Chromosome Structure and the Mechanics of Mitosis and Meiosis

I. Mitosis in Lilium[1]

BY

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With Plates XVIII and XIX

I. INTRODUCTION

THE classical researches of Morgan and his co-workers have placed the chromosome theory of heredity on a firm basis. But cytological evidence in support of these genetical studies has not yet passed the stage of speculation. Divergent views are still current regarding the internal structure of the chromosomes, the time and mode of their division, and the cytological mechanism of crossing over. With the magnifications now possible with our high-power microscopes the finer details of internal structure of the chromosomes in living nuclei are still beyond correct resolution. We have therefore to examine materials that have been treated with suitable reagents and compare these observations with those of other organisms at the same stage, treated similarly as well as with a variety of reagents. New and improved cytological methods have enabled us to approach this difficult study in a manner less open to the criticism to which it has been subjected in the past. Moreover, the investigations of Kuwada and other Japanese botanists on living nuclei under dark field illumination have not only clarified many obscure points in chromosome mechanics, but also confirmed in several details conclusions reached by the more orthodox methods.

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The present investigation was undertaken with a view to examine the structure of chromosomes and correlate this structure with their behaviour in mitosis as well as meiosis. Lilium was selected as a suitable material as it possesses unusually large chromosomes. Although this genus has been extensively used for various cytological investigations, very little work has yet been done on the structure of its chromosomes. The present paper deals with the structure and division of the somatic chromosomes in Lilium. Similar studies on the meiotic chromosomes will form the subject of a subsequent paper.

II. Material and Methods

The present investigation is limited to three species of Lilium: *L. neilgherrense*, *L. Henryi*, and *L. tigrinum*.

Lilium neilgherrense Wight is a plant growing on the higher altitudes of south India. The material for this study was collected from Kodaikanal, a hill station in south India. The cytology of this species has not been studied hitherto, and as its chromosomes appeared very favourable for a critical examination of their internal structure, extensive collection of material was made in June of 1935 and 1936. The other species are garden plants collected at Kodaikanal through the courtesy of Mr. M. Maiden and Dr. F. H. Gravely. The structure of chromosomes has been studied mainly from sections of young ovaries, anthers, and root-tips of *L. neilgherrense*, while the other species were used for purposes of comparison only.

Root-tips and young ovaries were fixed in the following standard fluids and their modifications: Flemming weak and medium, Hermann's, Merkel's, Bouin's, La Cour's 2BE and 2BD, Smith's S1 and S2, Taylor's and Navashin's fluids. Time for fixation varied from twelve to twenty-four hours. For the fixation of root-tips Sharp's (1929) 'method twenty' and Nebel's (1933) method were tried with success. Most of the preparations were made according to La Cour's (1931) schedule, and these were equally satisfactory in regard to internal structure of chromosomes. An exhaust pump was used to facilitate rapid penetration of the fluid. Washing was done in running water for more than six hours and then completed with three or four changes of tepid water. Dehydration was done in alcohol through a close series starting with $2\frac{1}{2}$ per cent. Microtome sections varying from 6 to 40 μ thickness were cut and stained in iodine-gentian violet.

Young ovaries for the study of somatic chromosome structure and embryo-sac development were fixed after trimming the sides of the ovaries with a sharp blade and then treating them in Carnoy's fluid or 20 per cent. ethyl alcohol. They were then cut into 2 mm. thick pieces before transferring to the fixative. Some of the ovaries thus treated were left in Navashin's fluid for two to four days. On staining with gentian violet it was observed that material treated in this manner showed more rapid and clearer differentiation in clove oil than the material treated by either Sharp's or

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Nebel's methods. Very clear preparations showing chromonema spirals were thus obtained.

III. Number and Morphology of the Chromosomes of *Lilium neilgherrense*

The diploid number of chromosomes in this species of Lilium has been determined for the first time as twenty-four. Pl. XIX, Fig. 20, shows a metaphase plate from a root-tip cell showing twenty-four chromosomes. Slight variations in the relative lengths of chromosomes have been observed in materials collected from different localities in Kodaikanal. In some of the root-tips examined, a fragment chromosome was seen. In Pl. XIX, Fig. 20, two chromosomes show secondary constrictions, though this feature was not observed in materials from which oogenesis and meiosis were studied. Except for these minor structural variations, the morphology of the twenty-four chromosomes is similar in materials collected from different localities. In Pl. XVIII, Fig. 19, the haploid complement of twelve chromosomes, seen at the metaphase of the first post-meiotic mitosis at the micropylar end in the embryo-sac, is drawn seriatum to show the comparative size and morphology of the chromosomes. There are two sub-median attachment chromosomes denoted by the letters S1 and S2, one J chromosome, and nine sub-terminal attachment chromosomes denoted by the letters A to I. The length of the chromosomes varies from 8·5 μ to 16·5 μ, while in root-tip mitosis the variation is between 12·5 μ and 25 μ. In oogenesis the organization of a triploid complement was seen at the chalazal end of the embryo-sac in the second post-meiotic mitosis as observed by Cooper (1935) in other species of Lilium. Three each of the S1, S2, and J chromosomes are seen more prominently than the rest (Pl. XVIII, Fig. 18).

IV. Description of Somatic Division

After the separation of the chromosome halves at late metaphase, each group of daughter chromosomes moves towards either pole. In deeply-stained preparations, as well as in those treated with certain fixatives, their internal structure is obscure. But in material treated with Benda's fixative and properly differentiated they show a clear spiral structure. This is most distinctly seen in those fixed in Navashin's fluid and treated as per schedule given above. Each anaphase chromosome shows two spiral chromonemata embedded in a common matrix (Pl. XVIII, Fig. 1). The structure and relation of the two spirals of each anaphase chromosome were studied very critically owing to their importance in the interpretation of subsequent stages and the mode of spiralization of each chromonema. Pl. XVIII, Fig. 1, shows an anaphase group in which the two coiled chromonemata are very distinct in each chromosome (see photomicrograph, Fig. 22). In most of these the two coils are entirely free from each other and run parallel, while in a few the two coils are twisted on each other. The degree of intertwining of the two spirals of

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each chromosome varies to some extent in different cells, and the crossing-points of the two spirals range from one to four. Pl. XVIII, Fig. 2, shows anaphase chromosomes drawn on a higher magnification. In these the spiral structure is brought out more clearly.

When the chromosomes present a side view in the optical field, due to the superposing of one chromonema spiral on another, a false appearance of intertwining at every turn of the spiral is seen. This false appearance of intertwining is, however, characteristically different from the true intertwining shown in Figs. 2 *a*, *b*, and *c*. Kuwada and Nakamura (1933) have shown that when in a double spiral the component spirals are separated from each other and are shifted in the direction of their longitudinal axis, they will give the false appearance of two chords twisted about each other. This is very well illustrated by photographs of wire models in their Text-fig. 1. In deeply stained preparations such parallel approximation of two spirals would give a moniliform appearance, so frequently reported in anaphase chromosomes.

In some chromosomes, particularly those of root-tip nuclei, the chromonemata exist in some regions as an apparently single spiral thread, while along other regions of the same chromosome the duality is clear. Nebel (1933), in his study of the chromosomes in the root-tips of Tradescantia, has found the anaphase chromosomes as having two free spiral chromonemata pushed into one another.

In a few cases, shown in Pl. XVIII, Fig. 2 *c*, the chromosomes showed very deeply chromatic borders with a few twists, but here the chromonema spirals are not visible. This parallel alinement of chromatic halves of anaphase chromosomes, without any suggestion of internal spiral structure, has been illustrated by Sharp (1929), Hedayetullah (1931), Perry (1932), and Huskins and Hunter (1935). The number of twists increases as the anaphase progresses (cf. Hedayetullah, 1931, and Perry, 1932).

Tassement polaire. It has been reported for several plants that the end of anaphase is marked by a clumping together of the chromosomes to such a degree as to obscure both their individuality as well as their internal structure. Grégoire, who first described this appearance, gave it the name 'tassement polaire'. In Lilium, though the clumping has been frequently observed in somatic mitosis, the individuality of most of the chromosomes and their internal structure could be clearly made out in sufficiently de-stained preparations (Pl. XVIII, Fig. 3). In Pl. XVIII, Fig. 4, the late 'tassement polaire' condition is shown. Duality and twisted aspect are particularly clear in the projecting arms of two chromosomes. The 'tassement polaire' clumping involves a maximum contraction of the chromosomes in length. From the zigzag or spiral, which the chromosome as a whole is forced to assume, it appears that the matrix substance contracts to a greater extent and that the more chromatic portion of the chromosome is less affected by it. But even under identical conditions of fixation, no polar clumping is observed at the meiotic telophase,

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and so it is unlikely that 'tassement polaire' is a mere artefact of fixation as Overton (1922) has assumed, though the clumping may be accentuated by fixation. The stage from the late anaphase to the completion of a nuclear membrane may be considered as the most delicate with reference to its reaction to fixatives.

Telophase. A thin nuclear membrane becomes distinct after the emergence of the chromosomes from the 'tassement polaire'. At this stage a septum is formed in the middle of the cell and two daughter-cells are thus organized. The nucleus then assumes a more central position.

At the early telophase the nucleus is kidney-shaped, but as the telophase progresses the nucleus enlarges in size and assumes a more regular shape. It is difficult to distinguish early telophase chromosomes and their structure. But by mid-telophase (Pl. XVIII, Fig. 5) the structure of each chromosome becomes more clearly visible. Each chromosome is now seen to contain two spiral chromonemata which are closely associated at certain regions. The more significant telophase alterations which the chromosomes undergo are an elongation of the chromosomes and a relaxation of the anaphase spirals after their maximum contraction at 'tassement polaire'. At mid-telophase (Pl. XVIII, Fig. 5), in chromosomes where duality has not been completely obscured by the close association of the two spiral chromonemata, it is found that the chromonemata are twisted on each other, the twists being more than were observed at the anaphase. Pl. XVIII, Fig. 6 *a–c*, show the twisted dual aspect as well as regions where the chromonemata are so closely associated as to appear as a single spiral. In Pl. XVIII, Fig. 7, the late telophase condition is shown. The nucleus has assumed a regular shape and the chromosomes are seen as apparently single stranded spirals. Hsu Siang (1932) has figured the telophase chromosomes in *Lilium tigrinum* as having two chromatids twisted on each other at three or four points, while one chromosome shows only a half-twist (see his Fig. 19). A comparison of this with his figures of anaphase chromosomes shows that there is a reduction in the number of twists subsequent to anaphase. In *L. neilgherrense*, however, there is an increase in the number of twists during telophasic changes. Hedayetullah (1931) noted a significant difference in the structure of chromosomes at telophase in materials treated with Merkel's fluid and Flemming's or Hermann's fluids. In those of the former he found parallel duality with chromomeric appearance and occasionally one or two twists, while in those of the latter the two threads of a chromosome appeared uniform and intertwined at several regions. No such difference was noted in Lilium, though the spiral structure and duality of chromosomes were brought out clearly by certain fixatives while certain others obscured them. In Lilium, anastomoses or interchromosomal connexions were seen in the telophase nuclei in materials treated with osmic fixatives. In those subjected to the long schedule of Navashin fixation and de-stained to show the internal structure, the chromonemata alone were visible at this stage, and no interchromosomal connexions were seen. Since

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it is found that prolonged treatment with Navashin's fluid has a solvent effect on the matrix alone, it is likely that the so-called anastomoses are merely strands of achromatic matrix connecting adjacent chromosomes. Telezynsky (1930) has recorded the presence of lateral anastomoses in living material both in the telophase and the prophase. Sarbadhikari (1927) and a few others interpret them as outgrowths from the body of the chromosomes. Kaufmann (1926) considers them to be outgrowths of highly active, mobile chromatic threads.

Interphase. The nucleus that passes on from telophase to a state of inactivity as regards visible chromosome behaviour has been described as entering a resting stage. In rapidly-dividing tissues, like the meristems of root-tips, the interphase between the telophase and the succeeding prophase may be expected to be of very short duration, while in tissues where nuclear divisions are less frequent this stage may be more prolonged. In these latter nuclei, which have apparently remained in the 'resting stage' for a longer period, the chromatic tracts are very difficult to follow. The nucleus at certain regions shows the presence of discontinuous chromatic dots, which sometimes show a spiral course, while at other regions faintly stained chromatic spirals, which are continuous for short lengths, could be made out. In the nucleus which is apparently emerging from the interphase (Pl. XVIII, Fig. 8) or has remained in that condition for only a short period, continuous portions of the chromosomes are more distinctly seen and the chromatic dots are less prominent. The karyolymph also retains the stain to a certain extent. The spiral organization of the chromosomes is distinct at several regions (Pl. XVIII, Fig. 8). Thus it is seen that at the interphase also the spiral structure is maintained as at the preceding late telophase. No evidence of duality is visible in any chromosome at this stage.

Early prophase. This stage is marked by the reappearance of the chromatic tracts which became less distinct with the passage of the telophase nucleus into the interphase. The spirals shown in Pl. XVIII, Fig. 8, become more clear and the whole nucleus presents an appearance similar to the late telophase, with the difference that some of the chromosomes are run into zigzags. Darlington (1935) states that the chromosomes which appear at the early prophase do not possess the regular spirals seen at the telophase, as these spirals are considerably relaxed before entering interphase. In Lilium the telophase spirals are not very much relaxed before entering interphase, and even the interphase nucleus shows evidence of the presence of regular spirals. In rapidly-dividing tissues the nuclei that emerge from interphase are hardly distinguishable from those that enter it. One method of identifying an early prophase nucleus from a late telophase nucleus is afforded by the fact that while the two daughter-nuclei, produced as the result of a division, show nearly identical changes in their development and in the structure of their chromosomes from early telophase to the onset of interphase, the duration of interphase may vary with each nucleus, as the result of which they may enter the next prophase at different times. In the meristems of root-tips,

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where very rapid succession of divisions takes place, there may be no real interphase, and some prophase nuclei may show even the straight arrangement of the chromosomes unaltered as in Sharp's (1929) Figs. 20 and 26. These need not then be interpreted as telophase as Darlington (1935) would have it. In Pl. XVIII, Fig. 9, of early prophase showing this aspect, no evidence of duality is seen in any chromosome, though in some preparations an uneven outline of the chromosome thread is evident. Pl. XVIII, Fig. 10, represents a later stage from a more deeply-stained preparation, showing the persisting telophase spiral (referred to hereafter as the major spiral). Duality is visible at several points along the length of the chromosome in this figure, but the outline of the chromosome is more or less smooth. Fig. 11 shows a nucleus at very nearly the same stage but is drawn from a more de-stained preparation. The chromosome duality is very marked. The two chromatids are seen twisted on each other, and the number of twists is more than what was observed at anaphase, though the chromatids are not twisted at every region where they are seen to cross each other. The chromosomes at this stage have been described by some recent authors, Koshy (1933), Hoare (1934), and Gregory (1935), as composed of two tightly twisted chromonemata with a very large number of twists. While there is the probability that such differences in the degree of intertwining of chromatids may be expected in different genera, Sharp's (1929) observations on the structure of large somatic chromosomes of several plants is significant. He has shown in his figures only a few twists between chromatids, as is observed in the present study. He has further stated that 'careful examination of the points at which the two threads cross each other strongly suggests that they are not simply intertwined, but that their relative position is such as would permit them to move apart to two sides of the enveloping matrix without becoming entangled'.

In Pl. XVIII, Fig. 11, each chromatid is seen organized into a very regular spiral of small gyres. At certain regions it is very clear while along portions of the chromosome, where duality is not visible, only a regular corrugation of the chromatid is seen. This latter aspect might give the false impression that the chromatids are tightly twisted on each other (right side, bottom chromosome in Fig. 11). The chromosomes appear thicker and shorter than they were at an earlier stage of prophase, and the chromosomes are less crowded. This contraction of the chromosomes at the prophase of somatic mitosis has been recorded by several authors. Kaufmann (1931), Koshy (1933), and Hoare (1934) have described the prophasic contraction as being due to a new spiralization in each chromatid at this stage, though they did not actually observe this spiral at any stage prior to metaphase. Kuwada and Nakamura (1934) observed this spiral formation in the living nuclei of Tradescantia, though they have not illustrated it. Darlington (1935) states: 'The process of spiral formation during shortening (of the chromosome) has not been observed but comparison of the chromosome before and after shows that the two processes occur together.' Hedayetullah (1931) and Perry (1932)

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observed only chromomeres in each chromatid at early prophase. Sharp (1929) has figured the uneven outlines of the mid-prophase chromosomes but has described it as due to the presence of chromomeres, though the chromomeric appearance is not so evident as in Hedayetullah's figures. Very clear evidence has been obtained regarding this stage, and it is seen that the corrugation in each chromatid of the early prophase marks the beginning of visibility of a new spiral which becomes increasingly clear from mid-prophase onwards (Pl. XVIII, Figs. 12, 13, and 14).

Mid-prophase. Concurrent with the increase in size of the nucleus, but more rapid in its pace, proceeds the shortening of the chromosome length mainly due to spiralization. The joint effect of these two forces, viz. chromosome contraction and nuclear enlargement, is to bring about a gradual straightening of the major spirals. With the disappearance of the major spiral (Pl. XVIII, Fig. 13) the apparent as well as real twists are considerably reduced in number and only a very few twists persist at late prophase and still fewer at metaphase. The chromatids in each chromosome run very nearly parallel and the minor spiral in each is very distinct. The chromatids increase in thickness up to metaphase. This appears to be due to the increase in diameter of the gyres of the spirals as well as to the deposition of more chromatic material. Sharp (1929) has described an increase in length of the chromosome associated with its mid-prophase straightening. In Lilium it was not possible to detect any actual elongation of the chromosome as a whole, though an apparent increase may be seen, presumably due to a difference in the time relation between the processes of new internal spiralization and the straightening of the major spiral.

Before the disappearance of the nuclear membrane, in some of the chromosomes duality is evident in each chromatid (Pl. XVIII, Fig. 14—arrow marks). It was seen from an examination of the late telophase and early prophase chromosomes that when two chromatid spirals become closely associated the duality of the chromosome becomes almost a matter of inference. Conversely we might expect, though this need not necessarily be so, that when duality is seen at certain regions in a chromatid spiral these regions are not the only places where cleavage has occurred, but that it is probably double along the whole length. This expectation appears all the more plausible when we consider the fact that the chromatid in which duality is evident is coiled into a spiral of smaller dimensions than the telophase spirals or the major spirals of the early prophase.

Hsu Siang (1932) has reported the absence of a matrix substance in the prophase chromosomes in *Lilium tigrinum*. In my preparations the matrix is seen to stain only very faintly during mid- and late prophase, though the matrix of the chromatids takes the stain more deeply. It may be that after the division of each chromatid in preparation for the chromosome separation at anaphase, the common matrix of the two chromatids accumulates more and more around each chromatid.

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Late prophase. This stage may be marked off from mid-prophase by a tendency exhibited by the chromosomes to recede from their peripheral disposition. Pl. XVIII, Fig.15, shows some of the late prophase chromosomes soon after the disappearance of the nuclear membrane and the nucleolus. The chromatids are seen very deeply stained while the matrix is very lightly stained. The spiral structure of the chromatids is clear in most of the chromosomes, while the duality of the chromatids is distinct only in certain regions. In some cases duality of the chromatids is seen for a length included by two gyres of the chromatid spiral, and it is significant that the two chromonemata in each chromatid are free at these regions. In Sharp's (1929) Figs. 44 and 45, of late prophase in Vicia and Podophyllum, similar duality and spiral structure are shown.

No chromosome shows more than three or four twists between the chromatids at this stage. Contrary to similar observations of Sharp and several others, Hedeyetullah (1931) has reported an increase in the number of twists between the component chromatids from mid- to late prophase. An examination of his figures suggests the possibility of his having confused early and mid-prophases with mid- and late prophases by attaching too much significance to variation due to the action of different fixatives. Further, Sharp (1929) has shown that when chromosomes at late prophase are viewed from the side it may present a false appearance of the two strands being twisted on each other, and such error in observation is more likely in chromosomes like those of Tradescantia, which are not large enough to allow any definite conclusions to be made as regards the interrelationship of the chromatids.

The chromosomes shorten still further and tend to aggregate in the middle of the nuclear chamber. With the movement of the chromosomes to the equator of the spindle they pass on to metaphase.

Metaphase. The chromosomes in the equatorial plate have their attachment constrictions all pointing to the centre, though the arms of the chromosomes lie in different directions (Pl. XIX, Fig. 20). At early metaphase the comparative lengths of the different chromosomes could not be made out, as in the limited space available the chromosomes are too long to lie in one plane. But as contraction proceeds further, they come to lie more or less in one plane. This aspect is shown in Pl. XIX, Fig. 20, which is drawn from a deeply stained preparation of a root-tip fixed in Benda's fluid. The daughter chromosomes are distinctly formed and in some cases are twisted on each other, while in others they are parallel. Duality of daughter chromosomes is not seen in this, but less stained preparations show it. The clearest preparations showing quadruple structure and the spiral organization of each chromonema in the daughter chromosomes were obtained from young ovaries and anthers subjected to the long schedule with Navashin's fixative. In some of these the quadruple structure was seen with diagrammatic clearness along whole lengths of chromosomes (Pl. XVIII, Figs. 16 and 17). As the data concerning the interrelationship of the two chromonemata of each daughter chromosome

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has important bearing on the interpretation of the spiral mechanism and mode of chromonema duplication, the structure of metaphase chromosomes was studied very critically.

Pl. XVIII, Fig. 16*a*, shows an early metaphase chromosome exhibiting duality in each chromatid. The two chromonemata (shown by arrow-marks) are free for a length including three gyres of the spiral. The chromonemata are much thinner than the chromatids. Figs. 16*b* and *c* show duality at the ends of chromatids. In Fig. 16*d* the chromonemata are seen as free and separate for the major length of the chromosome. In Fig. 16*e* the spiral nature of each chromonema and their free and nearly parallel arrangement are seen. Fig. 16*f* shows a rather exceptional feature in this material; while two twists are still retained between the daughter chromosomes in the longer arm of the J chromosome, shown in the figure, in one of them four twists are seen between the daughter chromonemata. The structure of the end half of the other daughter chromosome is not clear, but the duality is indicated by the forked end. In Pl. XVIII, Fig. 17, some of the chromosomes of a late metaphase are shown at a lower magnification than Fig. 16. Of the twenty-four chromosomes only fourteen are shown in the figure. The chromosomes at the sides have separated at the attachment region and show the early stages of anaphase separation.

V. Discussion

(*a*) *Structure of the chromosome.*

Critical examination of both fixed and living material in recent years has well established the view that each chromosome consists of a coiled thread (chromonema) embedded in a less-chromatic matrix. Although the presence of a coiled thread in chromosome make-up was observed as early as 1882 by Baranetzky, no serious notice of this aspect was taken till 1926, when Kaufmann reported that the chromosomes of Tradescantia contain double spiral bands in all phases of somatic and meiotic divisions. As a result of the work of Kaufmann (1926–36), Sharp (1929, 1934), and Kuwada and Nakamura (1933–7), which have been confirmed and elaborated by several contemporary cytologists, we have no doubt now that each chromosome is composed of two coiled chromonemata embedded in a less chromatic matrix. Darlington (1935, 1937) and Huskins and Smith (1935) and some other investigators hold the view that at certain stages of the nuclear cycle the chromosomes are made up of linear rows of small chromatic units called chromomeres, identifiable by their characteristic size and definite position in the chromosome thread. Belling (1931), by a special technique elaborated by him, demonstrated the presence of an ultra-microscopic particle near the centre of each chromomere. He also counted the number of chromomeres in various Liliaceous genera and obtained values ranging roughly from 1,000 to 2,500. The existence of chromomeres as definite morphological units has, however, been denied by Grégoire (1906), Sharp (1929), Bolles Lee (1920), Smith (1932), O'Mara

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(1933), Koshy (1933, 1934, 1937), Hoare (1934), Gregory (1935), Gates and Nandi (1935), Naithani (1937), Gates (1937), and others. It is sufficient to state here that while no evidence was available which would appear to throw light on the view of the existence of chromomeres as definite morphological units, the present study afforded convincing proof of the existence of coiled threads (chromonemata) in all phases of chromosome history. Certain problems of chromosome structure connected with the chromonema theory, particularly the mechanism of spiralization and the time and mode of chromonema duplication, are still very controversial, and are therefore discussed in detail in this paper.

(*b*) *Spiral structure and chromonema behaviour in mitosis.*

The supporters of the chromonema theory claim to have observed the spiral structure of the chromosomes at some or nearly all phases of the nuclear cycle. Even the adherents of the older 'alveolar' and chromomere theories of chromosome structure observed spiral organization of chromatic threads in some phases of the nuclear cycle though there have been considerable differences of opinion as to the mode of their origin.

At the earliest stage of prophase when the chromosomes could be seen distinctly they are seen as remaining in a spiral form. The two chromatids are so closely associated that duality of the chromosome is difficult to make out. And it was also seen that the spirals observed at the preceding telophase reappear without much alteration at the prophase. In view of a new visible spiralization in each chromatid at prophase, this persisting telophase spiral has been referred to as the 'major spiral'. At the early prophase in well-differentiated preparations the outline of the chromosome is not smooth, but appears corrugated. At a little later stage when the duality which became obscure at the preceding telophase reappears at the dual regions, in place of the corrugations a spiral structure is seen in each chromatid. By mid-prophase this 'minor spiral' becomes very distinct in each chromatid. By late prophase we find that the pitch of the spiral has increased and the number of gyres decreased, as a result of which the chromosomes appear very much shortened. Duality in each chromatid first became evident in favourable regions before late prophase.

How this spiral originates has now to be considered. The forces, internal or external, responsible for forcing the chromonemata into spirals could not be determined directly from a study of fixed materials. But when we get a clear picture of chromonema and chromosome division and correlate their external behaviour with their internal structure it is easy to show which is the more probable mode of spiralization.

The assumption of a spiral by a straight thread or of a new spiral within a pre-existing spiral could take place in one of two ways. The spiral can arise by the rotation of the two ends in opposite directions and without any internal compensating twists or spiral readjustments, the number of gyres of the spiral

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corresponding to the number of rotations of the ends. If we make such a spiral with a copper wire by winding it round a rod and then take out the rod and pull the spiral out straight without allowing any relative rotation of the ends, it will be found that the copper wire is internally twisted in the same direction as the spiral was wound, the number of such complete twists being equal to the number of gyres of the spiral. Thus, this mode of spiral formation involves an internal twist which is directly proportional to the gyre number. This type of spiral may be termed 'unbalanced spiral'. The second method by which a spiral could be formed is by the ends remaining relatively fixed without undergoing any rotation as in the previous case. Then in the course of spiralization an internal reversed twisting takes place, the number of such twists being equal to the number of gyres of the spiral. If this spiral is pulled out straight without any relative rotation of the ends, it will be found that there are no internal twists as in the previous case. Here the twisting produced by spiralization is balanced by a compensating twist in the opposite direction at each gyre of the spiral. This type of spiral may be termed a 'balanced spiral' (see Pl. XIX, Fig. 21 A and B).

The problem is; which of the two types of spirals is formed when a chromonema spiralizes, and whether the one type could change to the other or be derived from it.

In the history of the chromosome it is the chromonema that is seen to maintain a regular permanence, and hence a chromosome division normally involves a division of its constituent chromonemata. On genetical grounds it is necessary to take the division as qualitatively equivalent, and cytological observations show that it is quantitatively identical as well. In a spirally coiled chromonema if a cleavage takes place along the longitudinal axis of the spiral the chromonema will be cut into bits. As the chromonema in Lilium is found to remain as a spiral throughout the mitotic cycle, and as it is seen as an unbroken structure, the cleavage in this could have been only along the longitudinal axis of the thread forming the spiral and not along the axis of the spiral itself, i.e. the course of cleavage could only be spiral and not straight.

If the cleavage is equational and along the course of the chromonema thread, then it would give rise in a spiral to two spirals comparable in all respects. They will have the same number of internal twists as the parent spiral and the direction of twists will also be the same. In the unbalanced spiral, since the twists are in one direction only and that in the direction of the coiling of the spiral, the daughter spirals will be interlocked once for every gyre of the parent spiral. But in the balanced spiral, since the twists are reversed in each gyre of the spiral, the daughter spirals too will have this compensating twist and hence there will be no interlocking which will hinder their separation (see Pl. XIX, Fig. 21 A and B).

Thus, basing our conclusions on what appear to be very reasonable assumptions regarding the mode of cleavage, we come to recognize in the inter-relationship of the daughter spirals the cardinal difference between the balanced

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and unbalanced types of parent spirals. It follows that an examination of the relationship between the daughter spirals would enable us to say which manner of spiralization is really operative. At late prophase where spiral structure of the chromatid is very clear, it is seen that the two daughter chromonemata of each chromatid are free from each other where duality is distinct. At metaphase a distinct quadruple structure is observed and the two chromonemata approximate to the borders of the chromatid matrix and exist as two independent coils. Two entirely free parallel chromonema spirals have been observed in each daughter chromosome at metaphase and in each chromosome at early anaphase. Sharp (1929) in his study of the structure of large somatic chromosomes obtained clear evidence of a parallel association of the two chromatids in each daughter chromosome at metaphase in Trillium root-tips. His Figs. 52 and 53 show very clearly this aspect as well as suggestions of the spiral nature of each chromatid. Similar structures at somatic metaphase, without any indication of an internal spiral, have been observed by Hedayetullah (1931), Perry (1932) and Huskins and Hunter (1935). Thus it is seen that the daughter-spiral chromonemata produced by a split in the parent coil are free by metaphase and could separate without entangling.

In tracing the development of the minor spiral from very early prophase it is seen that duality in each chromatid becomes evident by mid-prophase though clearly seen only at late prophase. So it is improbable that cleavage could have been completely effected in each *chromatid* before early prophase. Thus it is seen that the cleavage would have taken place only after the early organization of the new chromatid spiral. The next point is how the daughter chromonemata spiralize, whether independently or together. The two chromatids in each chromosome were seen as more or less separated from each other and spiralizing independently as mid-prophase was reached, though at early prophase the first signs of spiralization were seen when the two chromatids were so closely associated as to obscure chromosome duality. But the spiralization of the two chromonemata in each chromatid is not comparable to this. In most cases we find the two chromonemata associated as closely as the two chromatids were at early prophase, and this condition persists up to early metaphase. If independent spiralization had begun in each chromonema simultaneous with, or soon after their organization, then the duality of the chromatids would have become more apparent before metaphase. Kuwada and Nakamura (1935, p. 318) have stated that 'if the split halves (of the chromatids) are still so intimately associated with each other that they are coiled into a single spiral, and if they are separated first later from each other when the coiling has proceeded to a considerable extent, they will appear twisted about each other in the later stages, as observed by many investigators'. The contrary is observed in Lilium, though the split halves are closely associated in a single spiral.

If an equational cleavage takes place along an unbalanced spiral or after cleavage the two threads spiralize together into such a spiral, they will be

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interlocked once for every turn of the spiral. If after cleavage in such a spiral the two halves spiralize independently, even then the secondary spirals will not get completely free as some twists will still persist. No such subsequent spiralization is visible in somatic mitosis in Lilium. So, since the two daughter chromonemata can slip out from its common spiral, the chromonemata should be considered as two free and independent spirals from the time of their organization. The mechanism of the unbalanced spiral does not account for such a relationship between the daughter spirals. If an equational cleavage takes place along a balanced spiral or after cleavage in a thread the two threads together or independently coil into such spirals, they will be entirely free from interlocking or intertwining. It follows, then, that the balanced spiral mechanism satisfactorily accounts for the structures observed in Lilium chromosomes.

The above interpretations have been based to a certain extent on the observation that the chromonematic constituents of each chromosome are defined one mitotic cycle prior to their separation into daughter chromosomes. It would appear, however, that if we could have more convincing evidence in support of the contentions of Nebel (1933) or Goodspeed (1935) that the chromonemata divide two or three mitotic cycles prior to their separation into daughter chromosomes, then the evidence on which the balanced spiral mechanism was shown to account for the observations in Lilium may not alone be sufficient to disprove the probability of the unbalanced spiral also giving rise to the appearances described. We could dismiss such a probability if we consider for a moment the interpretations of these authors. Nebel (1933) has stated that at no stage in the mitotic cycle do chromonemata entangle with each other, and this observation has been confirmed by the more extensive observations of Nebel and Ruttle (1936). Goodspeed, Uber, and Avery (1935) consider the somatic anaphase chromosomes of *Lilium longiflorum* as constituted of two parallel chromatids each having two chromonemata presenting an intertwined aspect, though he is not certain about their real inter-relationship. He agrees with Nebel (1933) in the view that four free parallel chromonemata are present in each telophase chromosome. Two interlaced chromonemata in each chromatid at anaphase could not become completely free by telophase even by a new spiralization in each strand. The chromosomes are seen to increase in length after anaphase, and if a new spiralization takes place at anaphase it would only shorten the chromosome length. The other method by which they could become free is by the untwisting of each chromonema. The spatial limitation of the telophase nucleus would prevent such a process being carried to an appreciable extent and moreover such untwisting is not observed at that stage. It is thus seen that the daughter chromonemata could not have been intertwined or interlocked and they were more probably free from their inception.

It was seen that the early anaphase chromosomes have two free spiral chromatids approximated to the borders of the chromosomes and in some

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cases these two chromatids were twisted on each other at two or three regions. In the late anaphase chromosomes an increase was observed in the number of twists between chromatids. At early prophase when the two chromatids were released from their close association it was seen that the number of twists between chromatids were still larger. There could be no doubt that this has been brought about by the changes undergone by the chromosomes subsequent to anaphase separation. Sharp (1929), Hedayetullah (1931), and Perry (1932), who have all figured free parallel chromatids at anaphase, have shown interlaced chromatids at prophase. Kaufmann's (1926) figures of the somatic anaphase chromosomes of *Podophyllum peltatum* show a maximum of three twists in one chromosome, whereas his figures of prophase show up to seven or eight twists in a few chromosomes (see p. 359; figs, 19–23). An examination of the figures given by Koshy (1933) and Hoare (1934) also reveal the same fact. Hedayetullah (1931) has stated that chromatid split and twisting of the split halves are nearly simultaneous processes and take place at metaphase. But he has described an untwisting of chromatids at telophase and leaves unaccounted for the twisted aspect of chromatids at prophase, which he as well as several others have observed. Darlington (1936), who has denied the duality of anaphase and telophase chromosomes, states that 'chromatid coiling at metaphase seems to be developed during prophase chiefly as a result of a strain imposed on the chromatids by spiralization, but subject also to other conditions that have not yet been ascertained'.

The twisting of the two free spiral chromatids on each other is comparable to the twisting of homologous chromosomes subsequent to pairing at the prophase of meiosis. Differential contraction might be responsible for the increase in twisting up to telophase. The prophase chromatids are more twisted on each other and the twists are mainly associated with the persisting telophase spirals, and so it appears possible that differential expansion also contributes to an increase in twisting between chromatids. Even after the close association of the two chromatids at mid-telophase chromosome elongation continues. If in the process of elongation the ends of chromosomes also rotate in opposite directions to a certain extent, there will be proportionate twisting between the two chromatids. This seems more likely as the increase in size of the nucleus prior to interphase does not keep pace with the elongation of the chromosomes. This satisfactorily accounts for the presence of chromatid twists at some of the gyres of the persisting telophase spirals and the twists seen at other regions are probably the twists brought about prior to telophase and subsequent to anaphase separation.

From early prophase the nucleus enlarges in size up to the dissolution of the nuclear membrane at late prophase. With this increase in volume of the nucleus and the shortening of the chromosomes due to spiralization, the chromosomes are more spaced out and also appear peripherally arranged by mid-prophase. With these changes is associated a straightening of the chromosome as a whole. This straightening also removes most of the persisting

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telophase spirals and as the twists are also seen reduced, it is presumed that this straightening involved a slight rotation of the chromosome also. This rotation with contraction, since it reduces the number of twists, must have been in the opposite direction to the rotation with elongation at telophase. Thus it appears that a reversal of the telophase changes, which led to an increase in the number of twists, removes nearly all the twists by metaphase.

It is seen in somatic mitosis that the minor spiral seen at early prophase appears as the major spiral at the prophase of the succeeding division. Thus the chromosome visibly remains as a spiral throughout the nuclear cycle in somatic mitosis and for a short period at prophase it is seen as a double-coiled spiral. At late metaphase two free parallel spiral chromatids are seen in each daughter chromosome. Due to the subsequent twisting of the chromatids, at all other stages in the somatic mitosis we get a 'spiral-twisted-on-spiral' structure (cf. Koshy, 1934, p. 112).

The next point for consideration is how the minor spiral originates and whether it is the 'ultimate spiral' or not. Physico-chemical investigations have shown that the molecules of some crystalline substances exist in the form of chains. Bernal and Crowfoot (1934), who made X-ray studies of the protein pepsin, have observed a 'molecular spiral' during contraction. They take the molecular chain as probably formed by a degeneration resulting from a linking up of amino-acid residues. They further suggest the probability of the molecules of the primary soluble proteins having their constituent parts grouped more symmetrically around a prosthetic nucleus. Astbury and Lomax (1934) are inclined to consider the initial unit as the molecular chain itself and the spiral arrangement of the pepsin molecules as a subsequent process which may only be an elaboration of the intra-molecular folding which Astbury (1933) observed in keratin transformation. It emerges from the above findings that the visible patterns are determined by the arrangement of the molecules themselves.

From evidence discussed earlier one is led to the conclusion that the mechanism of spiralization is associated with a compensating internal twist. Since the visible minor spiral is shown to depend on the ultimate molecular arrangement, the reversed internal twist should have originated in the latter itself. It may be that this twist is determined by the relative arrangement of adjacent molecules in a spiral with reversed twists with reference to a prosthetic nucleus (Bernal and Crowfoot, 1934) or a central axis. Darlington refers to this twist as the 'molecular spiral'. Koshy (1933), basing his view on Earl's (1927) conception of the gene-thread as the basis of chromonema, has stated that it is highly probable that the particles which compose the chromonema are arranged spirally along its axis.

It was seen that the major spiral results from the development of the minor spiral. The first visible spiral is the minor spiral and it may be the molecular spiral increased in dimensions as a result of growth. If the ultimate arrangement of molecules remains the same at all stages, then this growth would

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involve the formation of a new molecular spiral. So the new spiralization seen at the prophase of a nuclear division is in all probability determined by the organization of an invisible molecular spiral, which will become the minor spiral of the immediately succeeding division, and this in its turn becomes the major or relic spiral of the next division.

With the acceptance of the chromosome theory of heredity together with the conception of the chromonema as the permanent constituent of the chromosome it has to be conceded that the gene-string is located in the chromonema. Calculations of the average size of a gene give values from 20 to 70 m. An object of this size cannot normally come within the range of visibility with the optical instruments which we now use. The regularity in behaviour of the chromosome suggests that the genes are definitely organized self-reproductive units. This leads us to assume that they are of a higher order of complexity than single molecules. It is probable that definite portions of the molecular spiral represent particular genes. This conception of the molecular spiral tends to support Morgan's (1928) theory of the linear arrangement of the genes.

(*c*) *Time and mode of chromonema and chromosome division.*

Most of the earlier workers on somatic mitosis considered the chromosomes as homogeneous structures splitting at the prophase of the nuclear division in which chromosomes so formed separated. A very large number of recent investigators have shown the origin of split in each chromosome as occurring at or before the metaphase of the preceding division. But the time and mode of cleavage have been variously interpreted even by investigators who agree regarding the duality of structure. Kaufmann (1926), Sharp (1929), Telezynsky (1931), Tuan (1931), and Smith (1932) obtained evidence to show that the chromonema division takes place in the late prophase leading to a quadruple structure from that stage up to anaphase separation. Koshy (1933, 1937), Hoare (1934), Naithani (1937), and Atwood (1937) have shown that the chromonema division is a pro-metaphase process. Hedayetullah (1931) and Perry (1932) observed quadruple structure only at metaphase and hence assumed that chromonema split and chromosome division occur simultaneously at metaphase.

In Lilium it is found that each somatic chromosome consists of two chromonemata and the division of each chromonema is seen to precede the separation of each chromatid at metaphase. At mid-prophase duality is observed at certain regions and it becomes particularly clear at late prophase. Since the daughter chromonemata are associated very closely in a common spiral, the duality could not be made out along the whole length of the chromatid. At metaphase the chromonemata in each chromatid are seen distinctly separate and though there is no common matrix binding the chromatids, each chromatid possesses its individual matrix. In preparations in which the spiral structure is obscure chromatid duality is observed only at metaphase when the

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two chromonemata separate to the borders of the chromatid matrix. Hedayetullah and several others failed to observe the spiral structure of the chromosomes at this stage and it is no matter for surprise that they did not obtain any evidence of chromatid duality which would have been present in these spirals. Hsu Siang (1932) observed in the early prophase chromatids in *Lilium tigrinum* the individual chromatids having an irregular outline, 'strongly suggesting that they are each composed of two tightly twisted threads'. He observed a quadruple structure only in a few chromosomes, and that too only at metaphase. The irregular outline to which he refers only shows the presence of the internal spiral which he failed to observe.

It is to be expected, as stated earlier, that gene division would precede chromonema division. While gene-reproduction may be a rapid process, the synthesis and accumulation of chromatin and other by-products of its activity may be comparatively slower processes. The duality in the chromatid becomes visible only after the two daughter gene-strings have increased in size and separated sufficiently to allow them to be optically distinguishable. It is therefore not unlikely that cleavage might have originated at early prophase at least. Thus each chromosome may be considered as quadruple from early prophase. It is also probable that the onset of a normal nuclear division marks the beginning of reproductive activity in the genes and the initiation of processes leading to chromonema duplication may be considered as the normal cause of the transformation of the nucleus from the 'resting' to the 'kinetic' condition. Kaufmann (1936, p. 536) states that chromatid doubleness 'and the consequent quadripartite nature of each chromosome may not be evident until late prophase or early metaphase, although actual division apparently occurs much earlier, the split being obscured by the close approximation in pairs of the half-chromatids'.

Kuwada (1926), from a study of the structure of the somatic chromosomes of *Vicia Faba*, found that the anaphase and telophase chromosomes are constituted of single spiral threads which at the succeeding prophase become straight prior to longitudinal splitting. Later, Kuwada and Nakamura (1935) as a result of investigations in living staminate hair-cells of Tradescantia, have concluded that 'two chromonemata are contained in each chromosome in the anaphase and telophase but they are interlaced with each other and cannot be separated'. Kuwada (1933) was the first to suggest a mechanism of spiralization which would account for the separation of daughter spirals without entangling. Huskins and Smith (1935) have supported this mechanism of spiralization. Nebel (1933) has suggested that if reduplication of chromonema occurs in only one plane, passing through the main axis of the chromosome, no difficulty will arise in the separation of the daughter chromonemata. Darlington (1935) is inclined to consider that the mechanism of spiralization is determined by a twist in the ultimate chromonema spiral. Evidence from the present study, though contradictory to several of Darlington's views on chromosome structure, has shown it as highly probable that the visible new

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spiral at the prophase is determined by a reversed twist in the molecular spiral. The observation of interlaced chromonemata at anaphase and telophase led Kuwada and Nakamura (1935) to assume that cleavage in a spiral would give rise to two interlaced spirals as shown earlier by Koshy (1934). This would mean that Kuwada has abandoned his earlier explanation of the mode of spiralization, namely, that for each turn of the spiral there is a *twist of the two threads* about each other in the opposite direction, so that the two coiled threads may separate, without entangling or uncoiling, as occurs in the dyad of Tradescantia at late first metaphase in meiosis (see Kaufmann, 1936, pp. 542–5). Whatever may be the modes of spiralization or cleavage, in Lilium it is found that the interlaced aspect seen at late anaphase and telophase is not the result of cleavage in a spiral chromatid, as the early anaphase chromosomes have two free parallel spiral chromonemata and the interlaced aspect is seen only later.

Kuwada and Nakamura (1935) are inclined to the view that chromonema division takes place at the interphase of the division preceding the one in which the halves so formed separate. They have stated that the chromonemata are most shrunken at this stage and division could take place only then. No convincing evidence is put forward to show that chromonema division could take place only when it is most shrunken. Further, the observations on fixed materials tend to show that the chromosomes are apparently most relaxed at the interphase (Koshy, 1937).

Brief reference may here be made to the interpretations of some recent authors which widely differ from evidence obtained in the present study. Darlington (1935, 1938) is alone among recent investigators in holding the view that the anaphase and telophase chromosomes are structurally single. Critical cytological investigations on the structure of chromosomes beginning with the work of Kaufmann (1926) have convincingly shown that each somatic metaphase chromosome is four-partite and consequently duality a constant feature in chromosome make up. Darlington (1926) described a quadruple structure of the metaphase chromosomes in the pollen grains of Hyacinthus and Scilla, but later discounted such observations as optical illusions due to the 'hollow' nature of the chromosomes. After the lapse of more than a decade during which period critical experimental and observational evidence have accumulated in support of duality of chromosomes, Darlington (1938) has changed his ground and considers all cytological observations of anaphase duality as based on misinterpretations of the 'bubbles of differential refractivity' arising in the chromosome as a result of using fixatives with acetic acid. Though a variety of fixatives were tried in the present study, nothing that could be interpreted as 'bubbles' in the chromosomes or chromatids was seen in any preparation. Further, Gates and Mensinkai (1938) have shown that the duality observed in the somatic anaphase chromosomes of *Trillium sessile*, and illustrated with excellent photomicrographs (Gates 1937), is not an artefact induced by fixatives containing acetic acid, as similar

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structures have been observed in material treated with non-acetic fixatives. Preparations of Lilium showing optically homogeneous structures after deep staining show duality and spiral aspect when properly de-stained. In Pl. XIX, Fig. 22, the photomicrograph clearly shows two chromosomes in which two free and parallel spiral chromonemata are seen without any appearance even remotely resembling what is illustrated by Darlington and La Cour (1938; see Text-figs. 1 and 2, and Pl. XXIV, Fig. 15). If the intertwined aspect of the chromonemata so frequently reported in anaphase chromosomes is to be taken as due to the presences of 'bubbles', and not on account of any inherent duality, as Darlington would have it, how then are we to interpret similar intertwined appearances seen in prophase *chromosomes* (Koshy, 1933)? The general acceptance (cf. Gates, 1938) of the presence of a pair of coiled chromonemata in all phases of chromosome history has invalidated most of Darlington's hypotheses of chromosomes and the relation of meiosis to mitosis.

Goodspeed (1935) considers that anaphase and telophase chromosomes in *Lilium longiflorum* are composed of four chromonemata, an eight-partite structure being seen at metaphase, the division in each chromonema taking place at the interphase. Nebel (1933) and Nebel and Ruttle (1936) have shown that the telophase chromosomes in *Tradescantia reflexa* and *Trillium erectum* are each constituted of four free parallel chromonemata the division in each taking place at metaphase, two mitotic cycles prior to their separation. No evidence in support of quadruple structure at anaphase and telophase was obtained in the present investigation. In my preparations the dual strands composing each telophase chromosome are seen to approximate so closely as even to obscure its double nature and they are coiled into an apparently single stranded spiral. While fixatives may be responsible to a certain extent for the appearance seen at the various stages of the nuclear cycle it seems unlikely that variations within such wide limits would really occur.

VI. Summary

The chromosome number for *Lilium neilgherrense* is determined as $n = 12$; $2n = 24$. The morphology of the chromosomes is described.

Each chromosome at early anaphase in somatic mitosis consists of two free parallel spiral chromonemata embedded in a less chromatic matrix. Subsequent to anaphase separation a progressive twisting of the two spiral chromonemata takes place, giving rise to a 'spiral twisted on spiral' structure at all later stages.

At telophase the spirals relax and the two chromonemata become so closely associated that they are seen as only single-stranded spirals.

The spiral organization of the chromonemata is maintained at interphase also.

A new, visible spiralization takes place at early prophase in each chromatid

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and the first evidence of this is seen as a regular corrugation in the apparently single-stranded chromosome thread. When duality reappears at early prophase, the chromatids are seen as twisted more on each other than at the previous anaphase. This twisting is presumed to be due to inter-chromatid adjustments induced by chromosome elongation in a limited space at telophase.

Cleavage in each chromatid is initiated at early prophase and the plane of cleavage is equational along the spiral and is such that two free spirals originate from the parent spiral. By late prophase each chromosome becomes visibly quadruple.

Thus each separating daughter chromosome at metaphase has two free parallel spiral chromonemata.

The possible methods of spiralization are discussed and it is shown that the ultimate structure of the chromonema is in all probability a 'balanced spiral', i.e. a spiral in which the twisting caused by spiralization is compensated by a reversed internal twisting at every gyre of the spiral.

In conclusion, I wish to express my gratitude to Professor T. K. Koshy, under whose supervision this work was done, for valuable suggestions and criticisms during the course of this investigation. My grateful thanks are due to Professor R. Ruggles Gates, for the keen interest he took in the progress of this investigation and for helpful criticism in the preparation of this paper.

Literature Cited

Astbury, W. T., 1933: Trans. Faraday Soc., xxix. 193. (Cited by Astbury and Lomax, 1934.)
—— and Lomax, R., 1934: X-Ray Photographs of Crystalline Pepsin. Nature, cxxxiii. 795.
Atwood, S., 1937: The Nature of the Last Premeiotic Mitosis and its Relation to Meiosis in Gaillardia. La Cellule, xlvi. 391–406.
Belling, J., 1931: Chromomeres of Liliaceous plants. Univ. Calif. Publ. Bot., xvi. 153–70.
Bernal, J. D., and Crowfoot, D., 1934: X-Ray Photographs of Crystalline Pepsin. Nature, cxxxiii. 795.
Bolles Lee, A., 1920: The Structure of Certain Chromosomes and the Mechanism of Their Division. Quart. Journ. Micros. Sci., lxv. 1–32.
Cooper, D. C., 1935: Macrosporogenesis and Development of Embryo Sac of *Lilium Henryi*. Bot. Gaz., xcvii. 346–55.
Darlington, C. D., 1926: Chromosome Studies in the Scilleae. Journ. Genet., xvi. 237–51.
—— 1935: The Internal Mechanics of the Chromosomes, I. The Nuclear Cycle in Fritillaria. Proc. Roy. Soc. B, cxviii. 33–59.
—— 1936: The Internal Mechanics of the Chromosomes, V. Cytologia, vii. 248–55.
—— 1937: Recent Advances in Cytology. J. & A. Churchill Ltd., London.
—— 1938: Structure of Chromosomes. Nature, cxli. 371–2.
—— and La Cour, L., 1938: Differential Reactivity of the Chromosomes. Ann. Bot., N.S., ii. 615–25.
Earl, R. O., 1927: The Nature of Chromosomes. Bot. Gaz., lxxxiv. 58–74.
Gates, R. R., 1937: Double Structure of Chromosomes. Nature, cxl. 1013–14.
—— 1938: The Structure of the Chromosome. Journ. Roy. Micr. Soc., lviii. 97–111.
—— and Nandi, H. K., 1935: The Cytology of Trisomic Mutations in a Wild Species of Oenothera. Phil. Trans. Roy. Soc., ccxxv. 524. 227–54.
—— and Mensinkai, S. V., 1938: Double Structure of Chromosomes. Nature, cxli. 607.

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GOODSPEED, T. H., UBER, F. M., and AVERY, P., 1935: Chromosome Structure in *Lilium longiflorum*. Univ. Calif. Pub. Bot., xviii. 3. 33–44.

GRÉGOIRE, V., 1906: La Structure de l'élément chromosomique au repos et en division dans les cellules végétales (Racines d'Allium). La Cellule, xxiii. 311–53.

GREGORY, P. J., 1935: Cytological Studies in Safflower. Proc. Ind. Acad. Sci., i. 763–77.

HEDAYETULLAH, S., 1931: On the Structure and Division of the Somatic Chromosomes in Narcissus. Journ. Roy. Micr. Soc., li. 347–86.

HOARE, G. V., 1934: A Comparative Study of the Chromosomes of *Scilla nonscripta* during Somatic and Meiotic Mitosis. La Cellule, xliii. 7–41.

HSU SIANG, 1932: Structure of Somatic Chromosomes in *Lilium tigrinum*. La Cellule, xli. 165–78.

HUSKINS, C. L., and HUNTER, A. W. S., 1935: The Effects of X-Radiations on Chromosomes in the Microspores of *Trillium erectum*. Proc. Roy. Soc. Ser. B, cxvii. 22–33.

—— and SMITH, S. G., 1935: Meiotic Chromosome Structure in *Trillium erectum*. Ann. Bot., xlix. 119–50.

KAUFMANN, B. P., 1926*a*: Chromosome Structure and its Relation to the Chromosome Cycle, I. Somatic Mitoses in *Tradescantia pilosa*. Amer. Journ. Bot., xiii. 59–80.

—— 1926*b*: Idem. II. *Podophyllum peltatum*. Ibid., xiii. 355–63.

—— 1931: Chromonemata in Somatic and Meiotic Mitoses. Amer. Nat., lxv. 280–2.

—— 1934: Somatic Mitoses of *Drosophila melanogaster*. Journ. Morph., lvi. 125–55.

—— 1936: Chromosome Structure in Relation to the Chromosome Cycle. Bot. Rev., ii. 529–53.

KOSHY, T. K., 1933: Chromosome Studies in Allium, I. The Somatic Chromosomes. Journ. Roy. Micr. Soc., liii. 299–318.

—— 1934: Idem. II. The Meiotic Chromosomes. Ibid., liv. 104–20.

—— 1937: Number and Behaviour of Chromosomes in *Aloe litoralis*. Ann. Bot., N.S., i. 43–58.

KUWADA, Y., 1926: On the Structure of the Anaphasic Chromosomes in the Somatic Mitosis in *Vicia faba*, with special reference to the so-called Longitudinal Split of Chromosomes in the Telophase. Mem. Coll. Sci., Kyoto Imp. Univ., B, ii. 1–13.

—— and NAKAMURA, T., 1933: Behaviour of Chromonemata in Mitosis, I. Observations of Pollen Mother Cells in *Tradescantia reflexa*. Mem. Coll. Sci., Kyoto Imp. Univ., B, ix. 129–39.

—— 1934: Idem, III. Observations of Living Staminate Hairs in *Tradescantia reflexa*. Ibid., B, ix. 343–66.

—— 1935: Idem, VI. Metaphasic and Anaphasic Longitudinal Split of Chromosomes in the Homotype Division in Pollen Mother Cells in *Tradescantia reflexa*. Cytologia, vi. 314–19.

LA COUR, L., 1931: Improvement in Everyday Technique in Plant Cytology. Journ. Roy. Micr. Soc., li. 119–26.

MORGAN, T. H., 1928: The Theory of the Gene. Yale Univ. Press.

NAITHANI, S. P., 1937: Chromosome Studies in *Hyacinthus orientalis*, I. The Somatic Chromosomes. Ann. Bot., N.S., i. 129–46.

NEBEL, B. R., 1933: Chromosome Structure in Tradescantiae, IV. The History of the Chromonemata in Mitosis of *Tradescantia reflexa*. Cytologia, v. 1–14.

—— and RUTTLE, M. L., 1936: Chromosome Structure, ix. *Tradescantia reflexa* and *Trillium erectum*. Amer. Journ. Bot., xxiii. 652–63.

O'MARA, J., 1933: Division of the Generative Nucleus in the Pollen Tube of Lilium. Bot. Gaz. xciv. 567–78.

OVERTON, J. B., 1922: The Organization of the Nuclei in the Root-tips of *Podophyllum peltatum*. Trans. Wisc. Acad. Sci., xx. 275–322.

PERRY, K. M., 1932: Mitosis in *Galanthus nivalis*. Journ. Roy. Micr. Soc., lii. 344–56.

SARBADHIKARI, P. C., 1927: Cytology of Osmunda and Doodia, II. On the Gametophytic Tissue of Doodia. Ann. Bot., xli. 1–35.

SHARP, L. W., 1929: The Structure of Large Somatic Chromosomes. Bot. Gaz., lxxxviii. 349–82.

—— 1934: Introduction to Cytology. McGraw-Hill, New York and London.

SMITH, F. H., 1932: The Structure of the Somatic and Meiotic Chromosomes of *Galtonia candicans*. La Cellule, xli. 243–63.

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TELEZYNSKY, H., 1930: Le Cycle du chromosome somatique, I. Observations vitales sur les poils staminaux de *Tradescantia virginiana* L. Acta Soc. Bot. Poloniae., vii. 381–433.
—— 1931: Le Cycle évolutif du chromosome somatique, II. Observations sur le matériel fixé (racines d'*Haemanthus Katharinae* Back). Ibid. viii. 109–32.
TUAN, H. C., 1931: Unusual Aspects of Meiotic and Postmeiotic Chromosomes of Gasteria. Bot. Gaz., xcii. 45–65.

EXPLANATION OF PLATES XVIII AND XIX

Illustrating Mr. Abraham's paper on 'Chromosome Structure and the Mechanics of Mitosis and Meiosis. I. Mitosis in Lilium'.

All figures in Plate I were drawn at table level with the aid of a Zeiss camera lucida. Leitz 1/12 in. oil immersion N.A. 1·3 and 1/16 in. oil immersion N.A. 1·32 were used with Leitz periplanatic oculars. Unless otherwise indicated, the figures are drawn from cells of young ovaries fixed in Navashin's fluid according to the method described on p. 546. Figs. 1 and 17, ×2,200; Figs. 19 and 20, ×1,800; Fig. 18, × 1,500; all other figures have a magnification of ×2,650.

The photomicrographs[1] (Figs. 22–5) were taken from permanent preparations made from material fixed according to the long schedule of Navashin fixation and stained with gentian violet (Zeiss 100×15; bellows extension, 9 in.).

PLATE XVIII

Fig. 1. Early anaphase chromosomes showing two free parallel spiral chromonemata surrounded by a less chromatic matrix. In a few chromosomes, the chromonemata are twisted on each other.

Fig. 2 *a* and *b*. The chromosomes at early anaphase each showing two spiral chromonemata twisted on each other.

Fig. 2 *c*. Duality and twisted aspect of chromosomes from material fixed in Benda's fluid. Spiral nature of chromonema not visible. In the middle chromosome no twists are seen.

Fig. 3. 'Tassement polaire', before cell-wall and nuclear membrane are formed. Note duality and twisted aspect.

Fig. 4. Late 'tassement polaire'; duality and twisted aspect clear in projecting arms of chromosomes.

Fig. 5. Telophase nucleus soon after nuclear membrane was organized. Note duality as well as beginning of close association of the two spiral chromonemata.

Fig. 6 *a–c*. Early telophase chromosomes showing duality and twisted aspect as well as close association of the two chromonemata.

Fig. 7. Late telophase showing apparently single-stranded spirals. The more or less straight arrangement of the chromosomes is noticeable.

Fig. 8. Nucleus emerging from interphase. Note spiral structure of the chromosomes and discontinuous chromatic dots which show a spiral course.

Fig. 9. Early prophase. Chromosome spirals are more clearly seen but duality is not yet visible.

Fig. 10. Prophase (later than stage shown in Fig. 9), from a deeply stained preparation. Duality is visible in each chromosome at several regions. Chromosome threads appear smooth.

Fig. 11. Nearly same stage as above. Duality is conspicuous and each chromatid is organized into a minor spiral. Where duality is not visible chromosome thread appears merely corrugated. The two chromatids are twisted on each other, but at some points where the chromonemata cross each other one is superposed on the other.

Fig. 12. Nucleus near mid-prophase. Spiral structure of chromatids more clear.

Fig. 13. Mid-prophase nucleus showing the major spiral considerably relaxed and the minor spiral more conspicuous.

Fig. 14. Mid-prophase showing duality in a few chromatid spirals (see arrow-marks). The major spirals are seen mostly relaxed and the chromosomes show a straight arrangement.

[1] I am indebted to Prof. R. Gopala Iyer, Director of the Madras University Zoology Research Laboratory, for kind permission to take the photographs in his laboratory. My thanks are due to Dr. J. P. Joshua and Dr. M. K. Subramaniam for kindly assisting in taking the photographs.

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Fig. 15. Late prophase after nuclear membrane and nucleolus have disappeared (from a young anther cell). Twists between chromatids reduced.

Fig. 16 *a–f*. Chromosomes from early metaphase to late metaphase. Each chromosome half has two spiral chromonemata which are completely free in some cases. Details referred to in text.

Fig. 17. Some late metaphase chromosomes showing two free spiral chromonemata in each daughter chromosome. In a few chromosomes anaphase separation has commenced.

Fig. 18. Metaphase plate from the second post-meiotic mitosis at the chalazal end in embryo-sac, showing the triploid complement of thirty-six chromosomes—slightly spaced out in drawing. Three each of the S1, S2, and J chromosomes could be easily made out.

Fig. 19. Metaphase chromosomes of second post-meiotic mitosis at the micropylar end in embryo-sac showing the haploid complement of twelve chromosomes, drawn seriatum to show the morphology of the chromosomes (denoted by the letters S1, S2, J, and A–I).

PLATE XIX

Fig. 20. Metaphase plate from a root-tip cell (fixed in Benda's fluid). Note the morphology of the twenty-four chromosomes.

Fig. 21 A and B. Photographs of spiral models made from indiarubber pressure-tubing of narrow bore, painted white on one side. A tight fitting pliable copper wire is inserted into the tube to keep it in position. Fig. 21A shows the 'Unbalanced Spiral'; the longitudinal halves intertwine with each other at every gyre of the spiral. Fig. 21B shows the 'Balanced Spiral', with reversed internal twisting at every gyre of the spiral. The longitudinal halves could separate without entangling.

Figs. 22–4. (Photomicrographs of anaphase chromosomes). In Fig. 22 the spiral nature of the two free and parallel chromonemata in each chromosome could be seen. Only two chromosomes in the upper group are in focus; the others also reveal the same structure, some of the chromosomes in this group are drawn in Fig. 1. In Figs. 23 and 24 several anaphase chromosomes show duality.

Fig. 25. Metaphase chromosomes (slightly faded) showing duality in each daughter chromosome. Note chromosome pointing to 7 o'clock and the chromosome in the centre.

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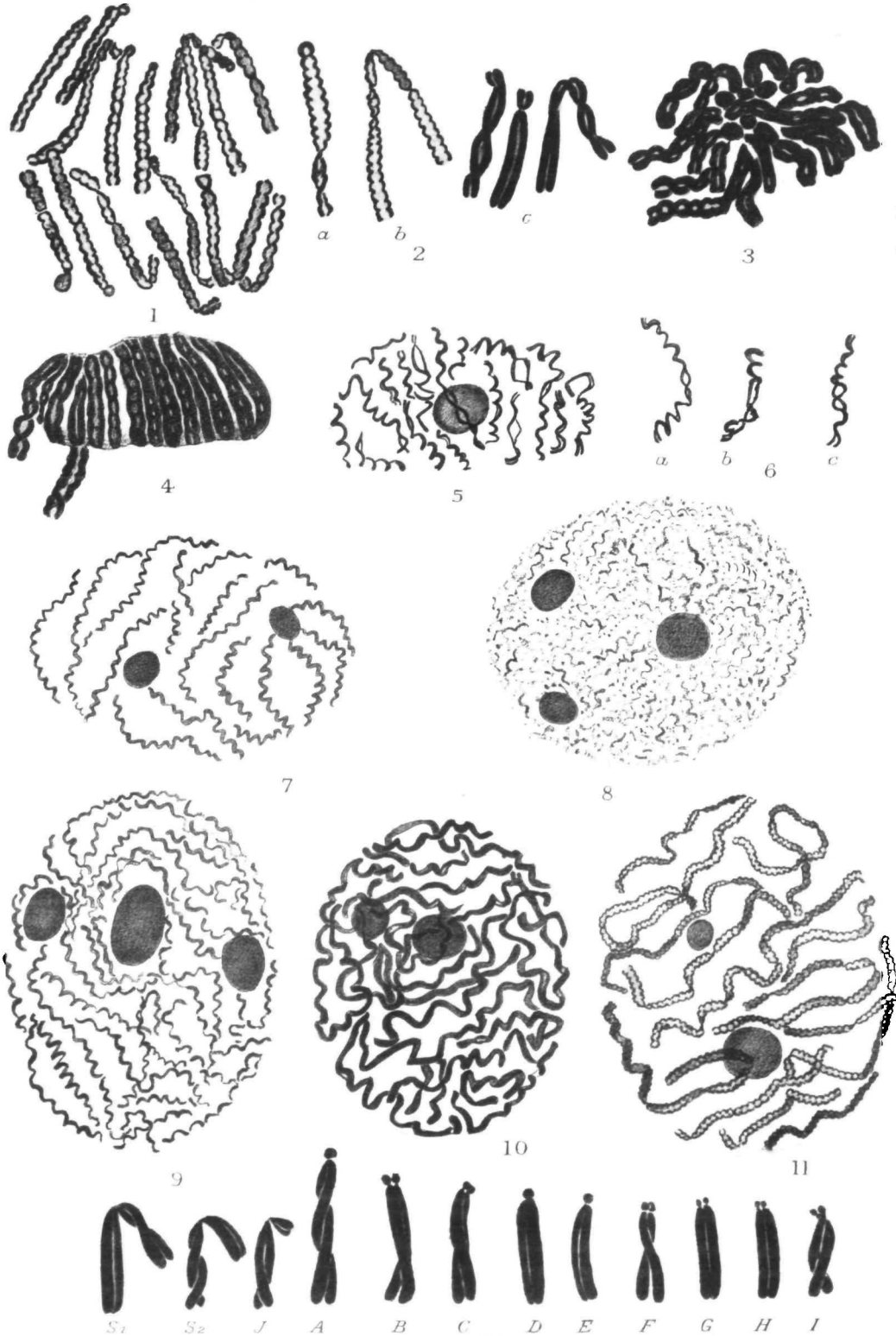

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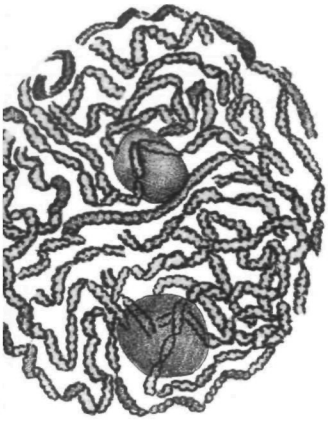

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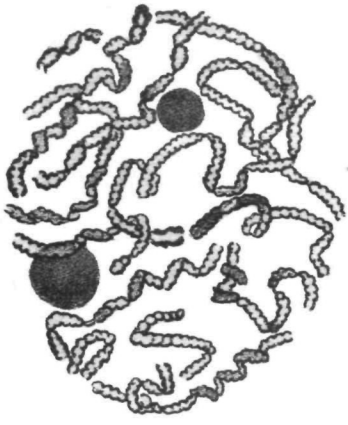

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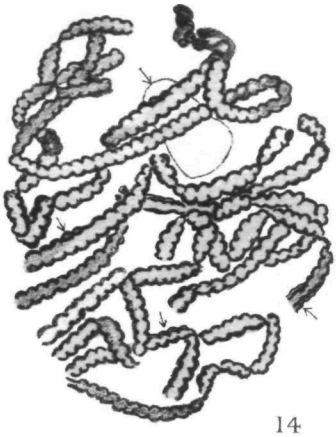

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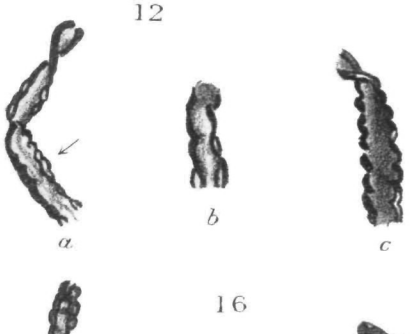

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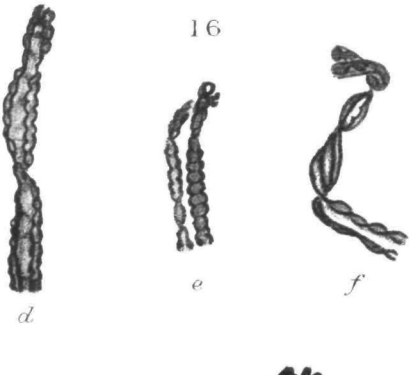

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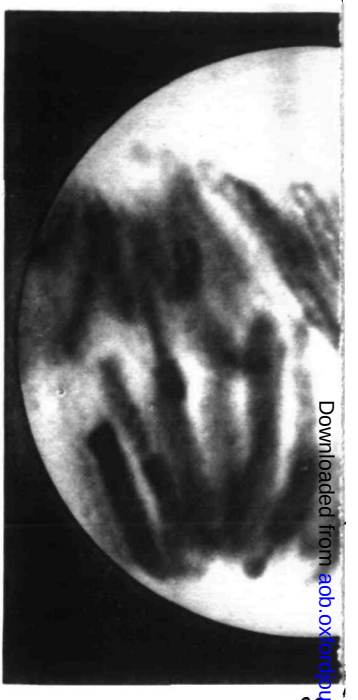

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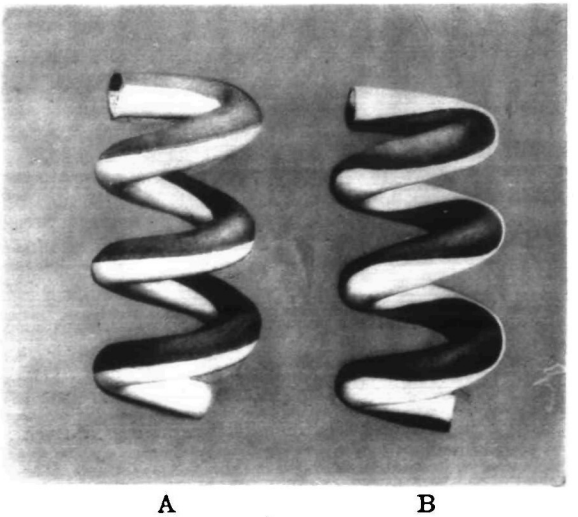

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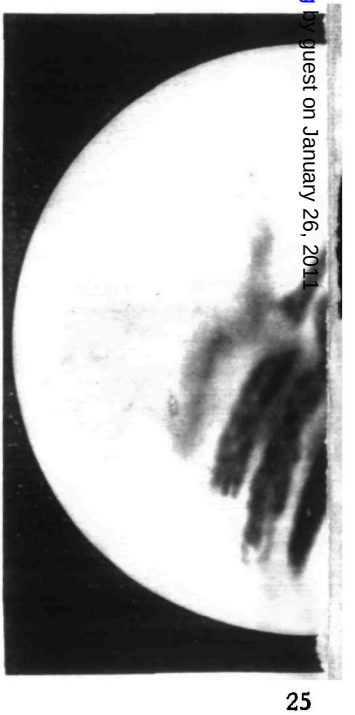

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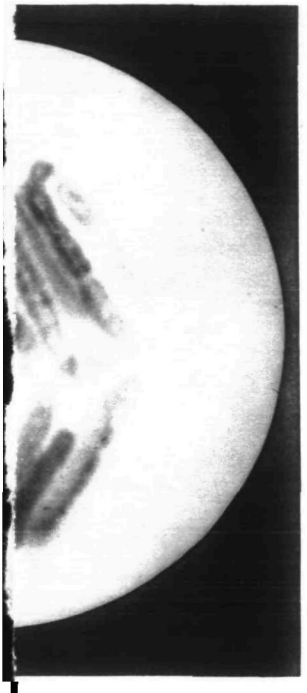

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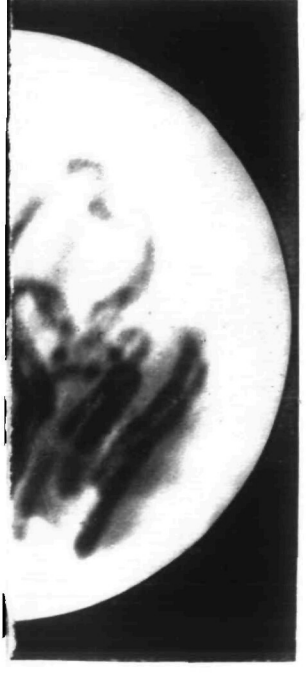

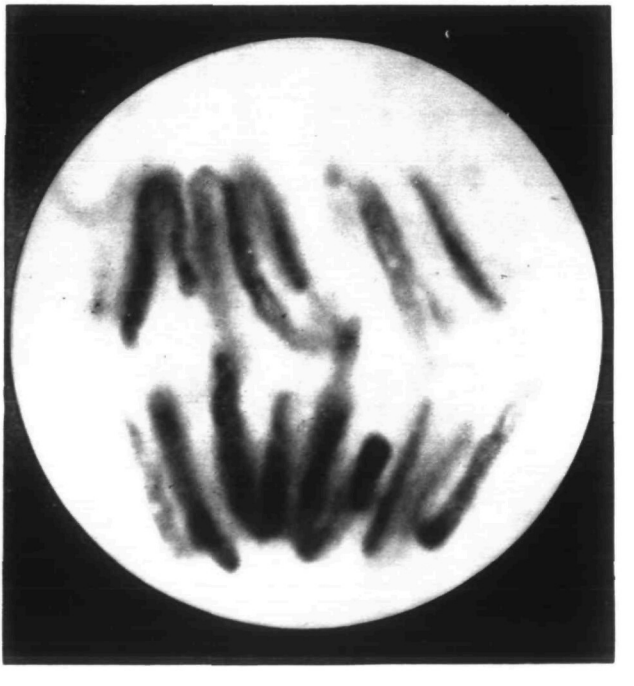

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Huth coll.

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