Behaviour of Chromonemata in Mitosis

IX. On the configurations assumed by the spiralized chromonemata¹⁾

By

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The studies of chromosomes by means of the polarization microscope seem to show that the chromonemata in the chromosome assume one configuration in a certain method of fixation and another configuration in another method (BECKER and KOZBIAL, 1937). In his critical review of chromosome structure²⁾, KUWADA (1939) has postulated a hypothesis of chromosome cycle in which it is assumed that there are three different configurations taken up by the chromonemata in the chromosome. The fine and coarse structures, experimentally shown by SINKE to exist in the nucleus and observed by NAKAZAWA in living material (both with KUWADA, 1939), are those structures which led him to this assumption. In this hypothesis it is assumed that the fine structure is due to the separation of the spiralized chromonemata in each chromosome from one another so as to present a fine but complex appearance in the nucleus; and the coarse structure due to a union being made between the two sister chromonemata of the separated four, the number of the chromonemata for one chromosome which NEBEL believes to be the actual one (cf. KUWADA, SINKE and NAKAZAWA, 1939, foot-note on p. 397). Looking back into the literature on telophasic chromosomes, KUWADA has further assumed that there should exist two types in the coarse structure. In one type, the united chromonemata take up the spiral or S configuration, and in the other the twisted or T configuration, the form which an orthospiral (KUWADA, 1939) can assume when drawn out. It has been also assumed that the twisted configuration is due to the chromosomes being in a more dehydrated condition than in the spiral configuration. It is the main object of the present

1) Investigation supported in part by grant from the Science Research Fund of the Department of Education.

2) In this critical review the authors' names, AISIMA under Fig. 5 on p. 222 and SINKE under Figs. 10 and 11 on p. 237, were left out by mistake, and are to be put respectively in place, each preceded by "After" and with parentheses. *Galtonia* in the 15th line from the bottom on p. 232 is also to be read for *Gasteria*. This opportunity has been taken to make these corrections.—Y.K.

investigation to see whether these hypothetical configurations of the chromonemata really exist in the resting nucleus or not.

Observation

SINKE (l.c.) has demonstrated that a coarse structure of the nucleus results from the nucleus of a fine structure by dehydrating it by means of hypertonic solutions. This suggests that a nucleus of the coarse structure will be transformed into one of a fine structure if it is put in a hypotonic medium. If, therefore, the two types of the coarse structure, one with the S configuration and the other with the T configuration, really exist, it is to be expected that a similar experiment, or the observation with a hypotonic solution as medium, will throw some light on the genetic interrelationship assumed between these two types of the structure. From this point of view, not only liquid paraffin but also tap water were used as mounting media in the following observations, staminate hairs of *Tradescantia reflexa* being used as material.

1. *Telophase.* In our previous observation with liquid paraffin, the main results of which were published in the "Behaviour" III (1934), we often noticed a structure in which the chromonemata appear as a series of dots, like a broken line, two such being found parallel or twisted about each other in some measure in the chromosome. In the preparation fixed with acetic acid, the anaphasic chromosomes also appeared to consist of two chromonemata with dotted or knotted appearance (Fig. 1). Spiral turns were also observed more or less clearly in living nuclei in telophase, and it was doubted at that time whether the structure of dotted appearance does not represent an optical section of a spiral.

Now it seems to us highly probable that the two strands with knotted appearance are not the chromonemata, but the chromatids which may contain two chromonemata in each (KUWADA, 1939). The chromatids may run parallel or be twisted about each other to

Fig. 1. Chromosomes in anaphase, fixed with acetic acid. Zeiss' apochr. obj. 1.5 mm $\times$ comp. oc. K. 12. Figs. 2-3, 5-8, 10-11: Zeiss' apochr. obj. 2mm $\times$ comp. oc. K. 12.

some variable extent. Recently SINKE¹⁾ has observed telophasic chromosome transformation with the same material using liquid paraffin as medium, and has reached the same conclusion, i.e. that in the telophase each chromosome may contain two strands mostly with the knotted appearance. In the renewed observation with liquid paraffin we also obtained the same results, and had an impression that the twisting between the chromatids becomes greatly reduced during the telophasic transformation in this material. In the early telophase, the matrix change being incomplete as yet, individual chromosomes are distinguishable from one another (Fig. 2*a*), but in the late telophase where the matrix change has great-

Fig. 2. Telophase, mounted with liquid paraffin. *a*. Early telophase showing chromosome distribution (1.40 p.m.). *b*. Late telophase showing chromatid distribution (3.21 p.m.). At 1.52, the chromosomes showed some internal structure, but the chromosome distribution was still clear. At 2.12, the nuclear structure was no longer of the chromosome distribution, but of the chromatid distribution just as shown in *b*. Fig. 3. Telophase at 3.28 p.m., showing chromatid distribution. Mounted with tap water at 2.10 p.m.

ly progressed, the chromatids become uniformly distributed, and it is hardly possible to distinguish which two chromatids form the pair belonging to one chromosome, though the straight arrangement is still clearly maintained (Fig. 2*b*). The chromatids in this case present the knotted appearance, though the spiral configuration may also occur. In the observation with water as medium, we find also the knotted appearance of the chromatids (Fig. 3).

When the nucleus enters into the interphase the straight strands of the knotted appearance become partly wound, so that the straight arrangement in the telophase is lost, and the nucleus presents a diffuse structure. A closer observation and an inspection of the photomicrographs of these nuclei show that in both media, water

1) Unpublished.

and liquid paraffin, the strands are no longer of a mere knotted or dotted appearance throughout, but in part are coiled into irregular spirals such as those which we can obtain when a tightly twisted and stretched thread with fixed ends is loosened merely by approximating the two ends without releasing them or untwisting the thread. These spirals are of relatively long pitch and small diameter, of variable length, having an irregularly drawn out appearance (Fig. 4). This structure seems to explain one reason why the straight strands in the telophase look wound in the interphase. It seems that in anaphase the chromosomes are of a highly dehydrated state, and hence the chromonemata contained in them must also be in the same condition, so that they are forced to transform their spiral configuration into a tightly coiled, DS, or twisted, DT, configuration such as shown in Fig. 9, through their shortening and hence thickening as the result of dehydration. These configurations, especially the DT, will appear to be "dotted" or "knotted" in a superficial view. In the anaphase this structure is not visible in the living state on account of the dense, highly dehydrated matrix of the chromosome, but in the telophase the nucleus becomes gradually hydrated and the matrix substance undergoes the change that enables us to see the chromatids with this dotted appearance clearly. In the interphase the chromatids become hydrated to a certain extent, and the tightly coiled spirals with the dotted appearance are partly loosened to variable extents, to present corresponding spiral configurations of variable pitches and diameters, so that the whole chromatids appear to be wound irregularly. This is the TS configuration, an intermediate type between the T and the S. The nuclei of this structure present a diffuse appearance.

In the telophase the chromonemata may also present the spiral configuration (Fig. 5), in which case the anaphasic configuration should also be one maintaining the spiral configuration, DS. In the case of such a telophase the spirality may be more conspicuous also in the early stage of interphase than in the case where the knotted appearance is predominant in the telophase. This spiral

Fig. 4. *a.* Diagram of TS configuration with a turning point, in the nucleus of diffuse structure, showing light part presenting dotted appearance of the whole structure. Comp. Fig. 9, TS. *b.* An enlargement of a part of the resting nucleus of a *Tradescantia* staminate hair cell from the original plate of Mr. NAKAZAWA which has been reproduced in Fig. 9 *b* of KUWADA, 1939. Comp. the chromatids indicated by vertical arrows with *a.* The horizontal arrow indicates a turning point.

configuration of the interphasic nucleus may be designated as CS in view of the fact that the nucleus is of a coarse structure. In this case, the chromosome territories are perceptible and the nucleus presents an aggregated appearance. This is the characteristic feature of the nucleus with the spiral configuration as contrasted with the nucleus with the TS configuration, which presents a general diffuse appearance. Strictly speaking, any one nucleus is not of the pure type of one structure, but of a mixed type in which one structure predominates over the others.

Fig. 5. Telophase. *a*, photographed at 11.26 a.m. *b*, at 11.41 a.m., showing spiral configuration. Mounted with tap water at 11.10 a.m. The spiral configuration in telophase is also observed in the preparation in which liquid paraffin is used as mounting medium. Fig. 6. Telophase, photographed at 6.52 p.m. Mounted with water at 1.50 p.m. Fig. 7. *a*. Resting nucleus of the coarse structure of twisted configuration, at 1.57 p.m. *b*. The same nucleus at 4.00 p.m. Spiral configuration is manifest, transverse striation being marked. *c*. The same at 5.10 p.m., showing a finer structure in which a reticulate appearance of the chromosomes is perceptible. Mounted with water at 1.50 p.m.

In one instance a peculiar case was observed. Hairs were mounted with water at 1.50 p.m. Some hours later a metaphase figure was found. In this metaphase the chromosomes were very thick and short, and appeared homogeneous in structure, being extraordinarily swollen. We assumed that the cell was in a very unhealthy condition and the mitosis would not proceed further. To our astonishment, however, it was found later that the mitosis had proceeded to telophase. The nuclei were photo-

graphed immediately (at 6.50 p.m.) and the observation was continued up to 7.50 p.m. During this period, little change was noticed in the structure of the nuclei, suggesting that the mitosis proceeded extraordinarily slowly. As seen from Fig. 6, it seems that the matrix change is as yet incomplete in these stages. This incompleteness in change of the matrix prevents accurate observation, but the chromosome structure appears to be finer than in the ordinary telophasic nucleus. From this case of incomplete matrix change, we are impressed with a suspicion that the anastomoses between chromosomes observed by some authors in living material may perhaps represent the parts of the matrix not yet undergoing the complete change (ABRAHAM, 1939).

2. *Resting stage.* Resting nuclei in the hairs mounted with water were watched under the microscope for several hours to see if any change in the structure of the nuclei is brought about in the water medium. So far as our observation goes, in only one case a remarkable change was observed with certainty. Fig. 7*a* is the photomicrograph of the nucleus showing its original structure, taken soon after it was mounted with water. In this nucleus, as will be seen from the photomicrograph, the knotted or dotted appearance (DT) predominates over the spiral appearance, and the general straight arrangement of the chromosomes is pronounced. In *b* which was photographed after about two hours, the straight arrangement is still maintained, but here the knotted appearance has disappeared and the spirality is rendered more clearly visible. This configuration represents the CS. In *c* which was photographed about one hour later still, the general structure is much finer than in *b*. This structure resembles very much that of the natural nucleus photographed by NAKAZAWA (Fig. 3 of KUWADA, SINKE and NAKAZAWA, 1939) which we have called the nucleus of fine structure, consisting of chromosomes having the structure of a reticulate appearance and showing the general aggregate appearance. We may probably say that by this observation it is demonstrated that in water medium the DT configuration can be transformed into the CS, and further into at least a fine structure (comp. Fig. 9). The reasons which can be suggested as to why experimentally such a transformation of the nuclear structures in water medium as that mentioned above was possible only in so extremely rare a case may probably be as follows:—

1) From the fact which he observed (SINKE, 1939) that while in animal cells a heterogeneous nucleus is transformed into a hydrated nucleus when treated with water or a hypotonic solution, such a transformation does not take place in plant cells, the entrance

of water being prevented by the cell wall pressure, SINKE¹⁾ is inclined to the view that when the cells are treated previously with a hypertonic solution, this transformation becomes possible also in the plant cells, probably because of the fact that the cells undergo a certain change in their permeability as a result of plasmolysis. It seems probable that in our case, in which the transformations in water medium of the nuclear structure from one to another were observed, the cell had undergone some exceptional change in its permeability during manipulation.

2) It may also be considered that some internal change must necessarily take place in order that the nucleus become one of fine structure, if in this structure, which may be designated as FS, it is the characteristic feature that the chromonemata of spiral configuration in the chromatid, presumably two in number, are separate from each other. This point seems to be very important, because the typical fine structure appears to be of rare occurrence. The

Fig. 8. *a*. Resting nucleus mounted with water at 11.05 a.m. and photographed at 11.18. The mounting medium was replaced by 0.1 M cane sugar solution at 11.30, and again by 1/4 M at 1.45 p.m. *b*. The same nucleus photographed at 2.35 p.m.

DT structure of the nucleus with which we observed the transformation into a fine structure might have been a secondary structure derived from FS, if the fine structure into which we could transform the DT structure is really one to be represented by FS. Fig. 8 shows a case of this secondary transformation of the fine structure into a coarse structure, by dehydration. In *a*, the nucleus shows a reticulate structure in the lower half, which is delimitable from the upper half by an oblique line passing through the center of the nucleus

from upper left to lower right; and it also shows the coarse structure of T or TS configuration in the upper half, which is especially pronounced near the center. In *b* which was photographed after being dehydrated gradually by replacing the water medium with cane sugar solutions of graded concentrations not strongly hypertonic, the whole nucleus shows the coarse structure except the region near the periphery on the left hand side where the structure appears somewhat finer.

1) Verbal information.

SINKE¹⁾ has demonstrated that when a nucleus (staminate hair) of diffuse structure (TS) is put under the influence of high temperature, the structure is transformed into a coarser structure (probably DT), and when the hair is put back in the room temperature, this coarse structure changes gradually until it attains the structure of the CS configuration. It has been also demonstrated by the same author (1939, with KUWADA and NAKAZAWA) that a coarse structure (probably DT) brought about by dehydration of the nucleus of a fine structure is brought back to the original fine structure by hydration (comp. also the experiment by SINKE shown in Fig. 10 of KUWADA, 1939, in which *a* shows the original spiral configuration, and *b* shows the diffuse structure (TS) produced from *a* by dehydration).

From what is mentioned above, we may perhaps be able to say that the configurations, S and T, are mutually transformable. The configurations DT, DS, TS, CS and FS which we can presume at present are diagrammatically shown in Fig. 9. In these cases, it is assumed that the chromosome consists of two chromatids, each containing two chromonemata forming a double-stranded spiral. In the configurations DT, DS, TS, and CS, the two chromonemata

Fig. 9. Diagrams showing chromonema configurations, DS, DT, TS, CS and FS, on the supposition that the chromonemata in the chromatids, of which only one of the two in the chromosome is illustrated in each case, form double-stranded spirals (case IIIa in Fig. 12). Arrows show mutual transformability of the configurations. The transformation through route 1 is exemplified by Fig. 7, and that through route 2 by Fig. 8 and SINKE's experiments with hypertonic solution. The heating experiments of the same author also show that the transformation can take place through route 2 when material is heated, and through route 1 when it is cooled. These reversible transformations have experimentally been shown also by this author by treating material first with a hypertonic solution and then with a hypotonic solution or water. For route 3, compare Fig. 5. Route 4 may exist, but it is hardly possible practically to distinguish between DS and DT.

1) To be published later.

are united together to form a double thread and assume the twisted configuration T or the spiral configuration S, or an intermediate configuration TS; and in the FS configuration they are separated to form a "relational spiral". The nuclei in which the two chromonemata are united together are collectively called of coarse structure, and those in which they are separated are of fine structure which is designated by the letter F. The DT and DS are dehydrated configurations and the FS is a hydrated configuration in which the hydration is connected mainly with the matrix substance. The CS structure, which is also a hydrated configuration as contrasted with the DT and DS, can be further hydrated as a whole without being transformed into FS, or in other words, with the sister chromonemata remaining united together as they are in the CS, so that the whole structure may become less clearly visible. The CS structure, especially this hydrated CS which may be designated as HS very much resembles in appearance the FS, and it is often very difficult to distinguish between them.

The interpretation made above of the nucleus of the diffuse structure as consisting of the chromatids of twisted or irregular spiral configuration is supported by the fact that there is often a close resemblance in general appearance between the nucleus of this structure and the nucleus in the mid-prophase after the spiral stage, where the old spirals are being drawn out. There is of course a great difference in the thickness of the threads, but the resemblance is so remarkable that one might mistake the prophasic nucleus for a resting nucleus, if he were shown a photomicrograph of this nucleus alone.

If a nucleus of fine structure presents the diffuse appearance, this fine structure will be one which is to be represented by the symbol FT or FTS. If such nuclei are demonstrated to exist, it will be critical evidence to show that each chromonema is spiralized independently from others, because if a pair of chromonemata forms together a double stranded spiral, the two chromonemata cannot be separated from each other to present the fine structure F, when the spiral takes the drawn out form, being forced to form a strand of two threads twisted on each other like the DT configuration; hence the fine structure FT or FTS cannot exist in this case. Since no such single nucleus, clearly of the fine structure presenting the general diffuse appearance, has as yet been found, the various coarse structures are provisionally assumed to be derived from the structure FS as illustrated in Fig. 9, though it has not yet been determined with certainty whether this FS structure really exists or not.

In most cases where we observed the fine structure, it is the characteristic feature that the general straight arrangement is perceptible. The straight arrangement involves the perceptibility of the chromosome territories. The number of these territories shown in the median optical section of the apical cell nucleus of this material roughly corresponds with that of the chromosomes of the *virginica* staminate hair nucleus illustrated by BĚLAŘ (comp. his Fig. 2*b* with our Fig. 7*c*). In the case of the nuclei of the spherical shape, the straight arrangement does not generally exist, but the chromosome territories are perceptible just to the same extent as in the case where the straight arrangement is visible. The straight arrangement concerns only the chromosome arrangement, and is not of primary importance in respect of the nuclear structure. The condition which is of important significance is that the chromosome territories are perceptible and the nucleus presents an aggregate appearance. This condition is brought about by the retention of the spiral configuration, hence the chromosome territories are visible in any case where the spiral configuration is retained, and especially pronounced in the hydrated configurations HS and FS. This situation makes it very difficult to distinguish these two structures with fine appearance, although the FS structure is characterized by the reticulate appearance which is presented within each territory. The fact that the hydrated structures present the spiral configuration seems to show that the chromonemata of a straightened form such as the T configuration have a strong tendency to resume the spiral configuration, if there is no impediment. This means not necessarily that the chromonema spirals are "constructional" (MANTON, 1939), because the spirals can be drawn out when the matrix substance is affected by some artificial means (cf. MIMURA's experiment shown in Fig. 2 of KUWADA, 1939), but suggests that they are such spirals, for instance, as those which forcibly twisted threads can assume when they, in a confined space, strive to return to their original untwisted condition without rotation (KUWADA, 1937). If these spirals are forcibly brought to take a straightened form, and if in this condition, moreover, untwisting is not allowed, a great tendency of retaking the spiral configuration must be exerted. It seems that in bringing the chromonema spiral into this condition the contraction of the matrix plays a great rôle. In the interphase the chromosome matrix is in a swollen state, and we must here then probably assume a matrix of another kind—the chromatid matrix. In the case of the typical DT too, the straight arrangement may be apparent in the nucleus, if the telophasic arrangement has not been

greatly disturbed. These nuclei show, however, a diffuse structure of some kind, since the spirality is here concealed.

The chromosome territories become also visible when each chromosome contracts. In one case we observed that the nuclei of a hair showed the chromosome contraction to the extent that each territory was clearly visible, probably being in an unhealthy condition due to certain ill-treatment during manipulation. We were interested in this appearance of the nuclei, and preparing for photomicrographing, but when ready, it was found that the nuclei presented the diffuse appearance as beautiful as we see in the healthy nuclei, without showing any perceptible sign of the chromosome aggregation. This fact may be regarded as disproving the view of the nuclear structure as one system of colloid whether the system be simple or compound. It must show that each chromosome system is maintained in the nucleus without mingling, even though it appears superficially to be nothing more than one diffuse structure. The chromosome individuality must be maintained in the resting stage (cf. FUJII and YASUI, 1935).

3. *Prophase*. The mitosis may proceed normally in the water medium, but very often a change was found (cf. WADA, 1932, SIGENAGA, 1937) to take place, probably in the matrix substance, as a result of the abnormality in water relation in the cell, at a certain definite stage in most cases, which probably corresponds with the stage shown in Fig. 2*e* of BĚLAŘ (1929). At first, the spirality becomes more clearly visible in the chromosomes than in the corresponding stage in the normal mitosis (Fig. 10*c*, *d*). The threads of the spirals are thin, and the nucleus appears at first sight to be of a fine structure (Fig. 10*e*). The change proceeds further, and the nucleus is finally transformed to the structure which resembles the diffuse structure of the resting nucleus (Fig. 10*f*). The threads are now thicker than in *d* and *e*. The spirality is much less conspicuous in this stage, and it appears that the spirals in the earlier stage have been drawn out. It seems that this drawing out is connected with thickening of the threads which is in turn, it seems highly probable, connected with the growth of the divided chromonemata. If this interpretation is correct, it leads us to the conclusion that the splitting of the chromonemata must have taken place in an early prophase, hence the resulting nucleus is di-diploid. This nucleus is about as large as when it was in the spiral stage. The comparison of the nuclear sizes as a support of this conclusion should, however, be made between the nuclei at corresponding stages. The nucleus in question corresponds in stage with young interphasic nuclei produced through the normal mitotic processes. These interphasic

nuclei are much smaller than the nucleus which we are presuming to be di-diploid. If the phenomenon of the prophasic nucleus proceeding back to the resting stage is correctly interpreted above, we may regard this katachromatic change taking place abnormally in the prophase, as furnishing a mechanism of WINGE's "indirect chromosome binding"

(KUWADA, 1939).

In this connection, it is a very interesting fact that according to MC CLENDON¹⁾ dividing eggs are much more sensitive in plasmolytic experiments than unfertilized eggs. It seems not improbable that in interspecific hybrids in which there may be expected a certain disturbance in the normal physiological equilibrium to be established in the homozygous state (KUWADA, 1928), this sensitivity may be so great as to change the normal water relation in the cell and to result in the abnormality that in

the first division after fertilization the anachromatic process is extremely abbreviated, or at least the katachromatic process takes place before the mechanism of polar separation of daughter chromosome comes into action.

This abbreviated mitosis artificially induced may be regarded

Fig. 10. Abbreviated mitosis experimentally induced. *a.* Spiral stage at 1.25 p.m. *b.* at 1.45 p.m. *c.* at 2.35 p.m. *d.* at 2.55 p.m. *e.* at 3.35 p.m. *f.* 5.10 p.m. Mounted with water at 1.15 p.m.

1) Cited from SPEK (1920).

as an experimental demonstration of endomitosis (GEITLER, 1939). In both cases, the chromosome separation mechanism having lost its existence, they are essentially the same. The distinction between the two is only in the manner of occurrence and in the degree of chromosome development, one occurring occasionally as an abnormal case without full development of chromosomes and the other successively mitosis after mitosis with fully developed chromosomes; as a response to a certain temporary condition in the former case and to a continuous one in the latter, that is peculiar to the tissues in which the mitosis (endomitosis) occurs.

It may be expected that in these mitoses lacking the chromosome separation mechanism, especially those with incomplete chromosome development, sister chromosomes are intimately connected with each other, so that they may appear in the succeeding mitosis in pairs (LORZ, 1937; GENTCHEFF and GUSTAFSSON, 1939). BERGER (1937) has observed this pairing or grouping in a mosquito in which the endomitosis takes place during the larval period.

The case of myxoploidy which is generally explained as occurring as a result of two successive chromosome splittings taking place in one mitosis (case 2c of MIDUNO, 1938) should be explained as in reality two successive mitoses of which the first one is an abbreviated mitosis, because in mitosis the chromosome splitting is a more essential phenomenon than the chromosome separation, hence what should represent a mitosis would be the former rather than the latter. This conclusion is verified by the discovery of endomitosis. The fact of the "uniform nuclear control" of the chromosome reproduction, that is, the fact that the chromosome number increases in the series 2^n is also more simply explainable by the occurrence of abbreviated mitosis than by a mere assumption that it is brought about by "internal reproductions" (GENTCHEFF and GUSTAFSSON, 1939).

In one instance a very instructive case was met with (Fig. 11). The hairs were mounted with water at 10.00 a.m., and a resting nucleus of a diffuse structure (*a*) was watched under the microscope to see what change of the nucleus structure might occur under the influence of the water medium. About one hour later it was found unexpectedly that the nucleus was in the spiral stage (*b*). About 3 hours and a half after this, the nucleus proceeded to a very late prophase (*e*) passing the spireme stages (*c*, *d*) as in the normal mitosis. In the earlier spireme stage the nuclear threads appeared solid and single (*c*), but in the later spireme duality was visible in some of the threads (*d*). In the late prophase (*e*) the chromosomes were no longer of the solid structure, but showed a certain hetero-

geneity which suggests the internal spiral structure. Twenty minutes after this stage the chromosomes were all of a fine spiral structure (*f*), and after another 20 minutes the spiral structure was more evident than before (*g*). Then a pressure was given unintentionally. The nucleus shrank suddenly, and the chromosomes also shrank and became highly refractive (*h*). The observation was continued further for 3 hours and a half. The change was irreversible.

Fig. 11. Abbreviated mitosis with anachromasis proceeding further than in the case shown in Fig. 10. *a*. Resting stage at 10.15 a.m. *b*. Spiral stage at 11.15. *c*. Spireme stage, 12.10 p.m. *d*. 1.20. *e*. 2.40. *f*. 3.00. *g*. 3.20. At 3.35 a pressure was given unintentionally. *h*. 3.37. Mounted with water at 10.00 a.m.

Though in this case it was not possible to trace the further change of the nucleus, we have two important results. 1) The nucleus of the coarse structure directly enters into mitosis. Since most nuclei are of the coarse structure especially of diffuse appear-

ance, the first visible sign of mitosis in general must be a disturbance in the uniform distribution of the chromonemata, or the contraction of the chromosomes, which is immediately followed by a nuclear hydration as a step towards the spiral stage, as illustrated by the photomicrographs of NAKAZAWA (l.c.). 2) The chromosomes in prophase appear to be solid, but are in reality of the spiralized structure. The spiral is a very fine one with a far greater number of turns than the number which we can estimate in anaphase. This must be borne in mind in the study of the behaviour of chromonemata in mitosis. The number of turns must be reduced as the chromosomes become short and thicker. If, however, anaphasic chromosomes show chromatic spirals with comparatively too small a number of turns, it should be quite possible to assume that they are chromatid spirals.

In one case, a nucleus which appeared to be in the spiral stage was rendered in water medium of an apparently fine structure about 5 hours after being mounted, without showing any development in stage. The preceding stage was not observed, and it is open to question whether the nucleus was really in the spiral stage or not. This case is left for a further investigation.

Discussion

In the present investigation the following points were confirmed:—

1. In telophase, the results of observation with water medium coincide with those obtained with liquid paraffin in most cases.
2. Both knotted or dotted and spiral configurations of chromonemata exist in the telophase.
3. The knotted configuration in the resting stage can be transformed into the spiral configuration, and further into a fine structure.

The third point shows that the knotted and spiral configurations are not to be regarded as representing fundamentally different structures. They must represent the same structure in different configurations. We must then assume that the knotted configuration is in reality a twisted configuration (T) as a form of the spiral configuration (S). Thus the “knots” or “dots” seen in the telophase and interphase do not represent chromomeres in any sense, but the spiral turns in the spiral configuration. These conclusions seem to serve to explain certain points relating to the spiral structure which are debatable to-day. We shall consider them below.

- 1) In metaphase and anaphase, the chromosomes are generally in a highly dehydrated state, and it is highly probable that in these

chromosomes the DS or DT configuration predominates over the CS configuration. The chromonemata of the DT configuration will appear as smooth strands or strands of knotted appearance or some such, when fixed with ordinary methods. If in the chromosome two such strands or chromatids are contained (cf. KUWADA, 1939) and lie parallel with each other, a longitudinal clear space may be seen between them. This has been observed repeatedly by earlier workers and was called the anaphasic or telophasic split. The anaphasic and telophasic splits can, therefore, be understood also from the view point of the spiral structure of the chromosome and not incompatible with this theory. If the two strands (chromatids) are twisted to some extent, and present a configuration of the relational spiral, the spiral is a chromatid spiral and not a chromonema spiral. There would be in this case a great possibility of taking it erroneously for a chromonema spiral, if the chromonemata assume the twisted configuration making the chromatids appear to be much thinner than when the chromonemata are of the spiral configuration. The number of the spiral turns of the chromonemata at definite stage should practically be definite in definite chromonemata, but the number of the spiral turns or the twists between the chromatids, being relic ones of the old spiral should be much less than in the former (i.e. chromonemata) and may be variable (KUWADA, 1939). Fig. 1 of ROY (1936) may be taken as an example showing these twists, variable in number, between the two chromatids containing the chromonemata of twisted configuration. Thus, by distinguishing between the chromatid spirals or twists and the chromonema spirals, we can understand the reason why the number of twists or spiral turns can be variable in the anaphasic and telophasic chromosomes.

2) This distinction between chromatid spirals and chromonema spirals must be made also in the case of meiotic chromosomes. The so-called major spirals are the chromatid spirals and the minor spirals are the chromonema spirals (KUWADA, 1939). In the case of large chromosomes, the chromatid spirals assume the spiral configuration in metaphase and anaphase, but the chromonemata may be of a twisted configuration or some such form, and consequently, they are usually fixed in these cases as strands with a smooth or corrugated surface, and hence also no spirality is visible in them. It first becomes visible only when the chromonemata are subjected to a certain treatment by which the transformation of the twisted configuration into the spiral is caused. The minor spirals really exist, but in a dehydrated condition their spiral configuration is transformed into the twisted configuration, so that their existence becomes hardly perceptible. It should be for this

reason that the minor spirals generally appear concealed in living material and in that fixed with ordinary methods. Various chromatid configurations reported in méiosis can only be harmonized when it is assumed that the minor spirals that are visible in one configuration are concealed in another (KUWADA, 1939).

Special treatments of chromosomes can disclose their structure, but the result from fixed material may be more true to the natural configuration which they assume at definite stages. If the non-visibility of the minor spirals in fixed material is simply taken as an artifact due to fixation, this is probably erroneous. The results obtained with fixed material seem more reliable than we generally assume. It seems highly probable, that if in any stage the spirality is not visible in fixed material, the configuration taken up by the chromonemata in the living state would be in this stage some other than the spiral configuration. The chromonemata must assume various configurations in living conditions, which are derivable from their spiralized condition.

3) From the results of SINKE's hydration and dehydration experiments, especially from those obtained with the erythrocyte nucleus of *Triturus pyrrhogaster*, it seems to us that the fine structure of the nucleus is most simply explained by the assumption that the chromosome contains four chromonemata in it as believed by NEBEL (cf. KUWADA, 1939). It is, however, essential actually to determine whether this interpretation is correct or not. The determination is of course not an easy task, but the importance of the investigation of the structure in question in connection with the question of the number of chromonemata contained in one chromosome may be considered here briefly (Fig. 12). In the following considerations, it is assumed that the chromonema spiral is an orthospiral (the unbalanced spiral of ABRAHAM, 1939).

I. One chromonema is assumed. In this case the daughter chromosomes (chromatids) are separable at anaphase only when the two daughter chromonemata are coiled each independently in the spiralization to form two independent chromatids. The anaphasic chromosome is of the simplest structure containing only one single-stranded chromonema spiral in it. This corresponds to DARLINGTON's view of the chromosome structure.

II. Two chromonemata are assumed. In this case, two cases can be considered. a) The two split halves of the chromonema are coiled both together like one thread. The anaphasic chromosome contains a double-stranded chromonema spiral. b) The two daughter chromonemata are coiled each independently so as to produce two

Fig. 12. Diagram showing five possible cases of chromosome cycle on the supposition that the longitudinal splitting of the chromonemata takes place in the early prophase and that the chromonema spirals are of the orthospiral type. 1, spiral stage; 2, late prophase; 3, anaphase. For IIIb 3, compare it with the diagram F of NEBEL and RUTTLE (1936). In IIIb 3, the half-chromatids are illustrated running parallel along each other in order to show each half-chromatid clearly, but the two chromonema spirals in each chromatid may be pushed into each other as in IIIb 1, so as to assume a more regular spiral configuration than shown in the diagram (IIIb 3). Further explanation in the text.

spirals. In this case the anaphasic chromosome contains two single-stranded chromonema spirals or chromatids.

III. Four chromonemata are assumed. We have here again the two cases *a* and *b*. *a*) The anaphasic chromosome consists of two chromatids each containing one double-stranded chromonema spiral. *b*) The two chromatids of the anaphasic chromosome contain two single-stranded chromonema spirals or half-chromatids each. This case corresponds to NEBEL's view of the chromosome structure.

In all these cases it is assumed for the sake of simplicity, that the old or relic spirals have been completely drawn out before anaphase, and consequently in the accompanying diagrams the anaphasic chromosomes are shown straight, and the two chromatids (II*b* and III*a*) or the four half-chromatids (III*b*) in the chromosomes are represented as lying parallel with each other. In the case III*b*, it seems probable that the drawing out of the old spirals causes the intertwining between the chromatids (the half-chromatids in III*b* 2) too, but such an intertwining is also not shown in the diagram (III*b* 3).

Now, we have strong evidence to show that the anaphasic chromosome consists of two chromatids (AISIMA, cf. KUWADA, 1939), and we have little room to uphold the possibility of the assumptions I and II*a*. It seems then sufficient to consider only the cases II*b*, III*a* and III*b*.

If case II*b* represents the real structure of the chromosome, we must abandon the explanation that the transformation from the fine structure to the coarse structures is brought about by the union of the two sister chromonemata, and reconsider the question of how the "fine" structure is caused. If, on the other hand, either case III*a* or III*b* represents the real structure, the explanation of the fine structure which we are attempting will remain without much alteration. The configuration presented by the chromonemata in this structure should, of course, be different in these two cases; in III*a* two sister chromonemata (secondary sisters of NEBEL, 1933) form a chromatid, coiling together, while in III*b* each chromonema forms a half-chromatid, coiling independently. In the latter case, the half-chromatids may also present the knotted appearance, and it may be expected that the nucleus of the fine structure with individual chromonemata of twisted configuration (FT) would exist, but in the former case it cannot, because in this case the chromonemata of twisted configuration cannot be single. So far as our observation goes, we have as yet met with no clear case where the fine structure showing twisted configuration is observed. This is certainly negative evidence, but we may provisionally exclude the

case IIIb from consideration for the moment.

The question may then rest on the point as to which case, IIb or IIIa, is the true one. In these two cases the chromosome structure is quite identical excepting that the chromonema spiral is single-stranded in the one and double-stranded in the other. Since in the dehydrated state of the matrix the double-stranded spiral would appear to be a single-stranded spiral, the distinction between these two types of spiral should only be possible when the matrix is hydrated. The investigation of the nucleus of the fine structure seems, therefore, to be very important in the solution of the question of the chromonema number in chromosomes. Some authors deny, from the results of chromosome fragmentation induced by X-ray treatment, the possibility of chromosomes each containing two or more chromonemata (DARLINGTON, 1932; GENTCHEFF and GUSTAFSSON, 1939), but such an argument does not necessarily hold. We should rather distinguish here between the chromosome division and the chromonema number. The chromosome among the components of which the matrix should be included may remain undivided irrespective of the number of chromonemata which it contains.

If the F structure really exists, and if it is to be represented by the symbol FS only, the case IIIa will remain after all one which represents the true cycle of the chromosome. It is, however, not an easy task to prove substantially the existence of the FS structure and especially that it and it alone has the appearance of the F structure. The most important point which we must bear in mind in this line of investigation is that there is a danger of mistaking an HS structure for the FS structure. The structure which we take for FS is that structure in which the chromosomes show a reticulate appearance presenting an aggregate structure of the nucleus as a whole. And yet, even we ourselves are impressed with a suspicion that we might have mistaken some HS structure for the FS structure, though a nucleus can never correspond perfectly to the type of any structure.

It is left for further investigations, to find whether the FS-structure actually exists or not, but it is an observed fact that a structure exists which appears very much finer than the structure presented by the nucleus in which the chromonema configuration is DT, DS, TS or CS. This apparent fine structure, of which the true structure remains as yet undetermined is called for the present simply fine structure without using any symbol such as FS or FT. The "fine structure" may be, therefore, a collective term covering different structures of a similar appearance, or a term indicating one of the other structures presenting a different appearance.

4) A few lines may be added here about the opinion of ABRAHAM (1939) on the type of the chromonema spiral. On the basis of his observation, the author has concluded that the chromonema spiral must be of the "balanced" type (anorthospiral). He admits that if 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" He has taken into consideration the case which corresponds to our case IIIb, but has drawn no attention to case IIIa which differs from the scheme that he has found in his investigation only in the point that the spirals which he assumes to be single-stranded are double-stranded, a point which is decidedly very difficult to determine. If in *Lilium* which he studied the chromosome cycle is such a one as we represent by the case IIIa, the chromonema spiral can be of the "unbalanced" type. The fact that the spiral configuration and the twisted configuration of the chromonemata are mutually transformable is, on the other hand, rather difficult to explain if the chromonema spirals are "balanced" spirals. If they are such spirals, the compensating internal twists will cancel the external twists, when they take the "twisted" (drawn out) configuration, and new spiralization must take place in each transformation into the spiral configuration. But, it seems highly probable that the spiralization takes place only at one definite stage in the chromosome cycle where it occurs under a certain complex condition which is peculiar to that stage.

5) The fact that in most staminate hair nuclei the twisted or the TS configuration predominates, clearly explains the reason why the elongated nuclei show the negative optical sign in respect to their long axis. According to BECKER and KOZBIAL (1937, cf. BECKER, 1938), the optical sign of fixed chromosomes (in mitosis) is negative, when they are thin and long, and is positive, when thicker and shorter. These results are in accord with our view that the optical sign is determined by the configuration of the chromonemata. When the chromonemata are of the twisted configuration and the chromatids containing them run parallel or are twisted on each other only loosely or slightly, the chromosome will show the negative sign, and if in these chromosomes the twisted chromatids assume the form of spiral, if hence the chromosomes are of the double-coiled structure, the optical sign will be positive. The chromosomes of the former case may be roughly said to be of the single-coiled structure. If in these two cases, single-coiled and double-coiled, the chromo-

nemata assume the spiral configuration, the optical sign will be reversed in both cases; positive in the case of the single-coiled structure and negative in the double-coiled structure. BECKER and KOZBIAL are convinced of the fact that somatic chromosomes are negative when they are thin and long, and positive when thicker and shorter. According to the authors, in the former case the chromosomes "do not show any distinct microscopic structure", while in the latter they "show a spiral structure according to the scheme of NEBEL, GEITLER and others". If, then, still shorter and thicker chromosomes are optically negative, how should we interpret the reason why they are not positive. The simplest explanation is this: Because in these chromosomes the chromonemata which are of the same configuration as those of the chromosomes of positive sign are coiled into major spirals. If in these chromosomes major spirals are microscopically observed, it may be regarded as being demonstrated by the polarisation microscope that what we have called the minor spirals are actually of the spiral configuration. If in these chromosomes the optical sign is positive, this sign must show that the minor spirals assume a twisted configuration in this case. The investigation with the polarisation microscope can, therefore, not only confirm the microscopical results by ordinary light, but also give some information on the finer structure which the ordinary microscope can not reveal with accuracy. We wish here to emphasize, however, that microscopically visible structures must be taken first into account in the discussion of a morphological problem, though the basic finer structures which are not directly visible are also equally important.

In concluding, we may say that in living condition the spiralized chromonemata may not always present the spiral configuration, but may also be of the twisted configuration. These configurations are mutually transformable, and from this fact it seems likely that the chromonema spirals are orthospirals rather than anorthospirals. It seems also probable that the reason why the new coils are visible in the spiral stage only through a certain special treatment rests on the ground that in the first process of spiralization no regular spiral is formed. A twisted configuration in the beginning of spiralization seems to develop later into the spiral configuration, a development which may result in the shortening and thickening of the chromosomes. Both in living and fixed materials, the spirality is visible only when the chromonemata present the spiral configuration, and not when they assume the twisted configuration. Special treatment can, on the other hand, transform the twisted configuration into the spiral configuration, and thus it can reveal the true

structure which may appear in different configurations. Strictly speaking, the result of the special treatment may be an artifact, but it is the artifact characteristic of the structure. One may call it an artifact, indeed, if he refers only to the particular stage or condition at which the structure appears otherwise, but one cannot call it such in a general sense, because the structure can appear physiologically in other stages or conditions in the same form as it can artificially as a result of a special treatment.

Summary

1. Spiralized chromonemata present different configurations. The configurations can be classified into two general types, the spiral configuration and the twisted configuration.

2. These configurations are mutually transformable from one to the other. These changes are experimentally demonstrated in the resting nucleus.

3. In the twisted configuration, the nucleus shows a diffuse structure, while in the spiral configuration it presents an aggregate structure, the chromosome territories being perceptible.

4. When staminate hairs are observed in water medium, the prophasic nucleus is transformed directly into the resting nucleus without passing metaphase and anaphase, an "abbreviated mitosis" which is comparable with the endomitosis. It is pointed out that in one mitosis the chromosome splitting never takes place twice, but that it only appears to do so, when one of the two successive mitoses is an "abbreviated" one.

5. From the fact of the spiralized chromonemata presenting different configurations, the problems of 1) the anaphasic or telophasic split, 2) the minor spirals, 3) the fine structure of the nucleus in connection with the problem of the chromonema number in chromosomes, 4) the type of the chromonema spirals, and 5) the optical sign of chromosomes are discussed.

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