VEGETATIVE CELL DIVISION IN ALLIUM.

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(with plates xi–xiii)

Before the controversies which have arisen over the occurrence of reduction in plants can be settled or the problems as to the nature of fertilization can be attacked, a detailed study of dividing and quiescent plant cells is made necessary. The vegetative or typical division of plant cells from which the factors of sexuality and reduction are eliminated must be understood before deductions can be made as to the significance of the mode of origin and distribution of the chromosomes occurring in atypical divisions.

Accordingly, this research was undertaken with the hope of adding to our knowledge of the processes of vegetative cell division, and also of paving the way for further investigation of these processes as affected by variation of external conditions. The material used is Allium root tips, already known as exceptionally favorable for the study of karyokinesis. It was also considered an advantage, in order to secure a more thorough survey of the subject, to employ material which had furnished the text of other publications.

TECHNIQUE.

The following solutions were used for the fixation of the cells: chrom-acetic-osmic, both the weaker and stronger solutions as employed by Flemming, chrom-acetic acid (chromic acid 0.9 per cent. and acetic acid 0.1 per cent.); the solution recommended by Carnoy as composed of one part glacial acetic to six parts absolute alcohol and three of chloroform; and finally a solution made up of saturated picric acid one part, sublimate one part, to two parts of water. Cedar oil and xylol were used as clearing fluids. The best differentiation of the chromatic substance was obtained with Heidenhain's iron alum haematoxylin in combination with some cytoplasmic stain. The triple stain, safranin
gentian violet and orange G, followed by clove oil as a clearing agent, gave the best results in differentiating the nucleoli.

CELL DIFFERENTIATION.

The transition of meristem cells into permanent tissue is to be noted not only in the changes taking place in the nuclei, but also in the cytoplasm. As the cells develop into permanent tissue, large vacuoles appear in the cytoplasm, and the nuclei retreat into positions next to the walls, becoming then elliptical. Among the foremost of the problems suggested by watching the cells of the meristem pass out from their embryonic condition was that of the part played by the chromatin and nucleoli in this transformation of an embryonic cell into a tissue element.

A median longitudinal section reveals the large central cells and elongating cells which constitute the plerome. These large cells have correspondingly large nuclei and vacuoles in the cytoplasm and a relatively smaller amount of chromatin (figs. 50, 51). In the differentiation of these cells the chromatic substance first diminishes, and with this diminution it gradually loses the power of forming the karyokinetic figures. The great increase of the nucleoli follows from the quantitative reduction of the chromatin, while the energy that would have produced new cells becomes diverted into the expansion of the nucleus and cell body. The central cells reach a maximum of enlargement and then enter upon slow disorganization.

The cells of the plerome elongating to form procambium are early conspicuous, being marked off from the large central cells of the plerome on the one side and the periblem cells on the other by their narrow dimensions and elongating nuclei (figs. 51-56). An examination of these cells when in the act of dividing shows that the same general changes take place as in the meristem cells. The spireme stage is to be frequently seen, as well as the separation of the chromosomes. No cell, however, was seen which showed the chromosomes drawn into an equatorial plate. Their passage to the poles seems to follow directly upon the breaking of the spireme (fig. 55).

The changes leading to the formation of the elongated cells must be sought in the meristem cells from which these are...
derived. Cells such as those shown in fig. 53, situated within the borders of the meristem region, whose shapes vary but slightly from the typical embryonic cell, have noticeable differentiations ushered in even before the membranes of the daughter nuclei are formed. In the cell shown in fig. 53 of the anaphase the daughter chromosomes can be seen pushing into the cytoplasm as the spireme coils are being formed. This is the first visible elongation of the nucleus.

It cannot be said, then, that the peculiar shape of the nucleus is an adaptation to the shape of the cell, as in this case no appreciable elongation of the cell body has yet appeared. The causes which lead to this differentiation must be inherent in the chromatic substance where it is initiated.

In figs. 52, 54 of the telophase the elongation of the daughter spiremes can be seen. These early elongations give to the daughter nuclei an amoeboid shape. Such appearances in the various stages furnish evidence that the chromatic substance is here of a fluid nature, with the power of independent motion, and not a substance passively drawn by contractile threads of kinoplasm.

When the cells by successive division have reached their maximum of elongation, further changes can be seen to have taken place within the nucleus. The chromatin, instead of being separable into groups of fours, as in the earlier stages of elongating cells and as described in this investigation for the meristem cells, becomes vacuolar, the chromatin irregularly massed, while the nucleoli, which up to this time have been undergoing a considerable increase in size, suffer fragmentation.

The observations then drawn from the anatomical differentiation in both types of cells found in the plerome point to the conclusion that the chromatic substance must play the chief rôle in the transformation of an embryonic cell into a tissue element.

The same general statements as to the part played by the chromatin in the transition of the meristem cells into periblem and dermatogen can be made. The shape assumed by the chromatic figure in karyokinesis determines the shape of the daughter cells. As the chromatic substance may elongate in the meristem
cell to form the elongated cell of the procambium, so it may assume a square figure and produce the daughter cell of the dermatogen.

In case of meristem cells destined to form the protecting cap, the chromatic substance gradually dwindles in amount without being accompanied by further increase of the cell body. The cytoplasm as well becomes reduced until finally both disappear.

The process of division of meristematic cells without regard to the problems suggested by differentiation will now be treated in sections.

THE ACHROMATIC FIGURE.

The enlargement of the nucleus due to the growth of the chromatin in the prophase of division is accompanied by significant changes in the appearance of the cytoplasm. In figs. 2–5, 8, 9, which show stages in the formation of the spireme, the cytoplasm appears to be uniform in structure. A comparison of the shapes of the nuclei indicates that the nuclei in the living cells must be amoeboid in these stages. As nearly as could be determined, the changes in the structural appearance of the cytoplasm in the immediate vicinity of the nucleus just precede or accompany the transverse breaking of the spireme coil into chromosomes.

The first indications of the achromatic figure are to be seen in the aggregations of cytoplasm which appear first and remain the densest at the poles of the nucleus. The shape which these aggregations assume, and hence the final shape of the achromatic figure, is dependent upon the shape and nature of the cell, as also possibly upon the fixative employed. The nucleus taken together with these cytoplasmic aggregations may be spherical, (fig. 24), or ovate (fig. 18), or elliptical (fig. 19). Occasionally these aggregations appear in such manner as to cause the chromatic figure to be obliquely oriented, and hence to alter the division plane from the usual one at right angles to the long axis of the cell.

Cells were seen in certain preparations, notably those fixed in chrom-acetic, in which the segments of a hyaline sphere seemed
to cap either pole of the nucleus (fig. II) of a cell cut somewhat obliquely, the cytoplasmic aggregations appearing to extend over and around these segments (see also fig. 24). In such figures the clear space presents the same appearance and the same staining reaction as the nuclear interior.

A study of the nuclei in the prophase stages did not furnish satisfactory evidence that such hyaline segments capping the nucleus are regular stages in the formation of the achromatic figure. Although such appearances are to be frequently met with in material fixed in chrom-acetic, similar nuclei in the prophase show no hyaline sphere segments, but merely enlargements with cytoplasmic polar aggregations. Hence a question can be raised as to the existence of the hyaline polar caps in the living cells. Is this appearance a normal phenomenon produced by a withdrawal of cytoplasm from the pole of the nucleus, and to be referred to the turgescence of the living cell which causes the formation of a plasma membrane around the nuclear membrane? Or has this appearance been caused by fixation, the nucleus having become distorted and the membrane split or swollen? Or, thirdly, are these polar caps only optical effects resulting from looking down upon sections of an irregularly shaped nucleus, the lower boundary being projected beyond the upper boundary and hence producing the appearance of polar caps? A section of a nucleus shaped like a truncated cone can be conceived as presenting such an appearance.

In order to solve this problem, examination was made of many preparations fixed and stained by the methods previously outlined. If the hyaline caps are not produced by fixation, or are not optical effects produced from looking down upon sections of an amoeboid-shaped nucleus, but, on the contrary, are normal phases in the growth of the achromatic figure, their presence should be established in similar prophase stages and in all fixations which otherwise preserve the character of the cell contents. Preparations fixed in chrom-acetic showed such phenomena frequently, but by no means so constantly as to warrant their being considered a normal feature of division. Figs. 19 and 20 represent a periblem cell drawn at upper and lower focus and
taken from a series fixed in picro-sublimate solution. No nuclei in the prophase showing hyaline caps at the poles were found in this series. In the drawing of the upper part of the nucleus the cytoplasmic aggregations may be seen at the poles. The nucleus has elongated in the direction of the long axis of the cell. Indications are also present of the ingrowing cytoplasmic fibrillae which later meet the linin strands that join the spireme coils to each other and to the nuclear membrane. Fig. 18 from a series fixed by the same method represents a ple-rome cell in similar stage where the nuclear membrane has pushed out at the poles into the cytoplasmic aggregations. Fig. 28 from the same series may be taken as a later stage, where the nuclear reticulum has completely joined with the cytoplasmic fibrillations. The stages in material fixed in Flemming presents configurations similar to those in the material fixed in picro-sublimate. An early stage from these preparations showing the pushing out of the nuclear membrane at the poles is seen in fig. 26; a somewhat later stage in fig. 27, where the cytoplasmic fibrillations can scarcely be distinguished from the nuclear reticulum. In fig. 6, from another series fixed in Flemming's solution in which the chromatin is well preserved, it would seem as though a vacuolization had taken place around the nucleus. There is no evidence of such vacuolization in figs. 17 and 25, taken from another series fixed in Flemming's solution, both cells being in the same section of the root tip. In both these cells the nuclear membrane can be clearly traced, marking out the nuclear space from the cytoplasm. Fig. 25 is of a stage later than that shown in fig. 17. Here there are no signs of hyaline caps, but there is unmistakable evidence that the achromatic figure is formed by the elongation of the nucleus accompanied by the ingrowing of fibrillations from the cytoplasm. Fig. 30, from a preparation fixed in chrom-acetic, is a still later stage, where there is but the vaguest trace of a nuclear membrane. The achromatic figure is well formed and indistinctly multipolar. The term "monaxial multipolar," as applied by Hof to distinguish this form from the type of multipolar spindle found in the dividing macrospore, better describes the achromatic figure at this stage.
When the chromosomes are being drawn into an equatorial plate figure, as also when they have begun to recede to the polar figures, the fibers are more frequently found converging to a single point, though multipolar monaxial figures are the more characteristic. Certain cells from material fixed in Flemming show but traces of the achromatic figure, such traces being confined to the linin connecting adjacent chromosomes (figs. 32, 33). Fixatives such as chrom-acetic accentuate the fibrillar character of the plasm, while that of Flemming accentuates the granular character, showing fewer intercytoplasmic spaces. Compare fig. 33 Flemming with fig. 37 chrom-acetic.

Thus the evidence from the preparations examined points to the conclusion that the achromatic figure in Allium is derived from both cytoplasm and the elongated nucleus; that the cytoplasmic fibrillae normally fuse with the nuclear reticulum without the intervention of periplast formation, and form a multipolar monaxial spindle which may secondarily become bipolar.

The observations of other investigators regarding the achromatic figure in the division of meristematic cells is as follows:

Rosen (8) stated that in the cells of root tips of hyacinth a homogeneous plasm collects in a thin hyaline layer about the nucleus destined to divide. This layer he found to be concentrated at the opposite poles in the form of two conical caps in which rows of fibrillae originated close to the nuclear membrane.

Hof (11) stated that in Ephedra kinoplasmic bodies forming caps appeared simultaneously at the two diametrically opposite points of the nuclear surface. The polar caps soon took on the shape of sharp pointed spheres. In the interior of these spheres delicate threads form which finally fasten to the nuclear surface.

Němec (9) describes the fundament of the spindle as being bipolar in orientation from the beginning, appearing as a hyaline formation surrounding the nucleus and in the form of caps at the poles. He designated such caps as “periplasts.” In another contribution upon the nuclear division of Solanum tuberosum he stated that, whereas in normal cells a hyaline periplast is formed as in Allium, in cells taken from wounded tubercles the threads grew out directly from the nuclear surface. He considered that
the reason for the failure of the hyaline periplasts to appear in the cells of the wounded tubercles is because of the greater size of the nuclei. Nuclei occurring in the tubercles experimented upon were 30 per cent. larger than those found in the growing points of root and stem.

His statement as to the cytoplasmic fibrillae in the cells of wounded tubercles growing out directly from the nuclear surface agrees with what I found in normal cells from the root tips of Allium fixed in Flemming and picro-sublimate solution. As to the appearance of the periplast, I cannot agree with Němec in considering it a normal feature in the process of vegetative cell division, and its absence caused by experimentation or otherwise disturbed conditions. In some cases its presence seems to be due to the action of fixatives or to the sectioning of an irregularly shaped nucleus.

Also as to the cytoplasmic aggregations which Strasburger and Hof consider as being made up of a specific substance, kinosplasm, the evidence derived from comparing the structural appearance of these aggregations with the appearance of the remainder of the cytoplasm in the various preparations does not seem to uphold such a conception. Their appearance rather justifies the view expressed by Wilson (5) and others that alveolar spheres, microsomes, granules, and fibrillae may be morphologically considered as but gradations of one structure.

CENTROSOMES.

In one case only, out of the many preparations of Allium examined, was there a substance visible which could be construed as a possible centrosome. This case is shown in fig. 34 of a nucleus in the spireme stage with a dumb-bell-shaped body lying a little at the right of the lower pole. No astral rays are present, there being only a clear space about the body inclosed by a plasma membrane. The fact that the body was discrete and that its shape might indicate its undergoing a process of direct division led me to think that here might be a centrosome which by reason of its temporary character had before eluded observation. The section was from material killed in chrom-acetic and
stained with eosin and iron alum haematoxylin. After this cell was seen the material similarly treated was re-examined for bodies which might be interpreted as centrosomes, but with negative results.

The centrosome-like body was stained red by the eosin, as was the cytoplasm. As the centrosome of animal cells in all cases that I have observed stains black by the iron alum haematoxylin method, like the chromatin, the staining reaction argues against the interpretation of this body as a centrosome. The presence of the nuclear membrane still intact, together with the nucleolus within the spireme, shows conclusively that this body cannot be interpreted as a nucleolus. Whenever a nucleolus cast out into the cytoplasm is treated with iron alum and haematoxylin it takes on an intensely black stain (fig. 36).

On the examination of other cells on the same slide the cytoplasm was seen to contain bodies with similar staining reaction. These, however, showed no signs of division and their position in reference to the nucleus was inconstant. Fig. 35, of two cells in distinctly different phases of division, shows these appearances in the cytoplasm and indicates that their presence is not to be considered significant, but is caused by some imperfection in preservation, or else is due to some abnormal conditions in the cells such as the presence of parasites. Such appearances were not found in other material treated by the same or different methods.

Guignard (2) maintained the existence of centrosomes as dynamic organs in the cells of higher plants; Schaffner (13) described them as existing in Allium root tips; but Strasburger (14) and many other investigators find no evidence of their presence and have concluded that the centrosomes described as existing in the higher plants are in reality nucleoli, microsomes, or fixation products. Ch. Bernard (16) affirms the existence of centrospheres in *Lilium candidum*, but admits that they are variable in appearance. His figures showing bodies in the cytoplasm with clear spaces about them resemble mine of nucleoli degenerating in the cytoplasm.

The results of this investigation upon Allium can furnish no
positive evidence of bodies in the cytoplasm which can be interpreted as centrosomes.

**THE NUCLEOLI.**

The nucleoli, generally two in number, can be seen in fixed preparations of quiescent cells lying within a clear space surrounded by the chromatic network.

The size and shape of the nucleoli vary with the size and nature of the cell. Elongated cells have elongated nuclei and nucleoli (*fig. 55*), while the large central cells of the plerome have large, more or less spherical nuclei.

This power of accommodation of the nucleolus to the shape of the nucleus, together with its vacuolization and amoeboid contour, are direct indications of its essentially fluid nature. Referring to *fig. 50*, the large nucleoli in a central cell of the plerome can be seen. As these central cells recede in their multiplication from the zone of greatest growth, the nucleoli become relatively larger, the chromatic substance less conspicuous and abundant.

From these observations two conclusions might be drawn. The nucleoli, enlarging either by intussusception or apposition of particles coming from the cytoplasm, by their own growth may rob the chromatin elements of the nutriment necessary to enable them to develop the chromatic figure. Hence degeneration results, the line of broken-down cells giving rise to the central cylinder. Or the nucleoli may be thought of as accumulations of a substance which results from the reduction and degeneration of the chromatin. The latter seems more probable, as all the evidence obtained from cells which retain to the last their capacity for division points in the direction of a nucleolus arising from the disintegration of chromosomes into individual chromatin granules.

Rosen (8) states that in the hyacinth the nucleolus melts away during the formation of the spindle. In one series of sections only obtained from the hyacinth he found nucleoli cast into the cytoplasm. He offers the two following explanations for the difference in these series: special conditions of growth and nourishment might bring about the expulsion of the nucleoli.
from the mitotic figure, or karyokinesis might vary in this particular point on account of the many varieties of the cultivated hyacinth.

This persistence of the nucleoli after the formation of the achromatic figure, which Rosen found in only a small portion of his hyacinth material, I found in all the material examined, from tips of Allium just started within the bulb and from those grown in water or solutions for some time. The duration of the nucleoli varies. I have never seen any signs of the presence of the nucleolus as a stainable body in the spindle space or in the cytoplasm after the daughter chromosomes have begun their movement toward the poles. *Figs. 2–30* show the condition of the nucleoli as the formation of the achromatic figure advances; *figs. 36, 37* are chosen from the many cases observed showing their dissolution in the cytoplasm. These go to prove that in Allium at least the nucleoli do not furnish to any appreciable degree the material for the achromatic figure, as Strasburger (14), Hof (11), and Nemec (9) have claimed.

Neither did any cells yield evidence, such as Nemec (9) gives in his work upon Allium, that the new nucleoli arise from the substances coming from the degeneration of the mantle fibers. The degenerating mantle fibers appear rather to be streams of granules which become diffused in the cytoplasm, to gather again at the line where the cell plate forms. In *figs. 45 and 46* are two periblem cells in stages closely succeeding one another and approximating the time for the appearance of the nucleoli. No aggregations of granules which stain like nucleoli could be observed in these cells or in any similar stages. If we consider the mantle fibers and the like in the living cells as streams of albuminoids and other substances issuing from the nuclear elements in their activity, then the apparent degeneration of the fibers merely indicates a diffusion of these dissolved substances and a lessening of their flow. These streams, after the chromosomes have arrived at the poles, are mainly directed in their course to the equatorial zone, separating the two daughter nuclei where the deposited substances lead to the building of the cell plate.

As stated in the section upon the chromatic figure, at the time
of the fusion of the chromosomes to form the spireme in the daughter nuclei, the spaces between the coils can be seen to be occupied by a diffusible and slightly stainable substance (figs. 42-46). This substance becomes denser at last, resolving into the bodies which are known as the nucleoli of the resting nucleus. I think that this substance results from the mere deposition of particles coming from the disintegration of chromosomes into tetrad chromatin granules and the reduction of the latter in size. These particles, of a more or less plastic nature, increasing in proportion to the reduction of the chromatin granules, are entangled in the chromatic network only to be released with the dissolution of the nuclear membrane at the next mitosis.

In connection with this view of the close relation of chromatin, linin, and nucleoli, it may be well to cite results of other investigators which accord in some measure with those secured in Allium.

In the case of Spirogyra, Meunier (4) and Mitzgewitsch (12) have claimed that during mitosis the nucleoli become converted into chromosomes and in the reconstructing daughter nuclei the chromosomes are transformed into nucleoli. Wisselingh (15) found that ten of the chromosomes arise from the nucleus, while the remaining two arise from the nucleolus. Hertwig (10) states in regard to the nuclear division of Actinosphaerium that the nucleoli may be classed as of two kinds, those containing chromatic material and those free from chromatin. The latter he terms plastin nucleoli. They originate as small vesicles within the chromatin bridges. His conclusion is the same as I reached in this investigation, namely that the material of the nucleoli was originally contained in the chromatin bridges. I would also add that in Allium the chromatin bridges are in turn derived from material originally contained in the chromosomes and result from their quantitative reduction.

Since Strasburger (14) holds that the nucleoli of the higher plants are converted into the kinoplasm of the achromatic figure, he considers that the plastin nucleoli described by Hertwig are not at all analogous to those found in higher plants.

Researches upon forms of the lower plants and animals indi-
cate that chromatin, linin, and nucleoli are of the same substance, the form and amount of which vary as the nucleus is quiescent or actively dividing. If then the nucleoli of the higher plants are not genetically derived from the chromatic substance, but, as Strasburger holds, come from the degeneration of the mantle fibers, then the function and mode of origin of these nucleoli must have undergone a radical change in the course of evolution. Strasburger attributes this difference in the function of the nucleoli in the higher plants from that found in lower forms to the absence of centrosomes.

The view expressed in this investigation that the nucleoli are accumulations resulting from the quantitative reduction of the chromatin is not only in harmony with the evidence derived from lower forms, but better explains the conditions observable in the plerome cells which have no use for material with which to manufacture achromatic figures.

THE CHROMATIC FIGURE.

The chromatic granules of meristematic cells that are in a quiescent state are distributed in the linin network, but lie at the periphery of the nucleus rather than uniformly distributed, while the linin strands can be seen anastomosing with the more centrally lying nucleoli. In these nuclei the granules appear so minute even with the one-twelfth inch homogeneous immersion that no satisfactory evidence was obtained as to whether they have a definite arrangement. That they do not always appear to be of equal size can be seen by inspecting the lower right-hand cell shown in fig. 1. This variation may be only apparent, due possibly to inequalities of fixation or staining, or in some cases to an optical appearance produced by looking upon several granules overlying one another in the successively superimposed strands of the network. In cells, however, where the beginning of the enlargement of the nucleus indicates an approaching division, the network appears to consist of strands each of which is double; the granules that lie opposite each other in each of the halves of the strand are connected by cross bridges of linin. Fig. 2 shows a cell cut crosswise, in which appear the first indications of the double nature of the strands of this network.
This condition is especially to be observed in a few of the strands which are more favorably placed near the periphery. *Fig. 3* represents a similar cell cut lengthwise. As the nucleus enlarges still more, these double strands become more evident and regular in their arrangement, until we have the condition shown in the two periblem cells (*fig. 4*). Here the strands pass around the inner surface of the nucleus in what appears to be a more or less regular and continuous spiral, while the nucleolus still holds its central position within.

While bridges of the linin substance appear at first sight to connect the granules in pairs only, it is possible that the conditions are more complicated even at this stage, and that the chromatic thread which seems to be composed of only two parallel threads is in reality made up of four such parts. Such quadripartite thread with chromatic granules regularly arranged might give the appearance of being only bipartite, owing to the fact that, when viewed in certain directions obliquely to the axis of the thread, the pairs of granules in the distant half might appear to alternate with those of the nearer half.

In Allium tips treated with Flemming fixative (weaker solution), and not overstained by the iron alum haematoxylin method, the linin connecting the granules is less conspicuous. The granules, although they can still be traced as lying in strands, are more isolated, and thus separated are seen to lie, not in groups of two, but in groups of four. The stages shown in *figs. 5 and 6* (Flemming fixative) are directly comparable with the stage in *fig. 4* (chrom-acetic fixative). The cell shown in *fig. 5* is a somewhat later stage of a periblem cell treated with Flemming fixative, while *figs. 7 and 8* show later stages from tips similarly treated. The effect of the osmic acid in combination with the chrom-acetic then, as seen by these figures, is to bring out with greater distinctness the arrangement of the granules into tetrads. The linin can be distinguished in the cells thus treated, but it is more obscure than in the cells treated with chrom-acetic alone.

It would be natural, from our preconceived ideas of the individuality of the chromatin granules and of the necessity of an
equal distribution of substance to the two daughter nuclei, to assume that this arrangement of granules into an apparently double strand results from the splitting of a single thread or series of granules, but the very minuteness of the granules in the stages preceding the formation of the spireme precludes any direct observation of such a division even if it takes place.

There are two ways in which one might explain the formation of quadripartite threads out of the network of the quiescent nucleus. We might imagine that in a row of self-propagating units each divides into two, the result being two parallel rows, and that then each of the resultants again divides into two, thus giving rise to the groups of four which are joined into strands by the linin. Judging, however, from what it is possible to observe in the quiescent nucleus and in the later stages of division, there is some evidence in favor of another explanation. The grouping of the granules into pairs, which are at least double and probably quadruple, can be seen in early stages back to the point where the granules are so fine and so closely massed together that they cannot be said to have any definite arrangement. It is conceivable, therefore, that such a tetrad condition may be permanent, the granules having an inherent tendency to group themselves into fours.

An examination of the large cells of the plerome, which by their extreme size and presence of large vacuoles indicates the loss of the power of division, yields evidence that the tetrad condition may be permanent. In fig. 50, taken from a large plerome cell which is later to break down to form a central vessel, and fig. 51, of cells elongating to form wood fibers, fixed in Flemming and stained by the iron alum haematoxylin method, the chromatic network has the appearance of strings of vacuoles, the borders of the vacuoles consisting of the chromatin substance. A comparison of these figures with those of the early prophase, such as figs. 7 and 8, shows similarity of structure. The condition seen in the plerome cells may be interpreted as the result of the linin connecting the successive groups of tetrads having become more stretched, the tetrad granules less conspicuous; the space in the center of each group of granules acquiring
the appearance of a vacuole, one of a series in a network. As the nucleus enters into a quiescent condition then, the spaces in the centers of the groups of tetrads become more marked, and the boundary lines of the chromatin granules more and more indefinite.

The history of the chromatin shows that up to the time of the formation of the equatorial plate the whole process can be summarized as consisting of the growth, aggregation, and fusion of tetrads into chromosomes; whereas the period from the formation of the equatorial plate to that of the daughter nuclei can be summarized as a process consisting of the disintegration of chromosomes into tetrads and the reduction of the latter in size. The times of the appearance and disappearance of tetrads then correspond with those of appearance and disappearance of a definite arrangement of the chromatin, and this holds for the nuclei which are about to divide as well as for those which have just arisen from division.

From the time when the double strands of granules are first evident until the spireme is conspicuous, there is an increasing stainability, condensation, and enlargement of the component granules. Figs. 9 and 16 represent successive stages in the growth of the spireme thread before it has broken up into chromosomes. The linin connecting the coils is seen to persist; but the connecting bridges are reduced in number.

These linin connections never completely disappear, although the quantity of linin varies with the stage of division. The linin strands which persist in the late spireme are more deeply stainable than the earlier connecting bridges. This suggests that this reticulum may arise, concomitantly with the growth of the chromatin, from the fusion of some of the earlier connections, while other connections are torn apart with the growth and consequent pulling apart of the coils. A cross-section of the spireme loop taken in early stages shows it to consist of four granules joined by the linin in the form of a square; a cross-section taken at stages immediately preceding that of its maximum size shows that the granules have approached one another in their growth. Its structure is thus seen to be, not that of a homogeneous single
or double thread, but that of a band composed of a series of chromatin masses joined together in rings, each ring being separable into four primary masses of chromatin. Later, at the time of the formation of the equatorial plate and when the chromatin has reached its maximum size, the four masses have by their growth fused with one another into a ring, the succession of rings resulting in the formation of a more or less continuous tube. In figs. 13 and 16, longitudinal sections of periblem cells giving surface views of the coils in the nucleus, some of the loops have been cut transversely and thus give end views of the spireme. Views of both the ends and sides of the threads are of course necessary for the formation of an opinion as to the real condition of the chromatic substance at this stage. From a comparison of such views it is evident that the thread is now a tubular structure, the walls being composed of the four more or less completely fused elements of the quadripartite stage. The more completely fused the chromatin masses, the straighter and more sharply defined is the edge of the spireme thread. Its contour varies with the age of the spireme and with the nature of the cell. In many cases (fig. 10) the spireme appears as a double homogeneous thread with clean-cut edge. In other cases (fig. 9) it has a moniliform appearance, the ring structure being more evident as the component chromatin masses are further apart. In the cross-section of the cell shown in fig. 7 all the sections of the coil are transverse to the direction of the spireme thread. Groups of the enlarging tetrads can be seen joined to one another by the connecting linin substance. When the chromatin is not overstained and a surface view of the spireme is obtained, its appearance is that of a longitudinally split thread, the split, however, never being sharply defined. Figs. 11 and 16 show this apparent longitudinal splitting, while in figs. 9 and 14 the appearance, instead of resembling a split, is like a series of separate vacuoles. The apparent longitudinal splitting results from looking down upon the succession of rings formed by the incomplete fusion of the tetrads. The rings are less dense at the junction of the component masses, so that a surface view is that of two dense chromatin masses joined by the less dense linin;
or if the linin has broken down as in later stages, the split is the result of looking down upon the vacuoles which were formerly inclosed by the tetrads. That the split is not sharply defined is due to the persistence of the less deeply stained linin substance, which still connects in an uneven manner the masses of chromatin.

The growth and extension of the chromatic figure imply a correlated extension of the nucleus until the nuclear membrane breaks as a result of the interior pressure. Previous to the disruption of the nuclear membrane, the coils of the spireme have been quite regularly arranged within the nucleus, as shown in figs. 14 and 15, following the same arrangement that is to be seen in the early spireme, fig. 4. With the weakening of the membrane or closely following its dissolution, and with the consequent changes in pressure, the coils of the spireme break transversely at the places where they were bent. This breaking does not imply that the segments of the spireme have become completely separated, for, as fig. 30 shows, the linin substance may still connect the segments of the spireme into a more or less continuous thread.

The number of segments thus arising seems to be inconstant, apparently varying from ten to thirty or more. There is a great deal of uncertainty, however, as to the number of segments, because many of them are cut in sectioning, and thus the apparent number is increased. A study of fig. 30, which shows thirty-eight such segments, would seem to indicate that none of the chromosomes there has been cut by the knife, because there exists linin between the successive segments of the bands. If a chromosome had been cut off square by the knife, its end would appear sharply truncated, with no linin substance connecting it with an adjacent chromosome. Such evidence as this and that presented by the cells shown in figs. 37, a and b, leads to the conclusion that in vegetative cells of Allium the spireme is not invariably broken into sixteen chromosomes, as has been maintained by other investigators, but that the number of segments is dependent upon the size and course of the spireme in the nucleus. This transverse breaking of the spireme into segments
can be of no significance in the distribution of the chromatin, as each chromosome is yet a tubular structure resulting from the fusion of the tetrads, unaffected by the breaking of the spireme.

After the nuclear membrane has become dissolved and the interchange of material between nucleus and cytoplasm is complete, the rings of chromatin split, causing the separation of the associated chromatin masses. Figs. 32 and 37 show stages where the separation of the associated chromatin masses is well under way, while fig. 33 shows a similar stage where some of the segments have been cut crosswise. The pairs of chromatin threads separate first from each other at the middle, while the ends may remain for some time attached.

In cells shown in figs. 37a, 38, we have the last stages in the separation of the chromosomes in the spireme segment. The ends of the chromosomes are still connected by the linin substance, though the separation is nearly complete. The structure of each chromosome at this stage, as seen both in surface view and in cross section, presents exactly the same appearance as that of the mother segment of the spireme, the only difference being that of size. After the splitting the chromatin closes up to form again the rings, thus making the tubular structure of the daughter chromosomes of the spireme. The end of each chromosome shows the ring formed of chromatin masses, the margin becoming indefinitely four-sided; the surface view gives the same vacuolar or longitudinally split appearance throughout their length. Fig. 38 is of a stage showing the beginning of their movement toward the poles. The ring-like form, as seen from the end, and the split or vacuolar appearance, as seen in surface views, are here clearly marked, while in fig. 41 the component tetrads can be traced. In fig. 39 the cell was cut in such a plane as to give a longitudinal section of the chromosomes at one pole of the diaster and a cross section at the other pole. It can thus be seen from these figures that the disintegration of the rings into tetrads proceeds with the drawing apart of the chromosomes and with the beginning of their passage toward the poles.

The chromosomes having arrived at the poles become coiled, while their ends begin to approach one another. Figs. 42-45
represent sections of a cell, one drawn at high, the other at low, focus, so as to allow a complete reconstruction of the chromatic figure. At the right-hand upper pole of fig. 44 it can be seen that one chromosome cut in cross section reveals the tetrad structure. The chromosomes can be seen now to have become more vacuolated. The adjacent chromosomes are being drawn together by the linin substance, which is more deeply stainable than the remains of the spindle fibers. A reconstruction of the sections of the cell indicate that in the fundament of the daughter nuclei a more or less continuous spireme coil is being formed.

An opaque diffusible substance slightly stained by haematoxylin and other nuclear stains can be discovered between the chromosome coils. It appears to become denser as the vacuole-like spaces in the chromosomes increase in number (compare figs. 42-46). This substance has not before been recognizable. The chromosomes as they pass to the poles lie in a perfectly clear field, and it is not until they begin to fuse to form the spireme that this substance makes its appearance.

There is not a true vacuolization of the chromosomes, since the spaces are defined in too regular a fashion. This regular formation of the vacuoles in the rings is well illustrated in fig. 46. What we actually have is a resolution of the chromosomes into the component tetrads, with a gradual decrease in size of the chromatin bodies, apparently accompanied by an increase in volume of the linin. The loss of material accompanying the reduction in the size of the chromatin bodies is correlated with the increase of the linin and also with the presence of the diffusible substance which is deposited within the coils. In fig. 47, a longitudinal section of a periblem cell, are shown the first indications of a discrete body which can be termed a nucleolus. In fig. 48, where there is a continuous spireme, this is seen to lie within the coils and to occupy the same central position that the nucleoli of the quiescent nucleus hold after the nuclear membrane is completed. This nucleolus is only a condensation of the opaque substance to be seen in earlier stages between the chromosomes. In my opinion, it owes its origin to the chromatic bodies, being but a waste product of their activity.
In fig. 4g, where the cell plate is nearly completed and the nuclear membranes are beginning to appear, the spireme coil is still to be traced. It is now seen to be composed of a double strand of granules which are connected with each other in pairs and with the adjacent strands by the linin. With the complete separation of the daughter nuclei by the cell plate these strands become finer and the grouping more indistinct, until finally all that can be discerned in the quiescent nucleus are the chromatin bodies in a network and the nucleoli.

A review of all the changes of the chromatic figure is now in order. A comparison of the stages which led up to the formation of the equatorial plate with stages extending from that point to the formation of the daughter nuclei shows that the first series represents a gradual growth of tetrads and their fusion to form chromosomes; these latter split, half passing to each pole and in its passage suffering disintegration into tetrads. The changes in the two series are identical, but take place in the reverse order. The conditions in fig. 2, a mother-cell preparing to divide, are directly comparable with those shown in the daughter nucleus represented in fig. 4g. Similarly, fig. 14 is comparable with figs. 45, and the chromosomes at one pole of the diaster in fig. 41 with those in figs. 7 and 8.

The following diagrams show the order in which the changes in the chromatic figure occur.

![Diagrams illustrating the morphology of the chromatic substance during karyokinesis: the diagrams in the upper series represent end views of a portion of the thread; those in the lower series, side views.](image)

In reviewing the literature of the chromatic figure in plant cells, no account of such an origin, structure, and development as has just been described for Allium was found. The literature on the botanical side has been mainly concerned with contro-
verses over the number of chromosomes, the various shapes that they assume during division, and the manner of separation.

The work of Brauer (3) on the spermatogenesis of *Ascaris megaloccephala* has a direct bearing upon the results attained in the investigation of Allium. He states that in the passive nuclei of the spermatogonia the first preparation for the formation of the chromosomes is to be seen in the arrangement of the irregularly distributed granules into definite lines. The distinguishing feature of these nuclei in comparison with those which show no preparation for division is the presence of a sharp line between the chromatin granules, whereby these become arranged into two rows. In regard to the spermatocytes, he states also that when two threads do not lie directly over one another, or when through other change in their position an insight into their arrangement is made impossible, a division of the granules is everywhere to be seen. When a polar view of isolated groups of granules is obtained, each group can be seen to consist of four granules which lie arranged near one another in the same plane, each one being marked off from the neighboring ones by a well-defined fissure.

Brauer raised the question whether the splitting of the granules was from the beginning double, or whether the two splittings followed each other. He states that the granules in the earlier stages are too small and too numerous to allow a settlement of this question, yet he could discover in individual cases isolated granules composed of four elements.

The same question can be raised regarding the origin of the quadripartite granules of Allium in the earliest stages of the active nuclei in which any definite arrangement can be detected. Here too the evidence is of the same incomplete nature. Brauer does not describe for the early anaphase a resolution of the chromosomes first into double strands and then into quadripartite strands, each composed of a series of granules. Hence he has not raised the question, which I have previously discussed, whether, instead of a process of division of granules into fours to form the strands of the spireme, it may not be that from the beginning we have to do with a fusion of granules in fours.
Rosen (8) considered the formation of the spireme as due to the fusion of chromatic granules, but failed to get a polar view of the thread showing its quadripartite structure.

Němek (9) states that when the chromosomes have arrived at the poles, the chromatin coils send out pseudopodia-like continuations which bind opposite sides net-fashion. The chromatic substance gathers next on the periphery of the chromosomes, and dissolves into granules which wander into the pseudopodium-like continuations. In regard to the cell division of Solanum, Němek states that at the time when the spindle surrounding the nucleus is finished, the nuclear membrane vanishes and varicosities are to be seen in the chromatic threads. He thinks that the longitudinal splitting must occur at this time, as he found in thin sections of chromatin pieces in pairs extending into the periplast.

Hof (11) has a brief reference to what is doubtless the same condition which I have described in Allium. In stages of the dispireme, where the daughter nuclei are found, signs of a longitudinal splitting are rarely to be seen in the threads. The daughter nuclei present a structure similar to that shown by the nuclei of the prophase, except that they do not yet show the beginning of the enlargement of the nucleus. He thinks that very likely, owing to the quick succession of divisions in the cells of the meristematic tissue, those cells in which the longitudinal splitting is to be seen in the dispireme stage are to be considered as preparing for the ensuing division without the intervention of a resting stage. He does not hold this splitting to be a characteristic feature of the anaphase, as I do. Hof figures but two cells with signs of longitudinal splitting in the anaphase.

The fact that after this apparent longitudinal splitting has occurred in the chromosomes of the dispireme all stages can be found showing gradual reduction in size of the chromatin bodies and increase of the linin, is evidence that this is not a temporary phenomenon. A criterion for removal of all doubt that these stages in the late anaphase may have been mistaken for those of the early prophase is the presence of the incomplete cell plate.
Meves (17) describes vesicular chromosomes occurring in Paludina. The peculiar behavior of these chromosomes in their relation with the centrosomes makes it difficult to draw any analogies with the conditions found in Allium.

The interpretations which I have made do not refute what is a fairly well established fact, that in the rapidly dividing cells of meristematic tissue the daughter nuclei in the late anaphase often proceed to divide without the intervention of a resting stage or the formation of a nuclear membrane. It only reasserts, what Flemming (1) and other investigators have held, that the chromatin undergoes after the metaphase the same changes as before the metaphase, but in the reverse order. The history of the chromatin in cell division can be characterized as made up of periods of growth, aggregation, and fusion, followed by periods of separation, disintegration, and reduction.

The literature now remains for consideration which bears upon the shape of the chromosomes and the manner of their separation.

Ishikawa (7) studied division in the epidermal cells of Allium buds. He found that coil to break up into sixteen pairs of chromosomes, generally of an equal length.

Belajeff (6) stated that in vegetative cell divisions, while the daughter chromosomes are yet bound to each other at their ends, a rhomboidal figure is formed. The U-forming daughter segments move apart and form daughter stars, one at each pole of the nuclear spindle. Belajeff maintains that this U-shape of the chromosomes is characteristic and peculiar to vegetative cell divisions, and that in the heterotypical form of division there are V-, Y-, and X-shaped figures which show longer and shorter arms.

Hof (11) finds that the chromosomes in the stage of the mother star in vegetative cell division have mostly the figure of J-forming threads, with arms of unequal length, although rhomboidal figures also occur.

Strasburger (14) criticises Belajeff’s characterization of the three types of nuclear division based upon the form of the chromosomes. Neither the manner of insertion of the chromo-
somes in the spindle nor the definite lengths of the chromosome branches are considered by Strasburger as furnishing satisfactory marks for contrasting typical and atypical division in metaphytes. Strasburger found in the pollen mother-cells of Lilium and also of other forms that the daughter chromosomes at the beginning of the metaphase undergo a second longitudinal splitting. This takes place at right angles to the first.

Guignard (2) came to the same view as did Strasburger in regard to a second longitudinal splitting in the pollen mother-cells of *Najas major*.

This second longitudinal splitting described as occurring in the atypical divisions of cells Strasburger would make a distinguishing feature of their division, marking them out from typical divisions by this rather than by the form of the chromosomes or by the manner of insertion. Belajeff does not assent to the view that there is a second longitudinal splitting, but holds such V's as consisting of two chromosomes bound together.

It is not within the province of the present investigation to go into the details of atypical divisions in plants. A comparison of Strasburger's figures with the structures seen in the typical division stages of Allium confirms me in the belief that even in the presence of this second longitudinal splitting, we have no feature which will distinguish atypical from typical division.

I think that this second longitudinal splitting, described by Strasburger and by other investigators as peculiar to atypical division, is the same phenomenon as that described by me as occurring in the typical division of Allium cells, namely, the apparent longitudinal splitting seen in the surface view at the beginning of the metaphase, and that it is due to the changing of the daughter chromosomes from tubular structures into the quadripartite threads. This change is but a reversal of the change which occurred before the metaphase.

Future research, I believe, will establish that the mechanism of cell division in both the typical and atypical forms is essentially the same.

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LITERATURE CITED.


EXPLANATION OF PLATES XI-XIII.

Fig. 1. Cross section showing stages after the spireme has broken into chromosomes, and a quiescent nucleus; cells killed in Flemming fixative.
Fig. 2. Cross section of meristem nucleus where the spireme is first becoming evident; cell killed six hours in Flemming (weaker solution); stained with iron alum, eosin, and Heidenhain's haematoxylin. \( \times 2440 \).

Fig. 3. Longitudinal section of periblem cell showing quadripartite structure of the early spireme; Flemming twenty-four hours; iron alum and Heidenhain's haematoxylin. \( \times 2440 \).

Fig. 4. Longitudinal sections of two dermatogen cells showing the early spireme; fixed in chrom-acetic; stained with eosin, iron alum, and Heidenhain's haematoxylin. \( \times 2440 \).

Fig. 5. Longitudinal section of a periblem cell showing the quadripartite structure of the early spireme; killed in Flemming's weaker solution twenty-four hours; stained with iron alum and Heidenhain's haematoxylin. \( \times 2440 \).

Fig. 6. Longitudinal section of two periblem cells showing quadripartite structure of early spireme; killed in Flemming's weaker solution; the upper cell showing the withdrawal of the cytoplasm from the poles of the nucleus, giving the appearance of hyaline caps; stained with iron alum and Heidenhain's haematoxylin. \( \times 2440 \).

Fig. 7. Cross section of periblem cell showing the quadripartite structure of the spireme; killed in Flemming's weaker solution six hours; stained with iron alum and Heidenhain's haematoxylin. \( \times 2440 \).

Fig. 8. Longitudinal section of a periblem cell showing the quadripartite structure of the spireme in a somewhat later stage; killed with chrom-acetic; stained with orange G and Heidenhain's haematoxylin with iron alum. \( \times 2440 \).

Fig. 9. Cross section of periblem cell showing a later stage of the spireme and the nucleus beginning to elongate; killed with chrom-acetic and stained with orange G in combination with Heidenhain's haematoxylin and iron alum. \( \times 2440 \).

Fig. 10. Longitudinal section of a periblem cell with the spireme of smooth contour; killed in chrom-acetic; stained with safranin, gentian violet, and orange G. \( \times 2440 \).

Figs. 11–12. A longitudinal section of a periblem cell drawn at upper and lower focus, showing the appearance of hyaline polar caps and ingrowing cytoplasmic fibrillations; killed with chrom-acetic; stained with safranin and gentian violet. \( \times 1750 \).

Fig. 13. Longitudinal section of a periblem cell; the cell is sectioned in such manner as to give at the same time an end and surface view of the spireme thread; the end view shows the quadripartite structure, while the surface view shows the fusion of the component granules into a continuous thread; the elongation of the nucleus can also be noted here; killed with Flemming's weaker solution; stained with iron alum and Heidenhain's haematoxylin. \( \times 2440 \).
Fig. 14. A longitudinal section of a periblem cell showing a late stage in the spireme, where it has a vacuolar appearance with prominent connecting linin bridges; killed in chrom-acetic; stained with Heidenhain's haematoxylin with iron alum. × 2440.

Fig. 15. A longitudinal section of a periblem cell showing the elongation of the nucleus; killed in chrom-acetic; stained with eosin and Heidenhain's haematoxylin with iron alum. × 2440.

Fig. 16. Longitudinal sections of two periblem cells in the late spireme stage; the lower nucleus shows the appearance of hyaline caps; killed with chrom-acetic; stained with eosin and Heidenhain's haematoxylin with iron alum. × 2440.

Fig. 17. Longitudinal section of periblem cell showing ovoid enlargement of the nucleus; killed in Flemming’s weaker solution; stained with eosin and Heidenhain’s haematoxylin with iron alum. × 2440.

Fig. 18. Longitudinal section of a periblem cell showing the elongated nucleus after the breaking of the spireme thread, also the aggregation of cytoplasm at the poles; killed in picro-sublimate; stained with Heidenhain’s haematoxylin and iron alum in combination with eosin. × 2440.

Fig. 19. Longitudinal section of a periblem cell with the cell surface in focus, showing the elongated shape of the nucleus and its relation to the aggregations of cytoplasm; killed in picro-sublimate; stained with Heidenhain’s haematoxylin with iron alum in combination with eosin. × 2440.

Fig. 20. The same cell with the central portion in focus.

Fig. 21. Longitudinal section of a periblem cell showing the beginning of the dissolution of the nuclear membrane; killed in chrom-acetic; stained with Heidenhain’s haematoxylin in combination with orange G. × 2440.

Figs. 22–23. Longitudinal section of a periblem cell showing the lower and upper part of the cell; killed in chrom-acetic; stained with safranin, gentian violet, and orange G. × 1750.

Fig. 24. Longitudinal section of a dermatogen cell showing the hyaline cap appearance; killed in chrom-acetic; stained with Heidenhain’s haematoxylin in combination with eosin. × 2440.

Fig. 25. Longitudinal section of a periblem cell showing the fusion of the fibrillations from the cytoplasm with the nuclear reticulum; killed in Flemming’s weaker solution 24 hours; stained with Heidenhain’s haematoxylin in combination with eosin, × 2440.

Fig. 26. Longitudinal section of two periblem cells after the spireme has broken up into chromosomes, showing the disappearance of the nuclear membrane and the ingrowing of the fibrillations of the cytoplasm; killed in Flemming’s weaker solution twenty-four hours; stained with Heidenhain’s haematoxylin with iron alum. × 2440.

Fig. 27. Longitudinal section of a periblem cell showing an early stage in the formation of the achromatic figure; killed in Flemming’s weaker solution twenty-four hours; stained with Heidenhain’s haematoxylin with iron alum. × 2440.
Fig. 28. Longitudinal section of a periblem cell showing early stage in the formation of the achromatic figure; killed in picro-sublimate; stained with Heidenhain's haematoxylin in combination with eosin. × 2440.

Fig. 29. Longitudinal section of a periblem cell showing the formation of a bipolar spindle; killed in chrom-acetic; stained with safranin and gentian violet. × 1750.

Fig. 30. Longitudinal section of a dermatogen cell showing the last vestiges of a nuclear membrane and the union of the fibrillations from the cytoplasm with the nuclear reticulum to form the achromatic figure; a nucleolus somewhat reduced in size can be seen in the spireme; killed in chrom-acetic; stained in safranin, gentian violet, and orange G. × 1750.

Fig. 31. Longitudinal section of a dermatogen cell in a later stage showing the chromosomes beginning to form into equatorial plate; killed in chrom-acetic; stained with Heidenhain's haematoxylin and iron alum in combination with orange G. × 1750.

Fig. 33. Longitudinal section of a periblem cell, in stage just following the preceding; some of the chromosomes have been cut transversely in the act of separation; killed in Flemming's weaker solution; stained with Heidenhain's haematoxylin and iron alum in combination with eosin. × 2440.

Fig. 34. Longitudinal sections of two periblem cells in the stages of spireme and diaster; the upper cell shows a centrosome-like body below and at the right of the nucleus; killed in chrom-acetic; stained with Heidenhain's haematoxylin with iron alum in combination with eosin. × 2440.

Fig. 35. Two cells from the same slide as the preceding showing similar bodies in the cytoplasm.

Fig. 36. Longitudinal section of dermatogen cell showing late persistence of the nucleolus in the achromatic figure; killed in chrom-acetic; stained with Heidenhain's haematoxylin with iron alum in combination with orange G. × 2440.

Fig. 37. Longitudinal section of periblem cell showing separation of chromosomes; killed in chrom-acetic; stained with Heidenhain's haematoxylin with iron alum. × 2440.

Fig. 38. Longitudinal section of periblem cell showing the passing of the daughter chromosomes to the poles, the vacuolar appearance and the beginning of their disintegration into tetrads; killed in Flemming's weaker solution twenty-four hours; stained with Heidenhain's haematoxylin with iron alum. × 4240.

Fig. 39. Periblem cell cut obliquely, giving both cross and longitudinal sections of the diaster; the apparent second longitudinal splitting is to be

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