of threads, which are somewhat tightly stretched from one knot or anastomosis to another. The whole thread now thickens somewhat, and there are seen other threads of very delicate texture running from the nucleolus to the thicker linin. Whether these thinner filaments are remains of the original linin which have not shared in the general increase in bulk it is not possible to say; they are at least similar to it in staining characters. But however this may be, they served to connect up the linin with the nucleolus at this stage. I am

¹ Kossel, Ueber die Nucleinsäure, Verhandl. d. Physiol. Gesellsch. z. Berlin, 1892. Zacharias, Ueber d. Chemische Beschaffenh. v. Cytoplasma u. Zellkern, Ber. d. deutsch. Bot. Gesellsch. 1893.

myself disposed to believe that they do represent linin structures rather than structures originating from the more fluid part of the nucleus. They may also be occasionally observed in the pollen-mother-cells of Lilies at an early period of their nuclear division. The straight threads which run from one anastomosis to another often appear to be composed of a double thread lying side by side; this peculiarity becomes more obvious at a later period, but, as will be seen presently, it can hardly, by itself, constitute the longitudinal fission of the chromatic elements of the nucleus, and it may further possess a totally distinct significance. The nucleus remains in the rudimentary and early phase of karyokinesis for a considerable time, and this is a feature very often met with in spore-mother-cells. Thus it would seem from the accounts given by Belajeff¹ and by Strasburger² that the pollen-mother-cells of *Larix* can continue in a somewhat later phase throughout the winter, only completing the process on the return of warmer weather.

The first sign in *Fossombronina* of renewed activity appears in the cytoplasm in the form of centrospheres. These bodies arise, or at least become first recognisable, simultaneously at *four* points on the periphery of the nucleus which are situated equidistantly from each other, one being opposite to each of the four lobes of the spore-mother-cell. They are exceedingly well marked, and in preparations stained with Heidenhain's iron-haematoxylin and afterwards treated with orange, they are so clear as to be suitable for demonstration purposes (Figs. 2-4). The central part of the sphere is rather hyaline, and a minute granule may often be distinguished in the middle of it, which I take to be a centrosome. The cytoplasmic radiations spread outwards, and may often reach the periphery of the cell. Occasionally they are seen to terminate in granules, which are sparsely scattered about in the cytoplasm. When a number of these granules lie near

¹ Belajeff, Zur Kenntniss d. Karyokinese b. d. Pflanzen. Flora, Ergänzungsband zum Jahrg. 1894.

² Strasburger, Karyokinetische Probleme, Pringsheims Jahrb. XXVIII, p. 166.

together, they are often united into a sort of reticular system by threads of protoplasm.

As time goes on the nucleus becomes angular, each angle corresponding to a centrosphere, and then a quadripolar spindle of an exceedingly well-marked type is seen as the further result of this angular extension. The nuclear wall is not broken, although it becomes greatly pulled out beneath each centrosphere, and thus the quadripolar spindle is thus far a nuclear distortion. The cytoplasm meanwhile alters its appearance, and becomes vacuolated in a manner highly characteristic for this and subsequent periods. The centrospheres later become more closely approximated in pairs, and although I have not seen the process of fusion it is certain that the first karyokinetic spindle is *bipolar*, and that a simultaneous division of the nucleus into *four* daughter-nuclei does not take place here, as it does in *Pallavicinia*¹. This fact, taken together with the approximation of the centrospheres in pairs, seems to point clearly to the merging of the original four kinetic centres into two, and it is highly probable that the ultimate axis of the bipolar spindle comes to lie in the direction of the resultant of those forces which are operating within the cell, and of which the centrospheres and the radiations are the visible expression. The particular parts of the protoplasm which are involved in the production of the radiations is of a different character from the intervening mass. Although during life the radiations are by no means rigid structures, they become so after death, and I was able to observe in one cell, the side of which had been broken off in cutting it, that the radii extended, unbroken, as stiff projections beyond the edge of the fractured cytoplasm.

Whilst the quadripolar protuberances of the nucleus have been in course of formation, the linin framework also undergoes further change. Chromatin begins to form abundantly within it; and especially so at the places where the thread-anastomoses are found. In fact ultimately it all is found localized in these spots, the linin shrinking up to them.

¹ Studies in *Hepaticae*. *Annals of Botany*, Vol. viii.

There are formed eight of these aggregation or condensation groups, and they ultimately give rise to the eight chromosomes characteristic of the gametophyte of this plant. The longitudinal fission of the chromosomes occurs during this stage. The aggregations just mentioned lengthen and are then clearly seen to be double. Indeed the longitudinal halves are often so distinct that I believed at first that a fusion of two separate chromosomes, and not the fission of one, was taking place. Each half is very clearly seen in favourable cases to be only a local thickening in the linin, the thin and comparatively uncoloured portions still connecting one chromosome with another. The swollen parts of the linin, to which the chromatin is chiefly restricted, then contract, and form the eight intensely staining bodies which are situated just within the wall of the otherwise non-staining nucleus. As they contract, the longitudinal character of the fission becomes more difficult to discern. The chromatin is often seen to be grouped in a singular manner at this stage (Figs. 5-7) when the chromosomes happen to be regarded from one end. Sometimes four such aggregations could be seen in each chromosome, but the number was not sufficiently constant to afford very secure ground for theorising. At the same time the appearance was such as to suggest that a *double* longitudinal fission may take place here, and thus the next (and final) division may be prepared for. Such a case has been described by Brauer for *Ascaris*, and some of his figures bear a striking resemblance to what is seen at this stage in *Fossombronia*. But the question can only be settled by a study of a far larger amount of material than I had at my disposal. The process is passed through so rapidly that several hundred preparations may be examined without finding the exact stage required. Finally all the unthickened parts disappear, and the chromosomes then appear as either solid structures or else as ring- or ellipse-shaped bodies lying within the nucleus. They become more dense still before the advent of the achromatic spindle, all signs of any longitudinal fusion become obliterated, and they gradually assume the

form of spindle-shaped rods. The nucleolus in the meanwhile fragments, and the particles become attached to the rudiments of the young chromosomes, often in a very striking manner¹; there exists a good deal of variety, however, in the extent to which this relation of nucleolus and chromosome-rudiment obtains. One thing is however clear, that as the chromatin increases in the latter, the nucleoli become less and less easily differentiated, although traces of them can sometimes be observed even up to the time of the differentiation of the achromatic spindle. This body appears with great suddenness, and this particular material is not very suitable for tracing out its genesis, but from the relation of the drawn-out nucleus with the centrospheres it seems highly probable that it is for the most part of nuclear origin. There is, besides the so-called 'nuclear sap,' the unused linin which does not enter into the constitution of the chromosomes, and also possibly a part at any rate of the nucleolus which might serve as material out of which it could be differentiated within the nucleus. My own observations have rendered me sceptical as to the existence of a special spindle-forming substance, and I regard it as not improbable that the spindle represents nothing more than hyaline protoplasm (using the term in its widest sense) which has become strained along 'lines of force.' Thus, for me, it is not a very important matter whether the spindle is of cytoplasmic or of nuclear origin, especially as we know that it *may* be either of entirely nuclear or of mixed origin, taken that is as a whole. To this question I shall return later.

The chromosomes, eight in number, lie along the spindle in the equatorial plane, and they very clearly exhibit a hump in the middle of each one of them, similar to the hump seen in the chromosomes of Lilies during the first mitosis in the pollen-mother-cell. It is not very easy to follow out the true course of events in *Fossombronia*, owing to the minuteness of the chromosomes, but I think I am justified in saying that the

¹ Cf. Strasburger, Die Controversen d. indirecten Kerntheilung, p. 23.

hump is brought about here, exactly as in Lilies¹, by a bending over on itself of the elliptical chromosome, and by the divarication of the limbs formed at the place of bending. Finally the chromosome splits across its middle, that is, across the hump, and the daughter-chromosomes immediately open out to form V-shaped bodies. The limbs are very short, and so the daughter-chromosome may easily be mistaken for a rod, but in a number of cases V-shaped chromosomes were certainly observed. Of course, this does not exclude the possibility of variations occurring, such as have been described² for Lilies and animal cells. In any case, it will be seen that the division of the chromosome is really longitudinal, and not a transverse one, as it would have been had not the chromosome doubled on itself to form the hump already referred to. This doubling over on itself, and the subsequent separation of the daughter-halves of the chromosome, can easily be understood by bending a wire ellipse in the manner described, and on pulling out the two ends thus formed, it will be found that the originally (approximately) parallel sides, which represent the two halves of the chromosome, are thereby separated from each other.

After the separation of the chromosomes, the two daughter-nuclei are formed, and a cell-plate appears in the connecting spindle. It is very delicate, and in spite of devoting considerable time to the question, I was unable to feel quite certain as to whether the spore-mother-cell is at once divided across by a wall into two cells. I believe, however, this is the case, and that in this respect *Fossombronia* conforms to the method of spore-formation, as seen in those other members of the Jungermanniae that were examined, in which this certainly takes place. The vacuolated character of the protoplasm, which causes it to be often thrown into plate-like films, greatly increases the difficulty of arriving at a definite conclusion. In one spore-mother-cell (Fig. 12) the division of the cell appeared to be deferred until after the second mitosis.

¹ Farmer and Moore, On the Essential Similarities existing between the Heterotype Nuclear Divisions in Animals and Plants. Anat. Anzeiger, Bd. XI, 1895.

² Farmer and Moore, loc. cit.

The daughter-nuclei rapidly divide again, and the axes of their spindles lie in different directions, so that when one is seen in profile the other is commonly viewed from the pole. I am unable to say how the chromosomes originate for the second mitosis. The whole process is passed through with great rapidity, and I did not, even from a large stock of material, secure any stages sufficiently early. But the appearance of the fully formed chromosomes, when lying on their respective spindles, imitates exactly that seen in the first mitosis. The presumption then is, that the second karyokinesis is also heterotype¹ in character, just as is the first. It is a matter of some importance to settle this question, and I hope to be able to do so when my plants fruit again. The heterotype character of the first mitosis is shared by every spore-mother-cell in its first karyokinesis, so far as it is possible to judge from the figures published by Strasburger and others. Moreover it is also characteristic of that division in the reproductive tissues of animals in which the nucleus comes out of rest with the reduced number of chromosomes. Now in animals the subsequent mitoses vary in character, and also may exhibit certain interesting and, at present, obscure peculiarities; often the next division, at any rate, is heterotype, but it may also be homotype. The divisions in the spore-mother-cell of Lilies belong to this latter category; the first mitosis is strikingly different, not only from all the preceding vegetative divisions, but also from the one which follows it within the pollen-mother-cell. The indifference manifested in the second mitosis in animals (and probably also in *Fossombronia* when compared with *Lilium*) as to whether it be heterotype or homotype, is of some theoretical interest; it proves that the apparent transverse division of the heterotype chromosome cannot be interpreted as the separation of a *pair* of V-shaped chromosomes which had become attached by their free ends to each other.

¹ A term introduced by Flemming to denote the remarkable form of karyokinesis met with in the division of the spermatocyte-nuclei of the Salamander. Archiv für Mikrosk. Anat., Bd. XXIX, p. 400 et seq.

After the final separation of the chromosomes, each of the four daughter-nuclei becomes invested with its proper wall, and the spore-mother-cell becomes divided into the four spores, the protoplasm still retaining its singular vacuolated appearance (Fig. 13). The protoplasm of each spore immediately surrounds itself with its proper membrane, the endospore being shortly afterwards also differentiated. The wall of the spore-mother-cell then becomes mucilaginous, and the same is true of the partition-walls, and so the spores are ultimately set at liberty. The solution of these walls is not easy to follow. I have met with the best results by using orange G, which stains the swelling membranes deeply, and thus marks them clearly from the proper spore-membranes. The centrospheres have also long ceased to be recognizable, and apart from the radiations which characterize them during the active periods of nuclear division, there is no feature, in these cells, by which I find them to be distinguishable. The vacuolated and rather granular protoplasm, indeed, would seem to preclude any positive conclusion as to their permanence being reached.

Pellia epiphylla.

The spore-mother-cells of *Pellia epiphylla* afford good material in which to study the changes which occur in nuclei and cytoplasm during the formation of spores. After the completion of the archesporial divisions, the spore-mother-cells become isolated from each other, and a considerable period of rest and growth intervenes before the two final mitoses take place. The effects of growth are manifested both in the cells as a whole, and also, subsequently, in the nucleus. As regards the entire cell, it conforms to, and indeed exaggerates, the type characteristic of the *Jungermannia* series of *Hepaticae*. At first more or less irregularly spherical, it soon becomes four-lobed, and these lobes increase in size, chiefly owing to radial extension, so that the spore-mother-cell ultimately comes to consist of four large sacs whose cavities communicate with each other by means of

a small central space common to them all. The nucleus is always found to be situated in this central common region (Figs. 17-20).

If the nucleus be examined at a stage in which the cell as a whole has reached rather less than one-third of its final size, it will be seen to possess a well-marked wall, within which is contained a very distinct linin thread. The latter is very much convoluted, and lies just within the wall, though some parts of the filament are also visible in the more central regions of the nucleus. It is very easily differentiated at this period, as it still possesses a considerable amount of chromatin which is distributed through its substance. There is also present one (seldom more than one) large nucleolus.

As the sporogonium increases in size the spore-mother-cells also become larger, but the process is one which takes considerable time as compared with the cells during what may be termed their archesporial period, in which both growth and division proceed rapidly. The linin next loses its definiteness, owing to the disappearance of its chromatic constituents as such, but *pari passu* the nucleolus becomes more clearly prominent. It now absorbs greedily those stains used for differentiating chromatin, although in its reactions to these substances it is not quite similar to chromatin. It shrinks somewhat in size and occupies the centre of the nucleus. Indeed at this period it is easy to mistake it for the real nucleus, for the extra-nucleolar portion now hardly differs from the cytoplasm save in its rather denser and more uniform consistency. This peculiarity is one which is shared by a large number of liverworts at the stage in question, and it is also a familiar one in the vegetative nuclei of many Algae.

In the next stage, that is, when the spore-mother-cells have reached their mature size, the nucleus again begins to alter its appearance preparatory to the two final mitoses; the nucleolus becomes less chromatic in character, and assumes a more peripheral position, whilst at the same time the linin thread is again clearly distinguishable. It is in nearly all cases seen to be specially aggregated and convoluted in the

vicinity of the nucleolus (Fig. 18). This aggregation is not, I believe, to be regarded as an artefact produced merely by the inadequate action of fixing-reagents. It is confined to these particular divisions in the reproductive cells, and is absent from all other dividing nuclei which may have been fixed in the same material and at the same time; thus it is not seen in the cells of the vegetative regions of either gametophyte or sporophyte, nor does it occur in the earlier archesporial divisions. Furthermore it is best seen in those cells which in other respects seem to be the best preserved. Again, the fact that the aggregated linin was differently orientated in even neighbouring nuclei seems to tell against the view which would regard it as a mere artefact. But I would expressly state that I did not observe the nucleolus flattened out against the nuclear membrane. This body was always free from the wall, though near to it, and thus there was no 'sichelstadium'¹ represented. This in itself is not perhaps important, since Zimmermann was unable to recognize it in all the spore-mother-cells which he investigated (e.g. *Hyacinthus caudicans*). Humphrey² has suggested that the *Sichelstadium* of Zimmermann and the *paranucleolus* of Strasburger may be due merely to the faulty penetration of reagents, but I do not think that either of these structures corresponds with the aggregation of linin I have just described. Moreover an identically similar appearance is seen in the homologous mitoses of animal cells, and my friend Mr. J. E. S. Moore informs me that it can, in some favourable cases, be discerned in the still living cell. At the same time it must be stated that at this particular epoch in the life of the cell, the protoplasm is very sensitive to the action of reagents. It is exceedingly difficult to fix properly, and it displays a tendency to break up into lumps on a slight provocation. Moreover it has suddenly changed its affinity for stains, and now greedily absorbs dyes such as safranin, which at other times but slightly affect it. Everything points

¹ Zimmermann, *Morphol. u. Physiol. d. Pflanzenzelle*, Bd. II. Heft 1, p. 9.

² Humphrey, *Nucleolen u. Centrosomen*, *Ber. d. deutsch. Bot. Gesellsch.*, 1894.

to the cell being in a very unstable condition, if I may so express it, and if it were not for the extreme regularity with which the aggregation phenomenon, above described, appears at this stage, I should have regarded it as merely an artificial contraction-effect induced by the reagents employed for fixing purposes. The close connexion of the linin with the nucleolus, and their relative alterations in staining capacity, suggests that in these cases the chromatin itself or some nearly allied substance may have been stored in the nucleolus and is now passing into the linin. At any rate it seems difficult to explain the juxtaposition of the two masses apart from some such hypothesis, since it does not seem to be related to the diffusion of reagents. It may be that it is connected with the formation of the spindle, the functional relation of the nucleolus to which has recently been suggested by Prof. Strasburger¹. But the fact that the spindle is not formed till long after this phase is over, seems to be opposed to such a view as applied to this particular case, although it by no means follows that the nucleolus may not *also* be connected with the spindle formation in other plants, or perhaps in this one too, at a subsequent period. The 'aggregation' condition very soon passes over, and the linin filament becomes both shorter and thicker. Owing to the small size of the object, it is very difficult to be quite certain whether a longitudinal splitting of the thread occurs at this period, but I think I am justified in stating that it does, but it is of a somewhat irregular character and not very well marked. At uneven intervals the linin is seen to be very much thinner than at other places, and there may nearly always be distinguished two thin filaments lying close together. In *Pellia* it is not nearly so easy to follow out the course of events at this stage as in *Fossombronia*, but what evidence there is, all points to the processes in both being in reality quite similar; and this is especially true of the succeeding changes. Eventually the chromatin is seen to be almost confined to

¹ Strasburger, Karyokinetische Probleme, Pringsheims Jahrb. f. wiss. Bot., Bd. XXVIII.

pecially-thickened areas of the linin, and these mark out the future chromosomes. In many instances it seemed as if the chromatin were grouped in four distinct patches in each chromosome-rudiment, as Brauer described it in the case of *Ascaris*¹. And in the event of this being general it would point to a double fission, whereby the chromosome elements would be thus early distributed for the two following mitoses. But I was unable to recognize this arrangement with such sufficient regularity or frequency as would lead to so important a conclusion being definitely drawn, though I regard it as a very probable one. Many chromosomes, however, at this stage showed more than four chromatic groups, and this would seem rather to diminish the value of those observations in which the fourfold grouping was distinctly visible, for there was no evidence to show that the former was merely a transitional stage of the latter; at the same time it might easily be due to the chromosome having been observed in an oblique position. Contraction of the chromosome still proceeds, and these bodies, eight in number, are clearly seen to possess the form of *rings*, or loops closed at both ends (Figs. 20, 21). At this period the nucleoli cease to be longer visible. The original large nucleolus almost always fragments, after becoming very much vacuolated, and the particles into which it breaks up are usually seen to be lying on or close to the young chromosomes. It does not of course follow from this that substance is directly passing out of them into the segments of the linin, although I think this to be not improbable. It is, however, possible that their proximity has a mechanical rather than any other relation, and that as they decrease in size, their substance at first diffuses into the surrounding nuclear sap, whatever may be its ultimate destination. The chromosomes become shorter and thicker, and their ring-like shape is no longer discernible. They are at first lying somewhat irregularly in the nucleus, but they gradually assume a regular arrangement in the equatorial

¹ Loc. cit.

plate of the nucleus. Nothing has hitherto been said as to the origin of the spindle, which must now be considered.

At an early period in the process of mitosis, a centrosphere appears at four points on the periphery of the nucleus, and these points correspond with the four lobes of the spore-mother-cell, just as in *Fossombronia*. The centrosphere contains a minute centrosome, and from it, though starting at a very short distance from it, are seen radiations which extend into the lobe in front of which it is situated. The small clear space around the granule may perhaps be caused by the fusion of the radiating filaments. Each centrosphere apparently exercises a pull on the nucleus, which becomes drawn out at each of the four spots, and forms a small protuberance into each lobe. Later on the radiations diminish, and the nuclear protuberances are less easily seen; in some cases they seem to fuse pairwise, probably by the mutual fusion of the centrospheres, but in other instances there is no doubt but that this quadripolar spindle persists through the first mitosis (Fig. 23). It must be clearly emphasized that the nuclear wall is quite visible to a late period, and that the quadripolar figure is entirely, in its earlier stages at least, of nuclear origin; only the centrosphere is cytoplasmic. But the nuclear wall can no longer be distinguished when the chromosomes reach the equator, and the achromatic spindle-fibres are completely formed. The achromatic spindle is often very short, but it varies a great deal in this respect. The difference seems to depend on the length of the arms of the quadripolar body. If they have extended far into each lobe of the spore-mother-cell, then the spindle is elongated, and it often is divided up, so that there is an apex in *each* lobe. But no simultaneous division into four nuclei follows, even in these latter extreme cases, and thus the four spindle-arms when present act in pairs. The chromosomes agree in shape with those met with in the first division of the pollen-mother-cell of *Lilium*, that is they exhibit two processes which lie along the spindle, and a humped portion which is directed outwardly from it. The same mode of division of the chromosome as has been

described in the case of the Lilies also obtains here, although it is more difficult to follow out the process on account of the minuteness of the structures in question in this plant. The daughter-chromosomes retreat as open V's with their apices directed polewards, and they aggregate as the two daughter-nuclei into two groups placed very closely to the cell-plate which forms between them. The cell-plate forms a cell-wall which exhibits very remarkable curves, the general effect of which is to enable it to meet the walls at the middle of the spore-mother-cell at right angles. In fact it appears, in this respect, to obey ordinary physical laws, since a comparison instituted between it and the behaviour of a soap film introduced into a glass model of the spore-mother-cell which I had constructed, revealed a most remarkable degree of correspondence between the two cases.

I am unable to say whether the two daughter-nuclei in *Pellia* enter into a condition of rest before they finally divide once more. This does happen in some liverworts, for example in *Scapania* and in *Lophocolea*; but if it also occurs in *Pellia* it must be very quickly got over, since I never saw any instances of it. In fact the whole process is very rapidly passed through, and intermediate stages are in any case difficult to get. But when the chromosomes of the second division are in the equatorial plate, and lying on the spindle-fibres, they resemble exactly the corresponding structures of the first mitosis. In this they are in marked contrast with Lilies. In the latter plants the second mitosis of the spore-mother-cell is strikingly different from the first one in all its more obvious features. But it does not therefore follow, in the case of *Pellia*, that a second *heterotype* division follows on the first one, because in this plant the homotype vegetative mitosis in the germinating spores are also hardly distinguishable from the heterotype mitosis when both are examined at the somewhat late stage in which I found them. The heterotype is, however, clearly recognizable in the younger condition, both on account of the very *early* longitudinal fission of the linin, and also by reason of the peculiar forms assumed by

the young chromosomes. In the absence of the early stages of the second mitosis it is impossible to be sure whether it conforms in general characters with the homotype form met with in the gametophyte, or whether it rather should be regarded as a second heterotype. There would be nothing strange in the recurrence of this latter form, since it certainly reappears in the *successive* spermatogenetic mitoses in many animals.

After the chromosomes have receded to their respective poles the cell-plate now formed is converted into a cell-wall, and thus the division of the spore-mother-cell is completed. Very shortly after these events, changes become visible in the outer and common wall of the spore-mother-cell. It becomes extremely thin, except at those parts where the lobes were in close contact, and in the meanwhile the inner, newly-formed coat begins to exhibit the spiny character which is met with in the mature spore. The peripheral parts of the membrane become more and more attenuated, and finally disappear, but the thickened portions which lie in the angles formed by the lobes persist for a time, and sometimes break away and are found lying free amongst the spores. The separation of these bodies is effected by the final disappearance of the primary division wall¹, but the period which elapses before this occurs is subject to some variation.

After the complete formation of the spores they may either rest for a time within the sporogonium, or they may proceed at once to germinate. And in the latter event the process may even commence before the separation of the spores from one another (Fig. 28).

The first sign of change is seen in the nucleus and surrounding protoplasm. Beautiful centrospheres² with abundant radiations appear on opposite sides of the nucleus, which is rapidly drawn out in the direction of the longest axis of the spore.

¹ This agrees with Strasburger's statements, *Zellbild. und Zelltheil.*, dritte Auflage, p. 157.

² Cf. Farmer and Reeves, On the occurrence of Centrospheres in *Pellia epiphylla*. *Annals of Botany*, Vol. viii.

The linin goes through none of the evolutions which distinguish the first heterotype mitosis, but becomes visible as a thickening thread which is divided into eight segments. These double upon themselves, and so resemble V's whose limbs are nearly, or even quite, in contact. They place themselves on the spindle with their angle directed centrally, and at once split longitudinally. But the fission, thus deferred beyond the time usually assigned to even homotype divisions, is not at first complete through the entire length. It commences at the angle, and the split portions at once diverge polewards along the spindle-fibres. Thus an appearance is produced which irresistibly recalls that presented during the final separation of the segments of the heterotype chromosomes, and it is only the differences in their respective earlier stages which differentiates the one from the other. Further, a comparison of *Pellia* with *Fegatella*, in which the spores also germinate while still within the sporogonium, conclusively shows that we are here dealing with a true, if somewhat aberrant, homotype mitosis. For the process of karyokinesis in *Fegatella* is almost diagrammatic in the regular and typical succession of the events and in the distinctness of the several stages.

The nuclei of the *sporophytic cells* exhibit sixteen chromosomes, instead of eight, when in the equatorial plate, and thus this plant furnishes another instance of the correctness of Overton's view as to the correspondence of the reduction in the number of these bodies with the periodic alternation of generations.

I observed a slight amount of variation in the number of the chromosomes in the nuclei of the germinating spores (gametophyte). Thus in two cases there were certainly *nine*, and not eight chromosomes as in the great majority of the nuclei, and in one case I could only count seven. This last instance was taken from a very good preparation, and I do not think that there was any reason to think one chromosome had become displaced or that it had been so obscured by another one that I missed counting it. But these facts do not

invalidate the general rule that the number of the chromosomes is normally constant. Cases are known both in plants and in animals of a diminished or increased number occurring as exceptional cases. Of course there are the further instances of the antipodal cells of Lilies described by Guignard, and of the late endosperm cells in *Pinus* mentioned by Dixon; these, however, occur in declining tissues, and are therefore perhaps not to be regarded as on the same line as variations occurring in the first stages in the existence of the organism.

***Aneura multifida*.**

The process of spore-formation in this plant recalls that observed in *Fossombronia*, except that the number of the chromosomes are more numerous. A very well-marked quadripolar spindle is seen during the earlier stages, and the end of each arm, which protrudes into the rather shallow lobes of the mother-cell, is occupied by an exceedingly well-marked centrosphere, with exquisite radiations extending from it. The division of the nucleus is successive, and an interval of rest occurs between the first and second division. These two mitoses are, so far as I could determine the point, exactly alike, and when seen in the equatorial plate the chromosomes often exhibit the ring form with greater or less distinctness (see Fig. 36). It would be very desirable to repeat these observations on *Aneura pinguis*, of which I have for two years endeavoured to secure sufficient abundance of fruiting specimens, hitherto without success. The nuclei are very much larger in this species, and are in every way better fitted for accurate and close observation than is the case with those of *A. multifida*. In the latter plant it was clearly seen that the number of the chromosomes is reduced to one half between the last archesporial and the first spore-mother-cell division, and also that the appearance of the chromosomes in the two cases respectively is very different, just as has already been mentioned for other genera.

***Fegatella conica*.**

The characters of the spore-formation as presented by this

plant differ considerably from those exhibited in the *Jungermannia* series. The spore-mother-cell is a flattened oval body, rather like a biscuit in shape. The nucleus is prominent and large and is enclosed in a coarsely granular protoplasm, from which it is separated by a very well-marked membrane. The spore-mother-cells increase very greatly in size between the last archesporial division and the two final mitoses which result in the formation of the spores. As the time for these last divisions approaches, the character of the protoplasm alters; it evinces a much greater readiness to take up stains, and is more difficult to fix than at other times. But the latter remark does not apply to the nucleus, which in this particular plant is easy to fix, and gives very clear preparations, even at this, the most critical and usually most difficult period.

It is a noteworthy fact that neither in this plant, nor in any member of the *Marchantia* or *Riccia* series which I examined, was a quadripolar spindle at any time observed¹. I think that this point is of some interest when taken in connexion with other differences which exist between these groups and that of the *Jungermannia* alliance. In all of the former sections (with perhaps one exception), the *absence of the lobed character* of the spore-mother-cell was noticed, and this is very marked when one bears in mind the frequency, perhaps the universality, of this peculiarity in the *Jungermannia* series. It is true that the flattened character of the spore-mother-cell, which is so striking a feature in *Fegatella*, is absent from other forms, such as *Marchantia* (two species examined), *Plagiochasma*, *Fimbriaria*, *Riella*, *Riccia*, and *Targionia*, in which the spore-mother-cells are spherical. The last-mentioned plant is of interest inasmuch as its spore-mother-cells are at first slightly four-lobed, and there is some doubt as to its agreement with the rest in the main features of its cell-division. Unfortunately, however, its protoplasm is excessively difficult to fix properly, on account, probably, of the large quantity of oil-drops which it contains. Thus, although I had a con-

¹ With the possible exception of *Targionia*, reckoning it with the *Marchantiaceae*. See *infra*, p. 499.

siderable amount of material at my disposal. I am unable to speak with confidence about the more delicate details of its nuclear division.

As the spore-mother-cell of *Fegatella* begins to prepare for its final divisions, besides the changes in the cytoplasm already alluded to, an alteration is seen in the structure of the nucleus. At first this body is not very easy to discriminate. The linin takes no stain other than that absorbed by the cytoplasm, and it is the nucleolus, with its intense capacity for absorbing dyes, which strikes the observer. But as time goes on, the nucleus loses its comparatively homogeneous aspect. The linin threadwork becomes plainly visible, and is arranged in an almost diagrammatic way with regard to the nucleolus. When this body happens to be so orientated as to be seen in the middle of the nucleus, the linin threads are seen to radiate out from it, like the lines of longitude from the poles of a globe. When seen from the side, a corresponding connexion is also clearly discernible. I take this to represent the same aggregation stage as is seen in *Pellia* and other forms, but in *Fegatella* the subsequent occurrences lend additional support to the view that the connexion of the linin with the nucleolus is not a merely accidental one. The nucleolus now fragments, sometimes two or three, but oftener a larger number of small nucleoli are seen, and in every case the linin filaments are associated with them. It may be urged that there can be no real importance attaching to this observation, since the large nucleolus of the pollen-mother-cells of Lilies and other plants is often not obviously connected with the linin. But even in the case of these latter cells, the nucleolus often *is* related to the linin, and Strasburger has drawn attention to it in his recent memoir. Moreover I am myself convinced, after an inspection of a large number of preparations, that there exists in Lilies also, a very important relation between the linin and the nucleoli during the earlier stages of karyokinesis. But *Fegatella* certainly exhibits this relation far more clearly than in any other plant with which I am acquainted, and it is seen not only during the differentiation

of the reproductive cells, but almost equally well during the germination of the spores.

At this period, that is, when the nucleolus is fragmentary, the longitudinal division in the linin thread is effected, but it is not easy to follow out the details owing to the obscuring effect of the nucleoli. The linin, which is now becoming increasingly rich in chromatin, swells up and finally breaks into eight chromosomes, but I could not at this time find any trace of the primary slit formed by the earlier longitudinal fission. The spindle is now formed, and the eight chromosomes lie along it. They resemble the chromosomes of *Lilium* at the corresponding stage, and each one consists of a hump or thickening, directed outwards from the spindle, and two limbs which lie along it. When they have reached this stage they may in favourable cases be recognized as being of a ring-like form (Fig. 61), though this is often difficult to determine. Eventually they divide across the middle, and the two daughter-halves retreat towards their respective poles. A cell-plate is then formed across the equatorial plane of the spindle, but it remains in a rudimentary condition, and does not effect a division of the cell. The two daughter-nuclei immediately divide again, and so far as I could determine the question, the second division is here also exactly similar to the first, in its later stages at least. There is the less doubt in this case (as compared with *Pellia*), since the normal vegetative mitoses are very different from those which we are now considering (cf. Figs. 70, 71). It must for the present be left an open question whether the second mitosis is prepared for during the formation of the young chromosomes for the first division. I failed to find any direct evidence for this hypothesis, though I especially looked for it. Sometimes, during the shortening up of the linin to form the chromosomes, a localization of the chromatin at four spots could be seen, but this did not occur with sufficient frequency to admit of any safe generalization being based on it. At the same time, a similar condition was sometimes observed both in *Pellia* and in *Fossombronia*, as mentioned above.

I hope, however, to be able to settle definitely this point, both in *Fegatella* and in those other liverworts in which I have so far failed to reach a position of certainty as to the exact mode of procedure. The omission here is due to the great difficulty of getting enough material suitably fixed at exactly the right time. This being so, it seems better to avoid speculation, which further observation may only show to be unsound. But whatever the exact mode of origin of the chromosomes previous to the final karyokinesis may be, there is no doubt whatever as to the further course of events. The eight chromosomes possess, as has been said, exactly the same shape as those in the first division, and when they divide at the equatorial plane, they exactly resemble the chromosomes of the first heterotype division. The daughter-chromosomes now retreat to the spindle poles, and radiations extend from each daughter-nucleus to the two others which are nearest to it, and, further, a spindle appears connecting the two nuclei which are situated at the obtuse angles of the rhombus formed by the peripheral spindles (Fig. 59). In this way *five* spindles are formed altogether, and in each one a cell-plate is formed. These all unite ultimately, and thus the spore-mother-cell is divided into the four special mother-cells. The elements of the nuclei have now begun to enter into a state of rest, and they do so in the reverse order in which they came out of it. The nucleoli reappear in all cases, several in number, and they are always associated with the linin thread which has been re-formed, by the fusion of the chromosomes. The filament shows frequent anastomoses, and perhaps this points to the fusion of the chromosomes not being so regular as is sometimes supposed. As the nucleoli increase in number and in total bulk, the linin becomes thinner and diminishes in its staining capacity. Gradually the nucleoli run together, and though vacuolated at first, they lose this character as the whole process nears its termination, until finally there only remains in each spore-nucleus a single large nucleolus¹, and a diffuse and somewhat indistinct tangled

¹ It is not meant to imply that the nucleolus becomes the receptacle for the

linin filament; these are all enclosed in a wall, and are bathed in sap. The nucleus as yet, however, is by no means as structureless as it (probably only apparently) becomes at the time when the spores are lying free within the sporogonium. The separation of the spores is effected by the disappearance of the outer wall of the enclosed tetrad, and by the degradation of an extremely thin film in each of the recently-formed dividing walls. It is very easy to miss the stage of disappearance of the outer walls, but it is quite clear if observed at the right time.

GERMINATION OF THE SPORES OF *Fegatella*.

The spores of this plant germinate while still enclosed within the sporogonium, as do those of *Pellia*, but the process does not begin so early as in the latter-named plant. The difference in character between the gametophyte divisions as contrasted with the spore-mother-cell mitoses is most striking. The whole process accords with what we are accustomed to regard as the normal type of karyokinesis, and in this respect it compares favourably with *Pellia*, in which owing to a comparatively slight modification the proper relations are very much obscured. But *Fegatella* also exhibits a little variation, and it was by a comparative study of these differences that I arrived first at a clear idea of the structure of the gametophytic chromosomes in *Pellia*.

The resting nucleus in the newly-formed spores contains one or more deeply staining nucleoli and a tangled thread of linin, which is especially grouped in their vicinity. The rest of the nucleus is filled with a clear nuclear sap and the whole is enclosed by a nuclear wall. As the nucleus prepares for division, the linin alters its appearance, though still very delicate, forming a convoluted thread which exhibits frequent anastomoses.

The thread is stretched across the nucleus in various chromatin. The researches of Zacharias seem to entirely negative the probability of any such crude hypothesis. [Zacharias, Ueber d. Nucleolus, Bot. Zeit. 1885.] See also below.

directions, and its general character recalls the framework of radiating filaments in a spider's web. The nucleolus is situated in the middle of these radiating threads, and the appearance is such as to strongly suggest an intimate connexion between the two structures. As the linin becomes more pronounced in character, the centrospheres are seen on the periphery of the nucleus at opposite sides. They are difficult to distinguish at this stage, on account of the feebleness of the radiations, later on they are however readily recognized.

The nucleolus now becomes very much vacuolated, and no longer stains with its former intensity, but as the nuclear sap remains quite unstainable, and the only structure which at this point suddenly begins to absorb dyes greedily is the linin, it seems not unreasonable to conclude that something has passed out of the nucleolus to the threadwork. At any rate one is otherwise driven to suppose that the material which leaves the nucleolus, and thereby causes its vacuolated appearance, alters its reactions to stains entirely as it diffuses out into the nuclear sap. After the vacuolation just spoken of the nucleolus commonly fragments, and finally disappears at the formation of the achromatic spindle.

Meanwhile changes have gone on in the linin filament. Certain portions of it, which are relatively somewhat elongated, have become thickened, and now greedily absorb the ordinary nuclear stains. These thickened portions are the rudiments of the young chromosomes, and they certainly owe their present features to the increase of chromatin within the linin substratum. It is not easy to assign an origin to this chromatin which thus appears in these localized spots. The behaviour of the nucleolus already alluded to strongly suggests that it may have some share in the process, but I do not think it at all probable that it is *alone* concerned. Professor Strasburger¹ has suggested that the chromatin originates from the cytoplasm, in part at any rate, and I am inclined to attach importance to this possibility. Perhaps both nucleolus and cytoplasm may each furnish their respective

¹ Karyokinetische Probleme, Pringsheims Jahrb., Bd. XXVIII.

portions, which only assume all the characters of chromatin when they meet in the nuclear framework.

The regions in the linin which remain destitute of chromatin form the separation limits between the individual chromosomes. The latter bodies, however, still remain united by these delicate filaments even when they begin to group themselves under the nuclear wall. The chromosomes are also irregularly connected with each other by similar threads attached to other parts than the ends.

The origin of the latter filaments is obscure, but I regard them as being of linin origin¹. They become later indistinguishable from the threads of the achromatic spindle, though it is not possible, as yet at any rate, to state definitely that they form any component structural elements in this body. The young chromosomes are at first of an irregular shape, but later they nearly always assume the form of the well-known bent rods, and on the appearance of the spindle they become attached in the equatorial plane at its periphery with the free ends directed outwardly from it. Each chromosome is now divided longitudinally by a split in the plane of the whole bent body. Nearly always the split is complete, and the two chromosome-halves then become 'roped up' so to speak along the spindle, in such a way that the apex of each V is directed towards the pole. In nearly every case a well-marked sheaf of fibres is attached to this apex and seems to pull the chromosome towards its destination. The two sister-halves are commonly connected across the equator, from which they are now diverging, by connecting fibres. The latter fibres become much more numerous as the chromosomes recede from each other, and a cell-plate is subsequently formed across them in the usual way. Very often some of the connecting fibres stray away from the main spindle and become attached to nucleolar-like granules, which are very

¹ It will be recollected that the linin exhibits frequent anastomoses, and possibly the structures in question may owe their origin to these; at the same time it may be pointed out that young chromosomes often exhibit a ragged surface, even where anastomosis is not frequent.

abundant at this stage in the cytoplasm about the spindle. The appearance of these granules, and their relation with the spindle-fibres is such as to lend support to Strasburger's view of the nutritive relation of the nucleolus to the cell-plate.

Sometimes, however, the process is not so regular, as regards the behaviour of the chromosomes, and this especially applies to the mode of fission of these bodies. If they split through their middle portions, but not quite out to the ends, a complicated figure may result, and if further, the limbs of the V-shaped chromosome become closely approximated instead of widely separated, the same appearance met with in *Pellia* is produced. The apices of each half of the V (which may be regarded as split in the plane of this paper) then diverge towards their respective poles, whilst the ends of the two limbs are as yet undivided. It is obvious that in this way a figure may be produced which is essentially similar to the normal splitting of a heterotype ring, though the events which have preceded this particular state differ widely in the two cases. But it is of interest to note that this *delayed* fission (so to speak) may occasionally occur in *Fegatella*, whereas it is the ordinary event in *Pellia*.

It is remarkable that at the aster stage the radiations around the centrospheres die away completely for a time¹, and the centrosphere can no longer be readily distinguished. But just before, and during, the migration of the daughter-chromosomes to the poles, the radiations again become perfectly distinct. It is well known that the duration of the aster stage is relatively of considerable length, and thus perhaps a condition of comparative repose is temporarily established; this is, however, again broken during the final movements of the daughter-chromosomes to the poles, and it may be that the reappearance of the radiations, which certainly often extend to the periphery of the cell, is directly connected with this renewed activity, and renewed necessity for fixing the position of the poles. After the chromosomes have arrived at their destination, the daughter-nuclei become

¹ See also *Pellia epiphylla* for a similar occurrence (Farmer and Reeves, loc. cit.)

rapidly formed. The cytoplasmic radiations from the centrospheres entirely disappear, and with them the possibility of distinguishing either centrosphere or centrosome. The connecting fibres, however, persist for a short time, until the spore is completely divided by a cell-wall.

The most noticeable feature of the daughter-nucleus is the extreme clearness with which the stages by which it passes into the resting condition are shown. Very shortly after the appearance of the nuclear wall, the nucleoli are formed within it, and they are exactly similar to the small bodies already alluded to which are discoverable about the connecting fibres at this stage. They are at first small, and often two or three in number, and they exhibit the same relation to the linin framework as has been already described in the earlier phases of division. At first they are vacuolated, and this condition persists for a while after their fusion into a single large one, but gradually this appearance is lost, and *pari passu* the linin loses its chromatic constituent, or at any rate it is no longer distinguishable. The threads of linin now become excessively fine, but they are still quite clear within the nucleus, and they are stained brown, like the cytoplasm, by Gentian Violet and Orange G., whereas the nucleolus absorbs the Gentian Violet just as the chromosomes did when still rich in chromatin.

NUCLEAR DIVISION IN THE SPOROPHYTE.

I observed several cases of karyokinesis in the sporophyte of this plant, including cells of the archesporium and also of the wall of the capsule. I did not investigate them minutely beyond assuring myself that in both tissues there were sixteen chromosomes in each nucleus instead of eight, as in the gametophytic nuclei.

Plagiochasma, Riella, Marchantia, Fimbriaria.

Plagiochasma, in the general character of its spore-formation, recalls that of *Fegatella*, but it differs from it in possessing a spherical spore-mother-cell instead of a flattened one. The

spore-mother-cells, as in the other plants, rest for a considerable time before entering on their final divisions, and they also increase in bulk very considerably during this interval. The behaviour of the nucleus resembles that of *Fegatella* that it is not necessary to enter on details here, especially as I was not able to get an unbroken series of stages. The first mitosis does not result in a division of the mother-cell; a cell-plate is formed (Pl. XVII, Fig. 56), but it remains in a rudimentary state. Immediately after the first division, the two daughter-nuclei divide again (Fig. 57), and the resulting four nuclei take up positions similar to those occupied in a dicotyledonous pollen mother-cell (Fig. 58). The cell is then simultaneously divided into four daughter-cells, the protoplasm of which speedily becomes invested by a new membrane. The four cells then separate by the disintegration of the original wall together with the partition walls.

Essentially the same course is followed during the spore-formation of *Riella*¹, in two species of *Marchantia*, and in *Fimbriaria*², and they all differ from *Fegatella* in the spherical shape of their spore-mother-cells. It is a marked character of all these plants of the *Marchantia*-series that quadripolar spindles are *not* present. It is impossible to avoid the conclusion that this is connected with the difference in the shapes of their respective spore-mother-cells at the commencement of nuclear division, especially the presence or absence of lobes. *Targionia* is an interesting form in this respect. It does exhibit a slight amount of lobing, but unfortunately the spore-mother-cells are so exceedingly difficult to fix that at present we can hardly speak very confidently about it. But in several cases I saw what I take to be quadripolar spindles, and I do not think that I have been deceived by mere contraction figures; I hope, however, to re-investigate this plant when it next fruits. The great difficulty which arises in fixing the cell-contents arises from the frothy character of the protoplasm, which is filled with

¹ I am indebted to the kindness of Prof. Strasburger for material of this plant.

² Prof. D. H. Campbell kindly sent me material of this plant.

oil globules. At present, however, this is the only liverwort allied (almost certainly) with *Marchantia* in which I have observed any sign of a quadripolar spindle.

Scapania undulata.

The cells in *Scapania* after the last archesporial division become loosened from each other, and each one grows to a considerably increased size. As this increase becomes apparent, it is seen that the growth is not uniform, but that it is more active in four equidistantly-placed regions, and thus the cell as a whole assumes the four-lobed shape which is so characteristic of perhaps all the *Jungermannia* series of hepatics. The nucleus is situated in the centre of the cell, and consequently at the junction of the cavities formed by the four pouch-like outgrowths. As the spore-mother-cell approximates to its final size, the nucleus alters its appearance. During the earlier period, it is a rather diminutive spherical object, containing a small but readily stainable nucleolus. The extra-nucleolar portion, however, hardly stains at all, and it is difficult to make out any structure within it. But on passing out of the period of repose, the extra-nucleolar part becomes granular, and the linen framework is easily discernible. The nucleolus loses its compact and almost solid appearance, and often becomes vacuolated. At the same time the nucleus as a whole exhibits a change of shape. From being spherical, it passes into a kind of tetrahedral form with one angle directed towards the cavity of each of the four cell-lobes respectively. This appearance becomes accentuated as time goes on, until a quadripolar spindle is formed (Pl. XVII, Fig. 42). I was unable, in this plant, to observe with certainty the presence of well-defined centrospheres, and this seemed a little strange, as they are so obvious in some other liverworts (cf. *Fossombronia*). The duration of the quadripolar spindle condition is but brief, and it is eventually replaced by an ordinary bipolar spindle. But when this spindle is examined, it is seen to be always arrayed with regard to the symmetry of the spore-mother-cell. That

is, one of its poles is directed (apparently) into the cavity of a lobe, while the other is seen to terminate about a partition between the two lobes. And if the spindle end which occupies the latter position be regarded carefully, it will become obvious that it may sometimes fork, and that each limb of the bifurcation ends in an adjacent lobe. This is shown in Pl. XVII, Fig. 43. The apparent termination of the opposite end of the spindle in one point is, I believe, due to the fact that one sees the bifurcating limbs lying exactly one above another; that is, it is in reality similar to the lower end, but the limb which enters the fourth cell lies in the same vertical plane with its fellow. Thus it seems clear that the apparent bipolar spindle here is the result of a more or less complete coalescence of *four* original protuberances.

I am strongly of opinion that the spindle here produced is of *nuclear* origin. During the quadripolar condition the chromatin is seen to be distributed in the form of eight little aggregations, much like those in *Fossombronia*. These little aggregations (Figs. 41 and 42) are the rudiments of the future chromosomes, and there can exist no doubt but that these bodies form *closed rings*; when they are mature they take up a position just beneath the nuclear wall, and flatten out, so that they soon appear as mere rods. After the disappearance of the nuclear wall, they lie with their long axes directed along the spindle, and they then split across the middle as shown in Fig. 46. The daughter-chromosomes move apart, and a cell-plate is formed, which ultimately forms a membrane dividing the spore-mother-cell into two parts. Each of the two daughter-nuclei then divides once again, and the process is, so far as I have been able to follow it, an exact repetition of the first mitosis. Both of these two divisions are then *heterotype*.

Cephalozia bicuspidata.

The stage in this plant where a quadripolar spindle might have been expected was not observed, but during the first mitosis the forked spindle so nearly resembles that of *Sca-*

pania, that it seems reasonable to conclude that both have passed through a similar history. The chromosomes are much smaller. The same curious prophase was seen in this plant as has been described for *Scapania*, but it is not so obvious owing to the smaller size of the cells, and still more, to the increased number of the chromosomes. In the mature spindle there are about eighteen chromosomes, and as they are very small, it is exceedingly difficult to follow out the minute details. This much is however certain, that they consist of flattened rings, and that they divide across the middle, thus serving as another example of a heterotype mitosis. The second division is exactly like the first, and both differ markedly from the divisions which obtain in the gametophyte. I did not see any mitoses in the archesporial cells.

***Lophocolea bidentata* and *L. ciliolata*.**

The same features which characterize *Scapania* are also met with in *Lophocolea*, but in some respects the latter is a more advantageous object than the former, inasmuch as the spore-mother-cells mature more gradually, and thus several stages may often be made out in one preparation. The lobing of the mother-cell is a little more marked than in *Scapania*; the nucleus is situated, in both groups, in the centre of the lobed cell. It seems, however, a singular fact at first sight that the quadripolar spindle, so striking in *Scapania*, is difficult to distinguish here, although the tetrahedral shape of the nucleus becomes plain enough. I do not, however, attach very much importance to this, as experience shows that the stages at which this form of spindle is clearly recognizable may be very transient, and that in this respect much variation is shown amongst even closely allied forms.

The nucleus, during the earlier period, consists of a slightly staining ground-substance, which surrounds the nucleolus. This body stains sharply and intensely, but later on, it becomes vacuolated, and stains far less readily. This change occurs simultaneously with the initial differentiation of the chromosomes. These bodies originate as granular areas, each

of which is separate from all the rest, and rather resembles a little heap of coarse sand when treated with reagents which specially stain the chromatic elements of the nucleus. Later on, however, as the granular aggregations become more pronounced, the connecting groundwork of linin is also visible. Each chromosome-rudiment subsequently assumes an elliptical shape, which eventually passes over into the well-known rod-like shape, with a hump or swelling occupying the place where the fission finally takes place. After the separation of the daughter-chromosomes, a cell-wall is formed across the spore-mother-cell, and the two daughter-nuclei become enclosed in proper nuclear walls before entering on the final mitosis. The nucleolus also reappears within each of them. Owing to the very small size of the objects in question it was not found possible to trace certainly the earlier stages, but at the time when the aster condition is reached, the two dividing nuclei precisely resemble the original one in the spore-mother-cell. Thus it would seem probable that two heterotype divisions succeed each other here, instead of a difference obtaining between the first and second mitoses, as in Lilies.

After the completion of the process the spore-mother-cell is divided into the four special mother-cells, and the protoplasm within each one becomes surrounded with its own proper membrane. The spores are set free, as in other forms, by the disintegration of the original walls. It is very difficult to follow this in *Lophocolea*, but I have found either Bismarck Brown or Orange G. a good staining reagent for this purpose.

Frullania dilatata was also examined. It exhibited a very distinct quadripolar spindle, but the cell-contents are so exceedingly difficult to fix that I was unable to obtain any preparations of sufficient excellence to enable the entire process of karyokinesis being satisfactorily followed out. This is to be regretted, as the structure of the sporogonium in the Jubuleae section of liverworts is rather distinct from that of the others. But I saw nothing which would lead one to suppose that the process of spore-formation differs to any marked extent from that in the other *Jungermanniae* which I examined.

GENERAL CONCLUSIONS.

In endeavouring to obtain a general survey over the various processes which have just been detailed, one is struck at the outset by the difference existing between the behaviour exhibited by the cytoplasm in the *Jungermannia* and *Marchantia* series respectively. The (probably) constant appearance of four attraction-spheres in the four-lobed cells of the former, coupled with the absence of this peculiarity in the spherical or regular biscuit-shaped cells of the latter, can hardly be devoid of significance, and it seems to me it may have some bearing on the nature and function of these much-discussed structures.

The facts may be briefly summarized as follows. In the ordinary cells, whether of the archesporium, as in *Fossombronina*, or in the gametophyte, as in *Fegatella* and in *Pellia*, amongst the first signs of nuclear division is the well-marked appearance, at the two opposite poles, of a fan- or brush-like series of radiations. These can often be traced to a minute dense particle situated on the outside of the nuclear wall. Until the radiations become visible, there is nothing to distinguish the particular particle just mentioned from the many other granules of various sorts and sizes which coexist with it in the cell. As soon, however, as the radiation-systems are formed, they are seen in these cells to be definitely referable to certain focal spots, in the centre of each of which the 'centrosome' is usually to be discovered. But I did not always observe one body only, even during the early stages. Sometimes it was possible to speak of a *microcentrum* in Heidenhain's¹ sense of the term, for as many as three or four of these point-like granules might occur in the centre of each of the two systems of radiations. Just around the centrosome, or centrosomes, a clear hyaline space can be discerned. I believe this to be due mainly to diffraction, but partly, perhaps, also to the fact that a large number of hyaline radii are

¹ Heidenhain, Neue Unters. über d. Centrankörper und ihre Beziehungen z. Kern und Protoplasma. Arch. f. Mikr. Anat., Bd. XLIII.

converging to this point. The radii themselves often extend quite out to the periphery of the cell in the direction of the polar axis, whilst those on all sides of this axis exhibit the well-known system of curves produced by holding a horseshoe magnet below a paper covered with iron filings. Now it is not practicable in these cells with their granular contents to demonstrate the permanence of the centrosomes during those periods of inactivity when the radiations are absent. Thus I find that they become indistinguishable through a considerable part of the long interval which elapses whilst the nucleus remains in the aster stage, although they may often be quite plainly seen both before and after this period. Nor can they be recognized in the resting-cell. Even assuming them to be the permanent organs of the cell that Boveri¹ claims, they could scarcely be differentiated from the other granules scattered about in the protoplasm. Boveri and with him many other cytologists believe that they do actively direct and control the process of nuclear division, and these writers insist on their multiplication by fission alone—a necessary condition of the view of their morphological permanence. It may incidentally, however, be pointed out that their apparent formation *de novo*, which especially occurs during the maturation of the ova of certain animals, still demands a satisfactory explanation, regarded from this standpoint.

And when we turn to the spore-mother-cells in the *Jungermannia* series of *Hepaticae*, we are confronted with still other difficulties. Here we must assume that the centrosome either divides into four, simultaneously, or that the full number is reached as the result of a second bipartition, which might happen after the divarication of the first pair. And in either case it seems odd that the systems of radiations should defer their appearance until after the centrosomes have taken up their proper positions opposite each cell-lobe, seeing that the act of translation involves definite activity. This difficulty is also met with in a simpler form even in normal bipolar spindles,

¹ Boveri, Ueber das Verh. d. Centrosomen b. d. Befrucht. d. Seeigeleies. Verhandl. d. Physik.-Med. Gesellsch. z. Würzburg, Bd. XXIX.

for in ordinary plants there seems to be nothing which corresponds to the early spindle of animal cells, which arises between the two diverging centrosomes. But, especially in these four-lobed cells, it is difficult to conceive the nature of the emanations from the centrosomes which in some way are supposed to effect their transportation to their several peculiar destinations. No signs of any operating forces are to be *seen*, until the four systems of radiations suddenly start into existence in the places appointed to them by the configuration of the spore-mother-cell. And yet, if we assume with Boveri¹ that the centrosome is 'Ein der entstehenden Zelle in der Einzahl zukommendes distinktes dauerndes Zellenorgan, das, durch Zweitheilung sich vermehrend, die dynamischen Centren für die Entstehung der nächst zu bildenden Zellen liefert,' if we accept this descriptive definition, then it follows that we must also admit that the four daughter-centrosomes both initiate and direct their own transportation to their ultimate destinations in the cell.

For my own part, I find less difficulty in accepting the view that the centrosome merely acts as an 'Insertionsmittelpunkt,' a view which Heidenhain has so ably supported. It further appears to me to be most natural to explain the occurrence of these *normal* quadripolar spindles in the *Jungermannia* series as the result of the simultaneous activity of four independent cytoplasmic kinetic centres, which owe their existence to the peculiar conformation of the cell as a whole. There is evidence to show, in the early stages of even normal bipolar divisions, that the cytoplasm (kinetic centres) exercises a 'pull' on the nucleus. This is well seen, for example, in the nuclei of the germinating spore of *Pellia*. The spindle-shape assumed by the nucleus, previous to the disappearance of its wall, indicates this; and in those animal cells in which a central spindle is first formed remote from the nucleus, the same effect is seen in the roping-up, as it were, of the chromosomes, which thus become caught up and pulled on to the spindle.

And in the four-lobed cells of these Hepaticae, the pulling

¹ Boveri, loc. cit., p. 60.

strain exerted on the nucleus takes place in four directions, each of which corresponds to the localization of a mass of protoplasm which is comparatively isolated from its neighbours by reason of the four pouch-like outgrowths of the spore-mother-cell. Furthermore the appearance of centrosomes in these cells, wherever they can be best made out, is not of such a character as to inspire confidence in their claims to individuality. They vary both in size and in number, even within the same cell, and this irregularity they share in common with animal centrosomes, as for example in the tissues investigated by F. Reinke and by Heidenhain. But if they mark mere nodal points, as it were are granules which have been pushed or drawn into a position of stable equilibrium in the centre of those forces of which the radiations may not unreasonably be regarded as the optical expression, there seem to be no grounds for expecting them to exhibit uniformity, either in size, number, or texture. In all of these respects we do, as a matter of fact, find differences and variations, both in animals and in plants.

Again, it is by no means obvious, on the assumption of their individuality, why the centrosomes in these four-lobed cells should take up positions, and start systems of radiations, which in most cases are soon abandoned. In *Fossombronia* and in *Aneura*, in which plants I obtained the clearest cases of four equidistant centrospheres, these structures gradually approximate, and I believe (though I did not actually observe the process) that they finally coalesce in pairs to form the first karyokinetic spindle which, when mature, is *bipolar*, as in ordinary nuclear division. Those plants in which the poles of these spindles do not fuse to give rise to the bipolar condition, always disclose good reasons why this should not be the case, as for example in *Pallavicinia decipiens*¹, in which the lobing of the cell is carried to such a point that no room is left for a normal straight spindle to be formed.

Thus it would seem that in cases such as that of *Fossombronia*, the direction of the strains which are exerted on the

¹ Farmer, Studies in Hepaticae, Ann. of Bot. 1894.

nucleus is determined proximately at least by the cell-protoplasm; and that if circumstances admit of it, the original *four* separate strains are replaced by *two*, which act in the direction of the resultant. It may further be observed that a closer similarity exists between these quadripolar spindles, and ordinary bipolar ones, than might at first sight appear to be the case. It is usual for a cell to divide transversely to its own long axis, and it is a significant fact that the nucleus is acted upon by a larger mass of protoplasm in this than in any other direction. It is known that the order of succession of walls in spherical cells may sometimes vary, and it may well be that slight differences in the general symmetry of the whole cell determine ultimately the particular axis of such nuclear divisions; thus the geometrical considerations, so clearly enunciated by Sachs, harmonize also with the dynamics of cell-division as deduced from a study of the behaviour of the protoplasm during the process. The quadripolar spindle then is only a special case of ordinary karyokinetic phenomena; instead of two relatively large masses of protoplasm, there are four distinct aggregations, one in every lobe, each exercising an independent strain, and the directions of the strains may continue separate to the very end of the process, or not, according to the form and other special circumstances of the cell.

The existence of the centrosome is, for me, a secondary matter. I regard it as a mere insertion point, a granule or perhaps a condensation mass; and thus when it is present, it is immaterial whether its origin be nuclear or cytoplasmic. It is certain at all events that it may exist either within or without the nucleus. Thus in *Ascaris megalocephala*, var. *bivalens*, the centrosomes are extra-nuclear, whilst in the variety *univalens*¹, of the same species, the centrosomes, together with the whole achromatic spindle, arises within the nucleus. The same is true of the spermatogenetic cells of some birds, and from Fairchild's researches on *Valonia*, it

¹ Brauer, Die Spermatogenese von *Ascaris megalocephala*. Arch. f. Mikr. Anat., Bd. XLII.

would seem that in this plant, the centrosomes, if present, are also intra-nuclear. Their particular location depends on the arrangement and position of the systems of forces operating in the cell. I have already mentioned the fact that cases are known in animal cells, and particularly in those mitoses which accompany the maturation of the ovum, in which no centrosome presides over the process of karyokinesis by which the polar bodies are cut off. Brauer¹ has described such a case in *Artemia*, and when a centrosome is finally summoned into existence, it seems to arise as the result of a mere condensation of protoplasm.

So long as it was believed that the male and female centrosomes generally fused during fertilization, it was very natural (though not necessary) that considerable importance should be attached to these bodies, but we now know that as regards animals, this fusion does not take place, and that Fol's statements were based upon defective preparations. Sometimes the centrosome connected with the ovum², oftener that associated with the sperm nucleus³, alone persists, whilst the other one disintegrates and becomes lost, merged in the general cytoplasm. Another factor which enhanced the value of the centrosome depended on the discovery that during nuclear division these bodies also divide, and further that, in some cells at any rate, they persist through the interval which elapses between two successive mitoses. But in connexion with the latter point, it may be remarked that the divisions in these instances often succeed each other with such rapidity that the cell has not, in the interim, settled down to a state of rest. In other cases again, such as in certain cells described by Heidenhain⁴, it is not clear whether

¹ Zur Kenntniss d. parthenogenet. sich entw. Eies v. *Artemia salina*. Arch. f. Mikr. Anat., Bd. XLIII.

² Wheeler, The Behaviour of the Centrosomes in the fertilized egg of *Myzostoma glabrum*. Journ. of Morphology, Vol. x.

³ Julin, Structure et Développement des glandes sexuelles . . . chez *Styelopsis grossularia*. Bull. Sci. de la France et de la Belgique, T. XXV, p. 56 of the separate copy.

⁴ Ueber Kern u. Protoplasma. Festschr. für A. v. Kölliker, 1892.

the 'microcentrum' does not really represent stages in the disintegration of the original centrosome. The researches of Moore on *Branchipus*¹ are of interest in this connexion. During the earlier phases in the karyokinesis in the spermatocytes of this crustacean, granules of a fairly large size (the pseudosomes of Moore) are distributed through the protoplasm. They are connected together by cytoplasmic filaments so as to form a sort of net-work, but are clearly visible as separate particles during the earlier period in the existence of the achromatic spindle. Finally, they fuse up to form one gigantic 'centrosome,' and the spindle becomes henceforth normally bipolar. It would seem as if these separate particles were at first sufficient to start a number of centres of forces in the cell, but that these gradually drew together, and finally after the pseudosomes had been forced to coalesce, a single kinetic centre became a possibility at each end of the cell. The same phenomenon of multipolar spindles has been observed by several investigators² in the early stages of nuclear division in Lilies and other plants, though these usually pass over into the normal bipolar type at a subsequent period. Still any large mass in the vicinity of the spindle may serve to disturb the uniformity of the figure, as I have elsewhere described in *Lilium Martagon*³. These considerations, then, seem to confirm the suggestion that the quadripolar spindles of these Hepaticae are really only the result of the special conditions imposed by the configuration of the spore-mother-cell.

As regards the change which takes place within the nucleus, the chromosomes and the nucleolus have long attracted special attention. To the latter body indeed almost every conceivable function has been attributed. The reason for this is to be sought in the extraordinary difference in behaviour which the nucleoli of different nuclei exhibit during karyokinesis.

¹ Some points in the Origin of the Reproductive Elements in *Apus* and *Branchipus*. Quart. Journ. of Micr. Sci., Vol. xxxv.

² Belajeff, Zur Kenntniss d. Karyokinese b. d. Pflanzen. Flora, Ergänzungsbd. z. Jahrg. 1894.

³ Ueber Kerntheilung in *Lilium-Antheren*, besonders in Bezug auf die Centrosomenfrage. Flora, 1895.

In the liverworts examined by me, the nucleolus was associated with the chromosomes in an unmistakable and remarkable manner. In other plants (e.g. Lilies), on the contrary, this relation may be merely subordinate in character, whilst large portions of the fragmented nucleolus are cast out into the cytoplasm. This occurs in *Lilium Martagon*, and according to Zimmermann¹ it holds good also for a large number of diverse plants. Zimmermann's observations have been challenged by Humphrey², but I can safely state that in the account which Zimmermann gives of the process in *Lilium Martagon* he is certainly correct, and that the appearances shown in his figures may be seen in almost any well-preserved material which has been appropriately stained. There is also evidence to show that the nucleolus may behave differently in different tissues of even the same plant. Thus Guignard³ found that in the archesporial cell-divisions of *Psilotum*, when the chromosomes have congregated in the equatorial plate, the nucleoli, which have diminished somewhat in size, are cast out into the surrounding cytoplasm. But in the spore-mother-cells of the same plant the nucleoli are almost entirely dissolved within the nucleus, and at most only a few relatively small particles are cast out and continue to be recognizable in the cytoplasm. This latter observation seems at first sight hardly to harmonize with the course pursued by the same body in *Lilium*, but it must be remembered that in the latter plant the nucleolus reaches an enormous size, and that it does, as a matter of fact, lose a good deal of its substance (as is proved by the extensive vacuolation) before its remains are extruded into the extra-nuclear protoplasm.

In a considerable number of cases, the decrease of nucleolar substance is contemporaneous with the growth of the chromosomes, and I cannot regard this coincidence as a merely accidental one. I have been led to this view, not only by the study of the earlier phases of karyokinesis, but also by a con-

¹ Zimmermann, Beitr. z. Morph. u. Physiol. d. Pflanzenzelle, Bd. II, Heft 1.

² Nucleolen u. Centrosomen. Ber. d. deutsch. Bot. Gesellsch., 1894.

³ Guignard, Sur l'origine des sphères directrices. Journ. d. Bot., 1894.

sideration of the events which occur during the reconstruction of the daughter-nuclei. But I may at once say that I do not believe that the *entire* nucleolus is concerned in the process. For quite apart from the fact that the disappearance of the nucleolus does not, by any means, always coincide with the growth of the chromosomes, there are chemical considerations which render any such hypothesis untenable.

Zacharias¹ has clearly shown that the chemical constitution of the chromosomes, and certainly that of their chromatin component, is by no means identical with that of the nucleolus; for whereas the former contains nucleic acid in combination with other substances, this is wanting in the nucleolus which consists of plastin and albumen. But albumen also forms a constituent of chromatin, according to the statements of Zacharias and also of Kossel², who regards chromatin as containing, besides nucleic acid, a variable quantity of albumen. Zacharias³ has also pointed out that the male nuclei, which are so rich in nuclein, are usually destitute of a nucleolus, whilst the oosphere nucleus is poor in nuclein, but possesses a large nucleolus. The inference to be drawn from this observation seems to be, that the nucleolus, though not in itself containing chromatin, is able to furnish at least one, and that probably the albuminous constituent of this substance, and that when the chromatin diminishes, as happens during the final stage of karyokinesis, it again decomposes, and that the albuminous matter contributes to the rehabilitation of the nucleolus. This would perfectly agree with Kossel's observation that chromatin can be split up into albumen and other substances.

It seems hardly possible to explain the intimate relations which exist between the linin and the nucleolus, during the early and latest phases of mitosis in the liverworts, as a merely accidental circumstance. Not only their mutual positions, but also the relative differences in their staining capacity, all point

¹ Zacharias, Ueber den Nucleolus. Bot. Zeit., 1885; also Ueber d. chemische Beschaffenheit v. Cytoplasma u. Zellkern. Ber. d. deutsch. Bot. Gesellsch., 1893.

² Kossel, Ueber die Nucleinsäure. Verhandl. d. Berl. Physiol. Gesellsch., 1892.

³ Zacharias, Ueber Beziehungen d. Zellwachst. z. Beschaffenheit d. Zellkerns. Ber. d. deutsch. Bot. Gesellsch., 1894, p. 106.

to a transference of substance from one to the other, although this transference may take place in various degrees. Many other forms have been described and figured, especially by zoologists, which lend support to this view, and on the botanical side I may cite the observation of Strasburger on the embryo-sac of *Galanthus*¹, in which he describes and figures the fragmented nucleolus lying along the young chromosomes within the nucleus. I have myself repeated this observation on the snowdrop, and have obtained quite similar results.

But in attributing a nutritive function to the nucleolus I have left untouched the question as to what rôle (if any) is played by the other substances of which this enigmatical body is composed. Professor Strasburger has recently suggested² that the nucleolus is concerned in an important way in the formation of the intra-nuclear part of the achromatic spindle. This may well be the case, at any rate there can hardly be any doubt but that a considerable part of perhaps all normal spindles is differentiated from substances within the nucleus; and of course it must be so in those cases in which, as in *Ascaris megalocephala*, var. *univalens*, the whole spindle is intra-nuclear. I confess, however, that I feel sceptical as to the possibility, or even the *à priori* necessity, of deriving it from any one constituent of the cell, which one could regard as set apart to provide a 'spindle-forming' substance. It seems to me to be somewhat difficult to regard the spindle as a growing, organized structure at all, in the morphological sense of the term. I venture to think that the facts hardly warrant us in saying more than that the spindle-fibres are the visible expression of strains (and perhaps stresses) within the protoplasm. This protoplasm consists of, or at least includes, an extremely heterogeneous assemblage of substances which vary amongst themselves very much in such physical characters as cohesion, viscosity, extensibility, elasticity, and the like. If the kinetic centres are really *active* existences, and there seems no reason to doubt this, then such 'lines of force' as

¹ Strasburger, Die Controversen, p. 23.

² Strasburger, Karyokinetische Probleme. Pringsh. Jahrb., Bd. XXVII, Heft 1.

are seen in any well-developed spindle are exactly what might have been expected. Their resemblance to the line of force in a magnetic field has been repeatedly pointed out, and I am also one of those who believe that there is a real mechanical similarity underlying the appearances presented in both cases. I do not see any sufficient reason for postulating the existence of any special spindle-forming substance distinct from the general protoplasm of the cell. Just as the protoplasm of the limiting surfaces of cells assumes the form of hyaloplasm, however often the free surfaces may be artificially or naturally renewed, so also the spindle-fibres can equally well be differentiated, when the mechanical conditions of their existence arise. Given a homogeneous, extensible, cohesive framework, such for example as the researches of Bütschli have rendered probable for the structure of protoplasm, and it will be at once apparent that such a structure, when exposed to strains, must inevitably react differently from the surrounding mass of granules and other heterogeneous and disunited constituents of the protoplasm. And the same result will also follow, if we admit the distinction between hyaloplasm and the embedded particles which render its interior so turbid, without committing ourselves to any definite conception as to the architectural structure of the protoplasm. It is a marked feature in all well-developed spindles that the whole of the area lying between the two kinetic centres, and enclosed by the spindle, becomes cleared of nearly all the turbid protoplasm, which is, however, abundant enough in the regions outside the spindle. And during the reconstitution of the daughter-nuclei, after their walls have become differentiated, the granular portions are first seen to spread into the spindle area (whilst this persists as the connecting fibres) under the lee of the two daughter-nuclei, the equatorial regions remaining for some time clear of these minute granules. It may be urged that we have here to do merely with an additionally rapid metabolism, due in the one case to the strain, and consequently to the energy set free in the protoplasm, and in the other to the exigencies of the new cell-wall, and that this will

account for the clearance of the granules. But I think that any one who is familiar with the whole appearance presented by the spindle at the various stages of its existence in such a cell as, for example, the pollen mother-cell of a Lily, would admit that this will hardly supply a complete explanation of the appearances observed.

The general conclusion to which I have arrived is one which, so far as I understand him, is also held by Heidenhain, namely, that the achromatic spindle is the direct effect of stresses and strains acting in the cell from definite centres, that their arrangement obeys ordinary mechanical laws, and that those constituents of the cell, be they cytoplasmic or be they nuclear, which possess the physical consistency necessary for the purpose, may be requisitioned to take part in the formation of the spindle-fibres.

Finally, there remain the chromosomes themselves to be considered. A considerable amount of knowledge has been accumulated with respect to the structure and mode of origin of these bodies in plants, chiefly through the investigations of Strasburger and of Guignard. But it will have been already seen from the foregoing account that the earliest stages in the development of these bodies are not in all cases identical, nor do they conform to one type in the mode of their further evolution. This is especially true of the divisions in the spore-mother-cells, as contrasted with the mitoses which occur elsewhere in the same organism.

The features which seem to be distinctive of this spore-mother-cell division, at least in plants, are, first, the *early* period at which the longitudinal fission of the chromosome takes place, and, secondly, in the very peculiar and highly characteristic shape of the chromosomes when they reach their mature form on the spindle.

As regards the first of these two points, the liverworts, described above, do not quite agree with the process of chromosome-differentiation as seen in Lilies, in which most of the detailed observations in this process have been carried out. In these plants, and also, it would seem, in the Gymno-

sperms, the linin is discoverable at a very early stage as a much coiled thread, in which the chromatin particles are distributed. As the coil shortens and thickens, the chromatin is seen to be specially located in two lines along the edge of the filament, whilst the intervening clearer part of the linin marks the tract of the longitudinal fission. The chromosomes, thus made up of a linin basis, in which the chromatin is imbedded, eventually become isolated by the 'thinning-out' and final disappearance of the linin at certain transverse areas, and in this way the length of each chromosome is determined. But in Hepaticae the process is complicated by the existence of anastomoses, and the chromosomes are by no means delimited in so orderly a fashion as in Lilies. The longitudinal fission is often recognized only with difficulty, so much so that at one time I doubted its occurrence.

The appearance of the chromatic areas in the *Jungermanniae* is highly characteristic. The young chromosomes usually form more or less ring-like structures, the chromatin in which is frequently seen to be grouped in a manner which strongly suggests that a *double* distribution of the chromatin for the next *two* divisions occurs here (see Figs. 5, 6, 7, 8, 22, 38). There is, so far as I know, no *à priori* objection which can be urged against this. Brauer showed that it happens in *Ascaris*, and Flemming showed that a longitudinal division of the daughter-chromosomes of the one mitosis in the spermatocytes of Salamander occurred before going into rest, whereby the number of chromosomes for the final mitosis was already prepared for.

Later on, this curious appearance in the rings is lost, and the chromosomes contract and bend over on themselves, assuming the well-known humped appearance so characteristic of the corresponding mitosis in the pollen-mother-cells of Phanerogams. An explanation of the highly complex development which these chromosomes pass through before they finally divide on the spindle has been attempted elsewhere¹,

¹ Farmer and Moore, loc. cit.

so that I need do no more here than briefly recapitulate the important steps of the most normal type met with, bearing in mind, however, that variations on this may occur¹. The process, in outline, is as follows: The chromosomes in which the longitudinal fission has taken place, and which may be open or closed at both ends, doubles on itself. The two *sides* now begin to divaricate, beginning to do so at the place where the bending occurred. Thus the well-known T shape is produced, the vertical bar of the T representing the hump which is produced by the approximation of the two *ends* of the young chromosome, and which are directed outwardly from the surface of the spindle. Finally, the two halves separate along the spindle, and as a result of the bending already referred to, the daughter-chromosomes have from the first the shape of the letter V; each represents one of the original longitudinal halves of the mother-chromosome. Variations on this process may occur, but the essential feature, namely, the *longitudinal* splitting of the mother-chromosome, giving rise to the two daughter-structures, is common to all.

The second mitosis in the spore-mother-cell of the liverworts investigated is, so far as the minuteness of the objects admitted of its being traced, similar to the first, though the earlier stages were, however, exceedingly difficult to follow on account of the small size of the nuclei. On the assumption of a double splitting occurring during the first mitosis, it is obvious that the chromosomes in both divisions might well exhibit that identity in form which, as a matter of fact, is actually displayed.

In this respect the second karyokinesis in the Hepatic spore-mother-cell *differs* from that one corresponding to it in Lilies. The dissimilarity between the two consecutive mitoses in the pollen-mother-cells of Lilies and some other plants is most striking, and has not escaped the notice of those who have investigated these plants. I think I am justified in asserting that in the second division of the pollen-mother-cell

¹ Ibid.

of the Lily a true homotype mitosis occurs, and that it in every way resembles those mitoses in the vegetative cells of the plant, except in so far as the retention of the reduced number of chromosomes is concerned. I have observed the chromosomes lying in pairs, and arrayed on the spindle in such a way as hardly to admit of a doubt as to their having arisen by a longitudinal fission, exactly like that in the chromosomes of the endosperm nuclei, for example.

But it would appear that not all Phanerogams exhibit the difference between the two mitoses which is so pronounced in the Lilies. Thus Strasburger¹ states that in *Orchis mascula* the chromosomes of the two successive mitoses in the pollen-mother-cells are quite similar in appearance. Here, then, it seems that, as in liverworts and in some animals², the two mitoses are both similar, and approximate to the heterotype form.

A study of these divisions affords not the slightest evidence in favour of any reduction-division (in Weisman's sense) taking place. The only point at which, so far as I can see, it might conceivably occur, is in the period of rest which intervenes between the last archesporial division and the first spore-mother-cell mitosis, in which the reduced number of chromosomes is already complete. It might be suggested that the chromosomes of the last archesporial mitosis had come together laterally and in pairs, so that what has been here and by other writers described as a longitudinal fission in the chromosome really represents the partial lateral fusion, or perhaps re-separation of a pair of entire ones. The apparent division in this case would then really amount only to a sorting out of the two members of each pair, a process which would certainly fulfil the requirements of the 'Reductionstheilung' if it could be proved to take place. But the great similarity of the first heterotype in plants with the first heterotype division in

¹ Karyokinetische Probleme. Pringsh. Jahrb., Vol. xxviii, p. 192.

² Moore, On the Germinal Blastema and the nature of the so-called 'Reduction-division' in the Cartilaginous Fishes. Anat. Anz., Bd. IX; also, On the Maturation of the reproductive elements in Elasmobranchs. Quart. Journ. of Micr. Sc., 1897.

animals is opposed to such hypothesis, for in animals no 'reduction' is claimed at this stage. Moreover it seems clear that whatever significance the heterotype form of mitosis may possess, it cannot be concerned with a qualitative reduction, since in animals¹, and at any rate in some plants, *two* heterotype divisions succeed each other, and there is no reason for supposing that the real process is so essentially different in the two cases as to effect a reduction in the one and not in the other.

Thus it would seem that the heterotype divisions in plants, different as they are from the normal homotype mitosis in the rest of the cells of the organism, afford no evidence in support of any assumption of a *qualitative* difference existing between the daughter-chromes in each pair. The only 'reduction' is a numerical one, and it would appear that this must be due to an end-to-end fusion in pairs which occurs in the resting nucleus, as was suggested by Strasburger²; that is if the chromosomes really retain their identity in the resting nuclei.

But it is difficult to feel certain even on this point. Certainly the chromatin as such does not remain unaltered. It undergoes chemical change during the last phases of karyokinesis, which results in the loss of staining power and a large portion at least of its substance. Prof. Strasburger has recently suggested that the chromatin wanders into the nucleus from the cytoplasm in a dissolved condition, and he explains the 'Sichel-stadium,' previously described by himself and other authors under different names, as a condensation of this substance at one end of the nucleus. It is driven here owing to the one-sided penetration and diffusion of the reagents used in fixing. It is, however, not very easy to see how such a quantity of dissolved chromatin could exist in the nucleus without colouring the nuclear sap in those cells in which the fixing has succeeded better. But however this may be, it hardly seems to me to be probable that the chromatin

¹ Moore, loc. cit.

² On the periodic reduction in the number of the Chromosomes. *Annals of Botany*, Vol. viii.

as a *whole* enters the nucleus in this manner. It may well happen that the cytoplasm does furnish some constituent of that body which we call chromatin in the dividing nucleus, but in addition to this there exists another substance which forms the *essential* part of the chromatin, and it seems certain that this substance resides in the nucleus. The fact that nucleins are not found in the cytoplasm supports this suggestion. The sudden and large increase in bulk which the chromatin exhibits during mitosis is perhaps referable to the recombination of the substances which contain phosphorus with the albumen, which probably originate from the nucleolus, and perhaps partly also from the cytoplasm.

I have endeavoured in the above discussion to hint at some of the lines along which further investigation is much needed, and also to indicate the nature of some of the problems which await solution. I am conscious that in attempting to touch on some of these questions I cannot hope to avoid the charge of vagueness. This is, however, unfortunately a defect inherent in the present condition of the subjects involved, and I thought it best to put forward the difficulties with which I have been myself confronted, in order that perhaps others might be tempted to overcome them. So little ground has been as yet reclaimed, and of that little so much is still unsafe, that one can only with diffidence attempt to estimate the general bearings of any one set of observations, however true they may be for the particular objects investigated. Thus, even a short time ago, few would have believed that the achromatic spindle could be formed entirely within the nucleus, whereas we now recognize that the actual position which it occupies in the cell is of no importance from a general standpoint, and in the same way many observations which to-day seem to possess little significance may prove of considerable value to-morrow, as has already happened to the theory of the *general* fusion of the centrosomes during fertilization—

‘Multa renascentur quae iam cecidere, cadentque
Quae nunc sunt in honore —.’

I cannot do better than close this essay and these reflec-

tions with a passage from one of Boveri's papers: 'Ich meine deshalb, es sollte . . . nicht mehr jeder Autor, wenn er an einem bestimmten Object die eine Entstehungsart als sicher erweisen kann, nun denken, er habe damit alle anders lautenden Angaben für andere Objekte umgestossen und dürfe seinen Befund als allgemein gültig proklamieren.'

EXPLANATION OF FIGURES IN PLATES XVI, XVII, AND XVIII.

Illustrating Prof. Farmer's paper on Spore-Formation in Hepaticae.

All the Figures were drawn, using Zeiss 2 mm. apochrom. Microsc. with various oculars, and are reduced.

Figs. 1-16. *Fossombronia Dumortieri*.

Fig. 1. Spore-mother-cell with nucleus in resting condition.

Fig. 2. Same a little more advanced, nucleus preparing for division, three centrospheres at the angles, where the quadripolar spindle-rays afterwards appear.

Fig. 3. The same still later, the linin is forming aggregations, and the chromatin becomes prominent.

Fig. 4. The chromatin aggregations appear as flattened rings, well marked. The nucleolus still present, but vacuolated.

Figs. 5, 6. Variations which occur at the next stage, quadripolar spindle.

Figs. 7, 8. Slightly older cell. The nucleolus becomes much vacuolated. The chromosomes more marked.

Fig. 9. The first spindle.

Fig. 10. The divided chromosomes retreating, a rudimentary cell-plate shown.

Fig. 11. The second division. One nucleus is seen in pole-field, with its eight chromosomes. The dotted line indicates the position of the first division wall.

Fig. 12. The four daughter-nuclei, resulting from the last division. They are diagrammatically represented in one plane; in reality the pair with the cell-plate between them are alone in focus. The appearance of the division walls seems to be simultaneous, and not successive, in this particular cell.

Fig. 13. The spore-mother-cell after division. The protoplasm is very much vacuolated.

Fig. 14. A young cell of the sporogenous (archesporial) tissue preparing for division; the centrospheres well shown.

Fig. 15. Later stage of the same, with the chromosomes forming.

Fig. 16. A spindle from the same tissue, with sixteen chromosomes which have just divided (only seven are shown in the drawing). The astral radiations are hardly visible at this stage.

Figs. 17-35. *Pellia epiphylla*.

Figs. 17, 18. Spore-mother-cell with nuclei just preparing for division.

Fig. 19. Later stage nucleolus vacuolated, chromatin becoming more abundant, the nucleins drawn out into a four-armed spindle.

Figs. 20, 21, 22. Further stages in the development of the chromosomes. In Fig. 21 they appear distinctly as eight rings.

Fig. 23. The first spindle. There are eight chromosomes (four shown).

Fig. 24. Diaster stage seen obliquely.

Fig. 25. The second division. One nucleus only of the lower pair shown, it is seen in pole-field, and the fellow one is concealed. In the upper part, the other two daughter-nuclei are both seen in profile. The cell-walls have appeared dividing the spore-mother-cell into four chambers.

Figs. 26, 27. Other views of the process.

Fig. 28 *a*. The spores are formed, and are preparing to germinate.

Fig. 28 *b*. Slightly later stage, showing the marking on the proper wall of the spores, and the disappearance of the spore-mother-cell membrane.

Figs. 29, 30. Division of nuclei in cells of the stalk of the sporogonium, a typical homotype karyokinesis.

Figs. 31-35. Nuclear divisions in the gametophyte.

Fig. 31. Nucleus in early stage of karyokinesis.

Fig. 32. The same, showing a centrosphere at one pole.

Figs. 33, 34. Chromosomes in equatorial plate.

Fig. 35. Division of chromosomes.

Fig. 36. *Aneura multifida*; the division of the chromosomes in the first spore-mother-cell mitosis.

Figs. 37-39. *Cephalozia bicuspidata*.

Stages in nuclear division of spore-mother-cells.

Figs. 40-49. *Scapania undulata*.

Fig. 40. Spore-mother-cell with resting nucleus.

Fig. 41. Same preparing for division. The nucleolus has fragmented; the linin now forms a well-defined net-work.

Fig. 42. Nucleus drawn out in a quadripolar spindle. The chromosomes beginning to form.

Figs. 43, 44. Chromosomes form the equatorial plate.

Fig. 45. Polar view of nucleus in same stage as in 43. The nucleolus N still visible amongst the eight chromosomes.

Fig. 46. The division of the chromosomes.

Fig. 47. First mitosis, the cell-plate divides the entire spore-mother-cell.

Fig. 48. The second division of the spore-mother-cell.

Fig. 49. A slightly later stage than that represented in Fig. 46.

Figs. 50-53. *Lophocolea bidentata*.

Stages in the division of the spore-mother-cell.

Figs. 54, 55. *Lophocolea ciliolata*.

Fig. 55 shows the second division in the lower nucleus, and it is clearly of a heterotype character.

Figs. 56-58. *Plagiochasma*.

Fig. 56. Late stage in first division.

Fig. 57. The second division (the two lateral nuclei lie in deeper focus), diagrammatic.

Fig. 58. The manner of appearance of the cell-walls.

Figs. 59-75. *Fegatella conica*.

Fig. 59. Spore-mother-cell, with dense deeply-staining cytoplasm and nucleus with linin net-work.

Figs. 60, 61. Two views of the division of the daughter-nuclei.

Figs. 62-64. Different views of the spore-mother-cell after the division of the nucleus.

Fig. 65. Diagrammatic representation of the arrangement of the cell-walls (see Fig. 63).

Figs. 66-75. Germination of the spores.

Fig. 66. Nucleus with reticulum of linin, and two vacuolated nucleoli.

Fig. 67. The chromosomes have formed, two centrospheres are shown.

Fig. 68. Polar view at stage of the fission of the eight chromosomes.

Fig. 69. Profile view at a little later period.

Fig. 70. The same a little younger, the chromosomes just splitting.

Fig. 71. The daughter-chromosomes are retreating to the two poles.

Fig. 72. The daughter-nuclei forming. The cell-plate and spindle shown. Some of the spindle-fibres terminate in granular bodies.

Fig. 73. A later stage, the nuclear walls and nucleoli have appeared, the linin has lost its staining capacity.

Fig. 74. The first division of the spore completed, the daughter-nuclei are in a state of complete rest.

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FARMER.—NUCLEAR DIVISION IN HEPATICÆ.

Fig^s 1-16, Fossombronia. 17-25, Pellia.

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FARMER.—NUCLEAR DIVISION IN HEPATICÆ.

Figs 1-16, Fossombronia. 17-25, Pellia.

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Fig^s 26-35, *Pellia*. 36, *Aneura*. 37-39, *Cephalozia*. 40-49, *Scapania*.
50-53, *Lophocolea bidentata*. 54, 55, *L. ciliolata*.

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Fig^s 26-35, *Pellia*. 36, *Aneura*. 37-39, *Cephalozia*. 40-49, *Scapania*.
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FARMER. — NUCLEAR DIVISION IN HEPATICÆ.

Fig^s 56-58, *Plagiochasma*. 59-74, *Fegatella*.

Farmer, J. B. 1895. "On spore-formation and nuclear division in the Hepaticae." *Annals of botany* 9, 469–523.

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