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Behaviour of Chromonemata in Mitosis
III. Observation of Living Staminate Hair Cells in *Tradescantia reflexa*

BY

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With Plates XV-XVI and 1 Text-figure

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In his careful study of mitosis BĚLAŘ (1929) has given such excellent drawings and photomicrographs that no repetition of the investigation on those lines is needed, but there is still much need of observations by the method of dark field illumination and of observations in which the osmotic relation can be disregarded, for which reason the present investigation was undertaken.

Method

Stamens were carefully cut at the base of the filament so as not to hurt hairs growing near the base. Anthers were then cut off from the filaments, and the pieces of the filaments with hairs were mounted with liquid paraffin. Observations were made at a room temperature of about 19°–21° C.

It has been pointed out by TELEŻYŃSKI (1930) that liquid paraffin as a mounting medium has the advantage of supplying more oxygen to the material than water, and that the osmotic relation can be disregarded if it is usd. In order to test the suitability of it as the mounting medium the following observations were made :—

1) On Aug. 31, 1932, the tip cell of a hair was observed from 2.15 p. m. to 4 p. m. (metaphase to an advanced stage of telophase). After the observation this preparation was left as it stood, and the same cell was again observed at 4 p. m., Sept. 1, and at 3.30 p. m., Sept. 2. At each time the Brownian movement of microsomes in the cytoplasm was found very active in this cell.

2) On Sept. 10, the tip cell of a hair was observed from 1.15 p. m. to 3.12 p. m. (metaphase to telophase). This preparation was left as it was for later observations, and it was observed on Sept. 13. The cell with which the first observation had been made was lost, but the cells of other hairs appeared healthy, the circulation movement of the cytoplasm being active. The preparation was observed again on Sept. 16. In the cells towards the tip of the hairs the Brownian movement was inactive, but in those towards the base, the circulation movement was still active. In some of the hairs, cells were found dead.

3) On Oct. 8, the tip cell of a hair was observed from 2.14 p. m. to 4.44 p. m. (metaphase to telophase). This preparation was again observed on Oct. 10th, 12th, 13th, and 15th. In all these cases of observation not only the cell with which the first observation had been made on Oct. 8, but also other cells and those in other hairs were found quite healthy, both Brownian and circulation movements being as active as in fresh material.

From these results of observation it is seen that cells can live in this medium for a considerable time.[1] In no case, however, were cells found in which the mitosis was initiated anew.

In photographing, an arc lamp (5 Amps., 110 volts) with a water filter for heat rays was used as a light source. BĚLAŘ has pointed out that it is better in photomicrography not to use too small an opening of the iris diaphragm. We also found that it was better to use a larger opening than that suitable for the direct observation with the eye. As microscopical equipment, Zeiss' apochrom. imm. objective 1.5mm. or 2mm. with a central stop devised for use in the case of dark field illumination and a Zeiss comp. oc. K. 12 (15×), and for photographic plates Agfa's high speed plate "Isochrom-Platten" were used. The plates were exposed for $2\frac{1}{4}$ seconds in the case of the ordinary condenser being used, and for 3 minutes in the case of the cardioid condenser.

1) In 1933 another test was made. On Nov. 10th, hairs were mounted with liquid paraffin at 1 p. m., and they were observed on the 11th, 15th, 17th, 20th, 21st, 22nd, 29th, and 30th. Among others, three hairs, of which two were detatched from the stamen filament, and one remained intact, were observed with special attention. No abnormal symptom to be specially mentioned had been observed up to the 20th when it was found that in some of the cells the circulation movement of the cytoplasm was very inactive. On the 21st the movement had ceased in some, but in others it was still observed, though very inactive, even on the 30th. There was found no marked difference between the hairs detached from the filament and the hair remaining intact. Generally the microsomes were reduced in number as the time elapsed.

Observation

1. By the ordinary bright field illumination

In *Tradescantia* staminate hairs, the behaviour of chromosomes in mitosis has been most closely investigated by BĚLAŘ (1929), and illustrated by him with excellent photomicrographs and drawings showing the finest details. We are not intending here to describe the behaviour of chromosomes in mitosis, but of chromonemata which in every respect coincide with those perceivable in BĚLAŘ's illustrations. We shall begin with the interphase.

In young staminate hairs in which cells are capable of dividing, the nuclei are filled with chromonemata which run here sinuously and there present a coiled aspect (Figs. 7, 9b). These chromonemata are disposed in a very irregular manner, so that the nucleus looks at first sight as if it were of a mesh-work structure. Similar structures have been illustrated and described by BĚLAŘ (1929) and TELEŻYŃSKI (1930) in living staminate hair cells in *Tradescantia virginica* and by SHIWAGO (1926; see also his photomicrograph reproduced in Fig. 37 in GURWITSCH's monograph, 1930) in living leucocytes of frogs.

TELEŻYŃSKI states: "....: à la place des granules isolés, on voit des points de croisement des filaments, plus courts ou assez longs, souvent courbés dans différents sens. Les granules, visibles entre ces filaments, ne disparaissent pas subitement, lors de manipulation de la vis micrométrique, mais ils se déplacent, successivement, en lignes droites ou en lignes courbées en zigzags et en spirales; et le parcours de ces lignes ne dépend nullement de la direction de la lumière" (p. 384). SHIWAGO remarks: "In allen Fällen und bei allen Leukozytenarten waren im Kernraume, welcher, augenscheinlich, mit flüssiger, schwachlichtbrechender, und bei den erwähnten Beobachtungsbedingungen dunkel erscheinender Karyolymphe ausgefüllt ist, immer dichtere, starklichtbrechende, glänzende, scharf konturierte, dünne Fäden von einem beständigen Durchmesser zu sehen, die an der Kernperipherie gelagert sind und hier sich untereinander verwickeln nnd durchkreuzen. Dabei bilden sie kein wahres Netzwerk, noch Anastomosen, indem sie in den Kreuzungspunkten nicht zusammenfliessen oder verschmelzen. Mit besonderer Klarheit treten die letztgenannten Umstände in die Augen, wenn die Fäden nicht in Ruhe, sondern sich in einer Bewegung befinden, welche bei den beschriebenen Beobachtungsbedingungen oft sehr schnell zu sein scheint." (p. 681). And BĚLAŘ concludes: "....; ich vermute, dass die Chromatinkörnchen und Stäbchen des Ruhekernes optische Schnitte dieser Spiralfäden darstellen" (p. 130). Recapitulating his previous paper on chromosomes of *Paris quadrifolia*, LEE (1921) says: "I there found the chromosomes to be alveolated as described by GREGOIRE; but I did not find their alveolation to progress in the telophasic chromosomes to the point of breaking them up into networks. On the contrary, I found their alveoles to disappear, and the chromosomes to condense into thin spiral threads. But I did not find these threads to anastomose into a network in the resting nucleus, as described by BONNEVIE. I found nothing worthy of the name of a network, but only a tangle of the much elongated and attenuated spiral chromosomes. I found these persisting throughout the interphase, and at the next prophase forming typical chromosomes by shortening and thickening and at the same time again becoming alveolated" (p. 4). Recently NEBEL (1932) has stated: "The evidence of this paper strengthens the opinion of SHIWAGO (1626), BĚLAŘ (1930)

and others, that in the resting nucleus we still have before us the scarcely changed, coiled chromonemata, perhaps carrying at least some of their matrical coatings through the cycle. It would seem that in this stage the coiled threads are individually free, those of one chromosome possibly being held together at the insertion region only." Comparing a microscopic image of a resting nucleus with a photographic image of numerous artificial glass spirals immersed in glycerol and viewed in transmitted light he further says: "These figures illustrate the complexity of refraction images which may in some cases have led to the erroneous conclusion that a reticulum was present in nuclei of this stage" (p. 276).

In the present material no nuclei are found at all in which the structure mentioned above is obscure, so far as observation of young staminate hairs in which cells are dividing is concerned. The photomicrographs which SEIFRIZ (1930) has reproduced in his Figs. 14, 15, 17, 18, Pl. III as showing the alveolar structure of the nucleus seem to us rather to represent the same structure as that of the nuclei we have observed in the staminate hairs. In observing the ordinary acetocarmine preparation we several times met with the case where an accidentally drawn-out periplasmodium nucleus showed clearly the thread nature of its network-appearing contents (Fig. 8).

The first sign of the prophase is characterized by the clear presentation of beautifully coiled chromonemata (Fig. 1). In his study of living root-tips of *Allium cepa* LUNDEGÅRDH (1912, p. 243) has stated that "darin das Kerngerüst grobmaschiger als in dem Ruhekern ist," and that "die kleinen Tröpfchen des Ruhekerns sich zu grössern, in *spiraligen*[1] Zügen angeordneten, vereinigt haben," emphasising this characteristic as an "allgemeine Merkmal der Prophase." This stage has been more clearly observed in fixed material (SHARP, 1913; KUWADA, 1921) and excellently illustrated by BĚLAŘ (1929) both in fixed and living materials (his Text-fig. 2, *b*, *c*, and Fig. 3, Pl. III). SHARP (1929) has distinguished this stage as the "spiral stage." It is highly probable that not only in the somatic mitosis but also in the heterotype as well as the homotype mitosis the first stage of the prophase is characterized by the occurrence of this spiral aspect.[2] In plants this stage has generally not been mentioned in the meiotic divisions, but it seems probable that there are cases in which it has been overlooked or misinterpreted. A comparison of BEAL's Figs. 2 and 3, Pl. II (1932) with SHARP's Figs. 2 and 4, Pl. I (1911) seems to show that the nuclei illustrated by BEAL in these figures represent this stage, though it is otherwise interpreted by the author. SHINKE (1934) has observed this stage in the heterotype prophase in pollen mother cells

1) Printed in italics by the present authors.

2) In animals this stage can be regarded as corresponding to WILSON's stage c and stage d (unravelling stage). (Comp. Figs. 262, *c*, *d*, and 265, *G* in the "Cell." WILSON, 1925)

in *Sagittaria Aginashi*, and KATO[1] in the homotype division in pollen mother cells in *Tradescantia reflexa*.

In this stage the spirals are found rather straight than sinuous and oriented parallel to one another as they are in the telophase. If we describe the nucleus in this stage in terms of chromosomes, the chromonema and the matrix being taken together as a whole, we may say that "Die Chromosomen sind in den aller frühesten Prophasestadien verhältnismässig *dick* und *wenig*[2] gewunden" (BĚLAŘ, 1929).

According to KATO, in the homotype division in pollen mother cells in *Tradescantia reflexa* the spiral in this stage is the meiotic form, i. e. the form which is exhibited by the meiotic spiral formed of the chromonema coiled twice. It is a noteworthy fact that the spiral is single-coiled or of the somatic form when that found in the preceding telophase is of that form, as is commonly the case in the somatic mitosis, and that it is of the meiotic form when it is of that form in the preceding telophase, as in the case of the homotype division. A remarkable example has been given by SHINKE (1934) in the first division in pollen grains in *Sagittaria Aginashi*, showing that even in the typical mitosis the spiral may be of the meiotic form, if that found in the preceding telophase is of the meiotic form. The form of the spiral is, therefore, always the same in the two stages between which the interkinesis or interphase is interposed. It has no relation to the type of mitosis in which it appears; the two are quite independent. It is determined only by the form taken up in the preceding telophase.

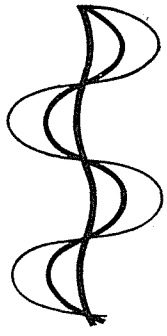
In one case (Oct. 15, 1932, room temp. about 20° C, Figs. 3–5) a nucleus in the spiral stage was watched under the microscope for 50 minutes from 12.14 p. m. to 1.04 p. m. when a certain change in the coiling state of the chromonemata was recognizable. As we did not observe from the very beginning of this stage, it seems likely that it lasts more than 50 minutes. This is the critical stage from interphase to prophase; it must be a stage of important significance.

The chromonemata then begin to thicken, and are gradually uncoiled or drawn out as they grow thicker, first to run zig-zag and later sinuously. In terms of the chromosome, it may be said that the chromosomes become "zunächst etwas dünner," "länger" and "stärker gewunden," "und die Spiralstruktur verschwindet" (BĚLAŘ, 1929, p. 83, 130, cf. his Text-fig. 2 *b–d*). The chromonema in this stage often presents the appearance through a certain length of being tightly

1) To be published later.

2) Printed in italics by the present authors.

twisted around itself. We should like to take this stage as representing the stage in which the new coiling is in progress. In the advanced stage the drawn out chromonemata or, more properly speaking, the spiremes, are considerably thick and more or less clearly show their internal coiled structure. BĚLAŘ's Text-figs. 2 *b–c* illustrate this process of change very clearly. In *b* which represents the stage we call the "spiral stage" the thickness of the chromosomes or the diameter of the chromonema spirals appears nearly the same as that of those in the telophase shown in his Text-fig, 4, *f*. In *c* and *d* the thickness or the diameter is gradually lessened in the order named, and at the same time the pitch of the spiral is progressively increased. In *d* the spiral is drawn out to such a great extent that it is no longer appropriate to denote it a spiral, but a zig-zag. There is moreover perceptible another change which can not be overlooked in these figures. This is the change in thickness of the threads drawn out from the spiral. The thickness of the threads grows in direct proportion to the degree to which they are drawn out. We mentioned above that the spiral stage continues for a pretty long time, during which the changes from *b* to *c*, and *c* to *d* are in progress. Perhaps an intermediate figure may be inserted between *c* and *d*. When we carefully examine the drawing *d*, we become aware of the fact that an aspect of internal coiling has been indistinctly drawn in the thread. In *e* the spiral shown in *c* has been entirely drawn out, but the internal coiling in the thread is shown so clearly that we can now readily perceive it. From inspection of these figures it may be concluded that the thickening of the thread is brought about by its coiling into a spiral, which takes place anew in the prophase.[1] The further the coiling progresses, the thicker and the shorter will be the thread. This shortening and thickening will result in uncoiling or drawing out of the old spiral which appeared in the spiral stage (cf. Text-fig. 1).



Text-fig. 1. Schema showing the drawing out of a spiral by the thickening of the thread.

In the late prophase the coiled aspect is not clearly recognizable (cf. BĚLAŘ's Text-fig. 2, *f*); the chromonemata seem to have swollen to a considerable extent. At a room temperature of about 20° C, it takes about two hours from the early prophase when the spiral aspect of the chromo-

1) The view that a new coiling occurs in the prophase has been put forward by some authors (see KUWADA and NAKAMURA, 1933, p. 137).

nemata is conspicuous to the late prophase. When the stage proceeds further, the chromonemata become considerably swollen and their coiling nature is generally hardly recognizable except where a certain indication of this may be found sporadically (Figs. 2, 10*b*). In the late anaphase a clearer spiral aspect can often be found in certain chromosomes or certain parts of chromosomes, though the chromonemata are generally still in the enormously swollen state (Fig. 3, cf. BĚLAŘ's Fig. 14, Taf. III). At the temperature given above, it takes about one hour or one hour and a half to proceed from the late prophase to the late anaphase. Then the chromonemata begin to shrink and consequently become thinner, so that their coiling aspect is rendered again more and more distinct (Fig. 5). In the late anaphase (Fig. 4) the chromonemata are generally still in a considerably swollen state, but soon after the cell plate has been formed the chromonemata appear thinner, presenting a clearer spiral aspect; the further the stage proceeds, the clearer is the spiral aspect. On entering the interphase the spiral is generally loosened out and the chromonemata come nearer one another to form a network-appearing architecture as a whole, their parallel orientation to one another which has hitherto been conspicuous being lost (Figs. 7, 9 *b*). In Fig. 6 is shown a nucleus which was photographed about 3 hours and a half after the cell plate had been formed. In this figure the parallel orientation of the coiled chromonemata is still visible to a certain extent. It probably represents the end stage of the telophase.

2. By dark field illumination

The dark field illumination was made by the aid of a cardioid condenser. In this illumination the nucleus appears to be filled with shining minute microgranules. But a closer observation reveals that these microgranules are arranged in rows or threads which exhibit a coiling aspect in many places. In photomicrographs no such granular aspect is presented by these threads (Fig. 9*a*). It seems likely that over-exposure may have disguised the granular aspect and given it the thread-like appearance, but it is left for further investigation whether the granular appearance really represents the discontinuous structure of the chromonemata, or not.

In the spiral stage the chromonemata shine nearly as brightly as in the interkinesis, but later they seem to become less bright. In the late prophase the brightness is markedly decreased and in the metaphase and anaphase the whole chromosomes appear quite dark except for the surface which seems to shine only dimly (Fig. 10*a*).

In the anaphase the microsomes in the cytoplasm which are in a very active dancing movement come to show a tendency to gather in the equatorial region (cf. MANN, 1924; KUWADA, 1929; KATO, 1933). The gathering of the microsomes becomes marked first in the late anaphase and early telophase, soon growing to surround the daughter nuclei too, which appear quite dark. When the microsomes become markedly gathered around the nuclei, a fine-granular appearance comes to sight in the nuclei. The intranuclear microgranules shine rather weakly, and as they grow in number, the whole nuclei appear milky. A closer observation reveals that the milkiness is only a phenomenon brought about by the microgranules being out of focus. At the beginning no definite order is perceptible in the arrangement of these granules, but as the stage advances, it becomes clearer that they are arranged into threads of a more or less irregular form of spiral. These shining granular threads are observed more distinctly in the interkinesis.

When the fresh I-metaphase chromosomes in pollen mother cells in *Tradescantia reflexa* are observed under the microscope, they are very often found swollen to such a great extent that no individual chromosomes can be distinguished from others, and the mass of these chromosomes appears quite dark in the dark field illumination. This optically empty mass of chromosomes has been called the "dark pocket" by FUJII (1926). If these chromosomes are contracted by some agents such as acetic acid or some neutral salts, as was done by SHIGENAGA,[1] or by introducing CO_2 gas into the observing medium by the method of SAKAMURA (1927) (Fig. 11*a*), the chromonemata are contracted as well and show their spiral form. With a cardioid condenser these contracted chromonemata shine like those in the living nuclei in the staminate hairs.[2] In the staminate hairs, therefore, the chromosomes in metaphase and anaphase are dark, because the chromonemata are swollen extremely.

Conclusion

Summing up the results we obtained, we may say that in staminate hairs the chromonemata can be traced in the living state through all the stages in the chromosome cycle, except the stages from late pro-

1) Unpublished.

2) In the case where CO_2 gas was used as a contracting agent the observation with the cardioid condenser was not made, because of the fact that the apparatus (see SAKAMURA, 1927) is not suitable for use with the ordinary cardioid condenser.

phase to early telophase where they are generally swollen to a greater or less extent. In the metaphase the swelling is enormous, and in most cases the coiled aspect is almost entirely concealed. The spiral form is most conspicuous in the late telophase and at the beginning of the prophase (the spiral stage) where the chromonemata are considerably shrunken. In the interphase, they are in an even more shrunken state and present themselves even more distinctly than in any other stage, but their regular spiral form is rendered very irregular. At the beginning of the prophase the original form of the regular spiral in the telophase is restored again—the spiral stage. The mechanism by which the spiral is rendered irregular in the interphase and that by which it assumes again its regular form in the spiral stage of the prophase are as yet obscure, but in connection with this question it seems worth while mentioning that the form of the spiral found at the beginning of the prophase is determined, as mentioned above, by the form assumed in the preceding telophase, and is not specific to the type of mitosis, whether it is somatic or meiotic.

According to KATO[1], in *Tradescantia reflexa* the meiotic spiral in the I-telophase is uncoiled in the interkinesis into a sinuously running thread which is not smooth, but of a corrugated, or a wavy appearance. This waviness which he is inclined to take as representing the smaller coil of the meiotic, double coiled spiral is then transformed into the loosely coiled spiral of small diameter. In the next stage the thread resumes the wavy or corrugated appearance, the loose spiral form having been lost, and then it becomes clear that the corrugated thread runs in a zig-zag manner. At the beginning of the prophase it takes the meiotic spiral form again, probably passing the zig-zag transition stage. This observation was made with fixed material, and the double-coiled nature of the meiotic spiral which is perceivable in acetocarmine preparation was concealed, and therefore, direct identification of the wavy thread in the interkinesis with the spiral of the lower order in the I-telophasic chromosome was hardly possible, but it seems highly probable that they are identical. If they are, then, the behaviour of the chromonema observed by KATO shows that in the interkinesis the spiral of the higher order is unravelled, this being followed by the loosening out of the spiral of the lower order which is again contracted into its original condensed corrugated form previous to the restoration of the spiral of the higher order taking place at the

1) To be published later.

beginning of the prophase. In short, the processes taking place in the interkinesis are unravelling of the spiral and its recoiling proceeding in reversed order into the original spiral form. Both these phenomena are found to take place also in the interphase in somatic mitosis, though in this case the unravelling would proceed further than in the interkinesis in meiosis, as is inferable from the results of observation by KATO that in the interkinesis the chromonemata are of loosely coiled spirals of a more regular form than those found in the interphase, which is probably due to the fact that in the case of the interphase the chromonemata in the preceding telophase are coiled into the ordinary single-coiled spirals, hence the unravelling is easier, while in that of the interkinesis they are doubly coiled and less easily unravelled. The unravelling and the recoiling[1] in the interkinesis and interphase seem not to involve any structural change in the chromonemata, because in the somatic mitosis it is, as has been mentioned in the descriptive part, highly probable that such a change will soon occur to unravel the spiral appearing in the spiral stage. Then we come to the view that the unravelling and the recoiling observed in the interkinesis and interphase is in reality a simple loosening out and recontraction of the spiral. How is, then, the loosening out of the spiral brought about?

When we treat the chromosomes in the I-metaphase or I-anaphase in pollen mother cells in *Tradescantia reflexa* with ammonia vapour before staining with acetocarmine, the matrix substance is affected by the vapour, and the product is very similar in structure to the nucleus in the interphase (KUWADA and NAKAMURA, 1934). When we observe preparations prepared from root-tips treated with boiling water before fixation, we often come across a case where the chromonema spirals found in the markedly swollen matrix are greatly widened out, though they are generally broken to a greater or less extent. These results of experiments show that the loosening out of the spiral in the interkinesis or interphase can be brought about by the colloidal change in the matrix substance of the chromosomes. In the ammonia experiment we have seen that the spirals of the higher and the lower order are not both affected at the same time by the vapour, but that the spiral of the higher order is affected first, and that of the lower order next, both, then, being deprived of the capability of contraction through the action of acetocarmine into the original forms (KUWADA and NAKAMURA, 1934). If the affection by ammonia is less intense, the original

1) We may rather look upon this stage as the very beginning of the prophase.

form of the double coiled spiral can be restored again on addition of a drop of acetocarmine. The latter facts being taken together into consideration, the former seems to indicate that the two spirals have their own matrices respectively, by the colloidal behaviour of which they are drawn out. If in the case of the natural nuclei in interkinesis the matrices of the spirals of the higher and the lower order undergo a colloidal change in the same order as in the case of affection by ammonia, the spiral of the higher order will be first unravelled and then the spiral of the lower order, though in this case the smaller spiral seems to be, according to KATO, not so extensively unravelled in *Tradescantia* as in the experiment—a fact which indicates that the change in the matrix does not go so far as in the case of experiment. In the latter case too, the spiral of the lower order remains not completely unravelled, while that of the higher order entirely disappears from sight both in the natural interkinesis and the artificial imitation nucleus. The reason for this difference in easiness of unravelling between the spirals of the higher and the lower order is a question, but a certain idea can be obtained from a wire model, with which the chromonema spiral can be regarded as comparable in some measure if it is here considered that the chromonema is an elastic body, as is perceivable from its behaviour in response to the elongation in anaphase and contraction in telophase of the chromosome containing them. When a length of wire is coiled into a spiral, it is flexible, and the secondary spiral made of it can not maintain its regular spiral form without an external force preventing it from uncoiling. It is, therefore, to be expected that if the matrix loses its power of contraction, the chromonema spiral of the higher order will be more easily unravelled than that of the lower order. It is of course a question whether in both interkinesis and interphase the matrix which can no longer be distinguished from the karyolymph has formed the karyolymph or the nuclear ground mass in cooperation with some other substances such as the spindle substance, or whether it remains in its integrity, the apparent disappearance being due to the change in its refractive index caused by swelling. From the experiments with ammonia vapour, however, it is inferable that it undergoes a change to the extent that a drop of acetocarmine can no longer contract it.

The meiotic divisions may be regarded as consisting of two peculier divisions, of which one is a division which is inserted into the prophase of a typical division, and the other a division in the prophase of which another division is inserted. The interkinesis between

these two divisions must, therefore, be a peculier one that can by no means be regarded as quite identical with the interphase in the typical mitosis. While in the somatic mitosis there is known no case where the telophasic spirals remain not unravelled to be directly transferred into the metaphase chromosomes, it is known in the meiotic division, as is exemplified by *Gasteria* studied by TUAN (1931). The non-unravelling of the spiral in the interkinesis may be regarded as indicating that in this case the matrix substance remains without undergoing any noticeable change in its colloidal state. This is an extreme case, and it is to be inferred that in the intermediate cases there are varieties in the degree of change in the colloidal state of the matrix substance.

Our next question is how the recontraction of the chromonema into its original compact regular spiral form is brought about. We have two alternative explanations.

When the karyolymph, as one of the components of which the matrix substance might be counted, is rendered less viscous by the water absorbed from without, the external strain which forces the spiral to uncoil may be removed, so that the spiral contracts and is restored to its original compact regular form again, as, for instance, an expanded spring takes its original compact form again on being released from tension. This is one of the alternative explanations. The mechanism of the restoration of the telophasic spiral in the prophase in the somatic mitosis may be looked upon in that way, but that of the spiral in the homotype prophase must be more complex than this. We have no adequate explanation at present. We could perhaps explain the mechanism of the restoration of the spiral of the lower order in the same way as in the case of the somatic mitosis, but then, how is the restoration of the spiral of the higher order brought about? We have here no other better way of explaining it than to assume that a reverse colloidal change of the matrix substance takes place, i. e. a contraction of the matrix which can at the same time give rise to the contraction of the unravelled spiral into its original form—a mechanism which is comparable to that of the case where we draw out a wire spiral and compress it again into its original form. This is the second possible explanation. This explanation is applicable to the case of the spirals of the lower order too, and therefore it alone suffices to explain both cases of interkinesis and interphase, while the first explanation needs another assumption such as

that we have assumed in the second interpretation, in order to explain the case where the chromonemata are doubly coiled.

In the early prophase the old spiral is replaced by the spiral formed by a new coiling which becomes evident after the spiral stage. In this replacing stage it is required that the matrix substance be largely dissipated in the karyolymph, otherwise, the replacement of the old spiral by the new one could not be realized, but a double-coiled spiral would result. If, in the homotype prophase too, the matrix substance, especially that belonging to the spiral of the higher order is so abandoned, as we have seen in the ammonia experiment (KUWADA and NAKAMURA, 1934), the spiral of the higher order becomes free from a force preventing it from unravelling, and hence it will be gradually uncoiled, and thus the double-coild form is reduced to the single-coiled form. Although, as mentioned in the previous paper (1933), we first reach the conclusion, from the result of observation of the homotype prophase, that in the early prophase the old spiral appearing in the spiral stage is destined to be replaced by a new spiral formed soon after this stage, we have as yet actually observed neither in fresh material nor in fixed material that the old spiral of the lower order is drawn out as in the case of somatic mitosis, and we wish to reserve it at present as a question, whether the replacement demonstrated in the case of somatic mitosis takes place in the case of homotype division too or not, until further observation can determine it. If it takes place a greater abandonment of the matrix substance must be assumed than is required when it does not. In any case whether the replacement takes place or not, the chromosome thus formed is of the somatic form characteristic in those chromosomes that contain the ordinary single-coiled spirals, and its fully developed form will be accomplished by the new formation of the matrix substance subsequently taking place. Such a case is exemplified by *Tradescantia*. It seems, on the other hand, not improbable in such a peculiar type as the homotype division that in the prophase too, there are certain varieties of the behaviour of matrix as in the interkinesis. If in such a case the matrix substance is not completely abandoned, it will result in varieties of the case. In an extreme case the double-coiled form of spiral will be maintained, or, if the new coiling takes place, a triple-coiled spiral would be formed. In this latter case it seems probable, especially in the case of chromosomes of small sizes or, in other words, of chromonemata of short lengths, that the spiral of the lower order will be replaced by the spiral formed anew and that

of the higher order by that of the lower order; in the final result, therefore, the spiral would be a double-coiled one. The chromosomes thus formed are of the meiotic form both when the new coiling takes place and when it does not. Such a case may be regarded as exemplified by *Lythrum* studied by SHINKE (1934). If in both interkinesis and prophase no marked change occurs in the colloidal state of the matrix, the spiral will remain in its double-coiled state throughout these stages, as is exemplified by *Sagittaria* studied by SHINKE (1934). In this plant SHINKE has observed that the thickness of the threads forming the spirals is slightly increased and the number of turns of the spiral is slightly decreased as compared with those of the spirals in the interkinesis. This fact might be taken as indicating the occurrence of the new coiling in the prophase in a certain degree, but it does not necessarily mean that such a new coiling takes place.

In short, reversible colloidal changes of the matrix substance are regarded as the causes of the loosening out of the chromonema spiral in the interphase and of the recontraction of the spiral at the beginning of the prophase. In the interkinesis these changes take place in varying degrees. From the facts that in somatic mitosis the replacement of the old spiral by the new one takes place in the early prophase and that in certain plants the spiral of the higher order is drawn out in the prophase of the homotype division, it seems highly probable that the old matrix substance is abandoned and passes into the karyolymph at or before this stage. It is suggested by the sinuosity of the chromosomes which is often very pronouncedly exhibited especially in the homotype devision in certain plants that the old matrix substance may remain intact in a certain degree, but it is to be inferred from the straightening out of the chromosomes in later stages that it is abandoned sooner or later. It seems that there is no genetic continuity in the history of the matrix. It is likely that it is formed anew in the prophase (cf. ALEXANDER and BRIDGES, 1928). The chromonema perhaps has its own matrix, but the chromosome has none of its own. The so-called chromosome matrix must be a mass of the matrix of the chromonema brought together by its coiling.

The twisting[1] that we find of the spiremes (the chromonemata coiled anew) which are now double (as a result of the longitudinal split) is nothing but a trace of the old coiling. In living material it

1) This point we shall discuss more fully in a later paper.

is hardly observable that the spireme is longitudinally double (BĚLAŘ, 1929), because in these stages the chromonemata swell in some measure; but it becomes clearer when the material is caused to shrink by fixatives.

In metaphase and anaphase the chromonemata are often swollen and indistinct to sight, but the matrix stands out clearly from the surroundings. From the telophase to the interphase a reverse change takes place; the chromonemata become distinct and the matrix indistinct. The term "chromosome" is only a term given to the chromonema coiled into a compact spiral. The chromonema exists, unlike the matrix, throughout all the stages in mitosis. Its obscureness in the metaphase and anaphase is only an apparent phenomenon due to a colloidal change of its substance. Imitation nuclei artificially produced directly from chromosomes in metaphase as well as those from chromosomes in anaphase (KUWADA and NAKAMURA, 1934) prove that it really exists in those stages too, because the artificial nuclei are quite the same in structure, except for the lack of nucleoli, as the nuclei physiologically produced from those chromosomes in which the chromonemata are obscure. There is another change in the chromosome cycle which gives rise to the coiling of the chromonema into a spiral—a structural change. This point we shall discuss in a later paper of this series.

To recapitulate shortly: The chromonema is, though it may be swollen in metaphase and anaphase, in a tightly coiled state throughout all the stages in its life cycle, except the interphase, where it is found in a more or less loosened form. A closer examination of BĚLAŘ's drawings and photomicrographs shows that what we have mentioned above is all sufficiently illustrated by them. It is unnecessary to say here that the formation of nucleoli in the telophase, where a change in the chromosome system begins to take place, has an intimate connection with the problem at issue, but our knowledge on this particular body is too meager; nevertheless we have ventured to discuss the problem not considering at present its formation at all, because it generally disappears abruptly in the metaphase without making any decided display of its relation either morphological or physiological to the chromosomes.

We have discussed above the behaviour of chromonemata in the mitosis in rapidly dividing meristematic tissues where the so-called resting stage is not found. We have then the question, in what state are the chromonemata in the resting or vegetative stage of the nucleus?

If we stain with acetocarmine meristematic nuclei such as those in young pollen grains, or in young leaves or petals, in which the network-looking architecture of chromonema threads is clearly visible, we find that the threads are stained without showing any noticeable change in the architecture. The general aspect of the nuclear structure is quite the same both in these stained nuclei and living ones mounted with liquid paraffin. In the case of periplasmodium nuclei, some appear to be homogeneous in the fresh state and present the appearance of being filled with a jelly-like substance, while most of the nuclei clearly show a meshwork-looking structure such as found in the young pollen grains. When stained with acetocarmine, the former presents a structure which is very similar to that exhibited by the latter in its fresh state. Mr. SHIGENAGA showed one of us (K.) under the microscope that in the nuclei in the leaves of *Elodea* which look quite hyaline except for the nucleolus, a meshwork-like structure comes out as soon as a drop of acetocarmine is added. According to GUILLIERMOND (1932) the nuclei appearing homogeneous are only more or less opalescent in the dark field illumination, and according to PRICE (1914), in *Elodea* they are "only slightly milky." Both this weakness in brightness in the dark field illumination and the behaviour of the chromonemata towards the contracting agent have also been observed in the chromosome in metaphase. Apart from the difference in the colloidal state, there seems to be no fundamental difference between chromonemata in the metaphase chromosome and the interphase uncleus, and we are led to ask ourselves whether an interphasic nucleus in which the chromonemata are in the swollen state to a greater or less extent as is the case in the metaphase is not the vegetative nucleus.

It has been generally assumed that the nuclei in the metabolic stage are more advanced in stage than those in the interphase. This idea seems to be based on the conception that the chromosomes from which the interphasic nucleus is formed have a structure quite different from that of the latter. As a representative opinion that led to this conception may be mentioned the view that the nucleus of a reticulate structure is formed by alveolation of chromosomes of a solid structure. We now know that this opinion is wrong. "The telophasic transformation does not involve an actual "alveolation" of the chromosome; hence this term should be abandoned" (SHARP, 1929, p. 351). In appearance the metaphasic chromosomes and the interphasic nucleus look entirely different from each other in structure, but foundamentally they are of the same structure, composed of chromonemata. The

only difference between them, which has no direct connection with the colloidal change in the matrix substance, is in the point whether the chromonemata are in a shrunken or a swollen state; in either case the chromonemata must exist without suffering any fundamental structural change. It may be supposed that they can be subjected in a given stage to such a colloidal change as that found in the metaphasic chromosomes, if in that stage the conditions in the milieu are changed so as to cause this change of the chromonemata. If such a change in the milieu takes place in the interphase, the internal structure of the nucleus will become obscure. This nucleus we like to regard as that one which is generally called the metabolic nucleus. It seems that it is in an extreme case that the nucleus appears quite hyaline. In a less swollen state it would appear as a jelly mass, and in a still less swollen state it would be no longer homogeneous, but exhibit a certain indistinct structure. W. H. LEWIS and M. R. LEWIS (1924) seem to regard the linin thread or chromatic granules as "fixation, coagulation, or precipitation products" which "do not represent living structures". They may be such, but if these authors are of the opinion that all the visible structures are to be regarded as such, their view is in disaccord with our finding that the chromonemata are visible in living interphasic nuclei which are quite healthy.

If the facts are interpreted in this way, no advance in stage is involved in the transformation of the nucleus from interphase to the metabolic stage. It must, however, be kept in mind that the change in the nuclear material taking place in the vegetative or metabolic stage must be far more complex than in the metaphase and anaphase. A certain structure which is reversible may appear in the nucleus as a result of transitory resolution of nuclear material into component parts ("Entmischung") in response to the physiological change in the milieu (cf. NASSONOV, 1932) or in connection with the physiological function of the nucleus. Perhaps WILSON's statement (1919, p. 28) on the structure of protoplasm that "while each may be characteristic of certain kinds of cells, or of certain physiological conditions, none is common to all forms of protoplasm," may also be applied to the case of the metabolic nucleus to a certain extent. It should also be remembered that the nucleus is rendered structureless by simple mechanical stimuli and *vice versa* (BĚLAŘ, 1930; CHAMBERS, 1924; BANK, 1933).

When we watch under the microscope the behaviour of chromosomes (in the heterotype metaphase in pollen mother cells in *Trades-*

cantia reflexa) towards the change in the hydrogen ion concentration of the observing medium caused by introducing or driving out CO_2 gas after the method of SAKAMURA (1927), we notice that the chromosomes which, when CO_2 is driven out, seem at first sight to have disappeared from view have not completely disappeared; the swollen chromosomes are still very faintly visible (Fig. 11*b*). This fact seems to show that the apparent disappearance is largely due to change in the refractive index rather than fusion of the neighbouring chromosomes caused by swelling. If the invisibility of the chromonemata in the vegetative nucleus is caused by identity in the refractive index, it would not be strange to assume that the individuality of the chromonemata is kept even in those stages where they are invisible.

What we stated above about the structure of the metabolic nucleus is only a working hypothesis. Experimental data on various sides are needed to set it on a firm basis, data which will make it possible adequately to analyse the causes that have given rise to the divergent opinions, for the literature on which subject we would refer the reader to MARTENS (1927). An important question left for further investigations, is whether or not the chromonemata are of the discontinuous structure indicated by the genetical data.

Before concluding we shall briefly discuss the question, on what grounds the various opinions on the structure of chromosomes hitherto put forward are based.

1. Non-differentiated, solid structure. From the parallel investigation of BĚLAŘ with fixed and living materials it is clear why the fixed chromosomes in metaphase and anaphase generally show the solid structure. It is because they appear homogeneous in the living state, the chromonemata being considerably swollen. In this case the object is fixed in its natural state, or to put it briefly, the fixation is natural.

2. Vacuolated or reticulate structure such as reported by OVERTON (1922). OVERTON is of the opinion that the reticulate structure is brought about by vacuoles which appear as a result of absorption of liquid substance from without. No such vacuoles are, however, visible in the living state. It seems highly probable that this structure is due to an unnatural fixation of chromosomes of solid structure or chromosomes in which the chromonemata are considerably swollen.

3. Cylindrical structure with a core and a cortical part. This structure is often reported both in fixed and in living material (SCHUSTOW, 1913; LUNDEGÅRDH, 1912; BONNEVIE, 1908; CHAMBERS, 1924). From the specific affinity for dyes it is seen that the cortical part

represents the chromonema spiral and the core the matrix. In this case it could be assumed that the chromonema is swollen to a lesser extent than in the case where the chromosomes appear to be of solid structure. The fixation which brings about this structure must be the natural one.

4. Spiral structure. This structure is generally not visible in living material, but it is clear from the results we obtained that it exists. The fixation that brings about this structure is neither natural, nor unnatural. It may be distinguished from the natural and the unnatural fixation as one which is hypernatural.

5. Chromomeric or granular structure. In this case there is often involved a misapprehension due to optical illusions (cf. FUJII, 1926). Apart from such a case it is highly probable that there are cases where unnatural structures of this kind are brought about by an insufficient hypernatural fixation of the chromonema spiral (a combination of the hypernatural and unnatural fixations).

6. Axially vacuolated structure. This is the structure regarded by some authors as representing the longitudinal split of chromosomes, and by others as a transition structure of chromosomes being transformed into the interphase or the resting nucleus. This must be a product of insufficient natural fixation of chromosomes in which the physiological shrinkage of the chromonemata has set in. In many cases this structure has been reported to have been observed in the telophase. That is perhaps because of the fact that it is generally at this stage that the physiological shrinkage sets in. Much less often the same structure has been reported in anaphasic chromosomes, and rarely in metaphasic chromosomes. This may be interpreted as due to the fact that the shrinkage sometimes occurs even in these stages. CHAMBERLAIN'S (1925) opinion that the chromatin is a vacuolated substance seems to have been based on the results of a rather unnatural fixation in these stages.

In some plants, especially certain hybrids (cf. NAGAO, 1933), fixation of chromosomes is extremely difficult. This can be controlled to a certain extent by subjecting the material to a certain condition such as cold before fixation (KIHARA, 1927), the improved result perhaps being due to the principle that a rise of hydrogen ion concentration in the cell caused by an unusual environmental condition (cf. NASSONOV, 1932) results in shrinkage of the chromosomes. Generally speaking, the result of fixation not only depends upon the quality of the fixatives, but also upon the colloidal state of the

object to be fixed. If an object to be fixed is a sol, it will be liable to a great change in its structure. In an extreme case the product would be largely an artefact. If, on the other hand, the object is a gel, it may generally be fixed true. We have seen that when the swollen chromonema in the metaphase is naturally fixed, differentiation between it and the chromosome matrix is generally indistinct, but that in the telophase, where it begins to shrink, the differentiation is clearer, and in the interkinesis, where it is in the most shrunken state, it is clearest. This has been beautifully demonstrated by SHINKE (1934) in *Sagittaria* (comp. Fig. 12). If in the metaphase and anaphase too, the chromonema spirals are beautifully demonstrable by an ordinary method of fixation, the chromonemata must be in a certain shrunken state. In our opinion a clear differentiation of the chromonema from the matrix does not always involve the absence of the latter, but may show that the chromonema is in a shrunken state.

From what we have said above it seems that the reticulate structure of the fixed metabolic nucleus is the image of an insufficient hypernatural fixation of the nucleus in which the chromonemata are swollen to a considerable extent. When the fixation is more hypernatural or the swelling goes less far, the parts of the chromosomes where the chromonemata do not remain considerably uncoiled would be "visible as bands in the reticulum" (SHARP, 1911, Fig. 1, Pl. I) or as "deutliche Fädchenpaare" (SCHUSTOW, 1913, Fig. 19,[1] Pl. XIV). Figs. 265, *G* and 266, *C* given in WILSON's "The Cell" (1925), which both correspond to his Stage *c*, seem to suggest that in this stage of maturation division the chromonemata may be swollen. The omission of Stages *c* and *d* in vertebrates generally (p. 538) may be accounted for by the assumption that in these animals both chromonemata and matrix are considerably swollen in these stages. In the prophase of the heterotype division in plants too, the same might be the case to a certain extent.

Coming back to our main issue, we may conclude that the schema of the chromosome cycle given by BONNEVIE (1908) is right on the whole, but it seems that she was led to a wrong conclusion in certain points through not considering the phenomenon of swelling of the chromonemata which may take place in certain stages. What she has

1) In this figure pairs of coiled threads are seen in the "Kernnetz." With the pairing (doubleness) of the threads we shall deal in a later paper.

considered to be an internal differentiation of the spireme into the core and the cortical part could probably be regarded as being in reality the new formation of the spiral in the prophase, and her endoigeneous differentiation of the spiral in the telophase as nothing but a reappearance of the swollen chromonemata due to shrinkage. The anastomosis in the resting stage which she regarded as really existing also seems to be largely the display of a colloidal peculiarity of the nuclear material with regard to fixatives, although TELEŻYŃSKI's result of observation of living material may seem to show that this conclusion goes too far from reality.

Summary

1) Young staminate hair cells were observed in the living state, they being mounted with liquid paraffin. In the interphase the chromonemata which run sinuously or are coiled in part more or less irregularly are clearly seen in the nucleus. These chromonemata are so disposed as to present an appearance of a mass of disordered chromonemata. At the beginning of the prophase the regular spiral form of the chromonemata is restored, and each spiral becomes clearly distinguishable from others. Then the chromonemata are gradually thickened and are drawn out from their coiling to form the spiremes. When the spiremes are considerably thick, their internal spiral structure comes to sight again. This spiral structure is lost to sight generally from the late prophase to the beginning of the telophase when it comes again to sight. As the telophase proceeds, the chromonemata become clearer and clearer, but when the interphase is reached, the regular spiral form is lost.

2) Observations by the method of dark field illumination show that in the stages where the spiral structure is obscure, the chromonemata are enormously swollen. In these stages the chromosomes appear dark, while in the other stages where the chromonemata are visible, they are more or less bright.

3) Some general problems with regards to the chromosome cycle are discussed and various opinions on the chromosome structure hitherto put forward are criticized from the view point of the spiral structure.

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Explanation of Plates

Abbreviations:— 1.5mm., Zeiss apochrom. imm. objective 1.5mm.; 2mm., objective 2mm.; 4mm., objective 4mm.; K. 12, comp. oc. K. 12 (15×); P. 15, Leitz periplan oc. 15× (In the case where this eye-piece was used, Leitz Makam camera was used); ×5/7, reduced about five sevenths, and ×2, enlarged about two times the original negative.

Figs. 1—11, *Tradescantia reflexa*.

Fig. 1. Staminate hair. Spiral stage phot. at 1.52 p. m. This cell was observed up to metaphase (4.30 p. m.). Mounted with liquid paraffin. 1.5mm.×K.12×5/7.

Fig. 2. Staminate hair. Metaphase phot. at 4.43 p. m. Mounted with liquid paraffin. 1.5 mm.×K.12×5/7.

Fig. 3. Anaphase of the same cell phot. at 4.59 p. m.

Fig. 4. Late anaphase of the same cell phot. at 5.03 p. m.

Fig. 5. Telophase of the same cell phot. at 5.15 p. m.

Fig. 6. Staminate hair. Probably end-telophase phot. about 3 hours and a half after cell plate had been formed. Mounted with liquid paraffin. 2mm.×K.12.

Fig. 7. Staminate hair. Interphase. Mounted with liquid paraffin. 1.5mm.×P.15×5/7.

Fig. 8. Periplasmodium nucleus accidentally drawn out. Acetocarmine preparation. 1.5 mm.×P. 15.

Fig. 9. Staminate hair. *a*, *b*. The same cell in the interphase. Mounted with liquid paraffin. 1.5mm.×K.12×5/7. *a*. Bright field illumination. *b*. Dark field illumination.

Fig. 10. Staminate hair. *a*, *b*. The same cell in metaphase. Mounted with a 3% sugar solution. 4mm.×K.12.
a. Bright field illumination. *b*. Dark field illumination.

Fig. 11. Pollen mother cell. *a*, *b*. The same cell in I-metaphase. Mounted with a 3% sugar solution. 4mm.×K.12×2.
a. CO_2 introduced into the medium. *b*. CO_2 driven out.

Fig. 12 *a–c*. Pollen mother cells in *Fritillaria verticillata* WILLD. var. *Thunbergii* BAK. Paraffin section. Fixed with FLEMMING's weaker solution, the material being pre-

viously treated with CARNOY's mixture containing chloroform for 30 seconds, and stained with iron alum haematoxylin. 1.5mm.×K.12. *a*. I-metaphase. *b*. Interkinesis. *c*. II-metaphase. Explanation in the Text.

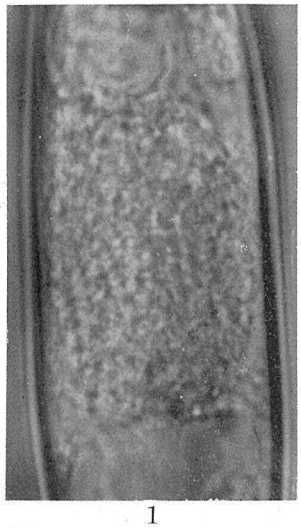
Errata

Vol. IX, No. 2, Art. 4, 1933.

(KUWADA and NAKAMURA, Behaviour of Chromonemata in Mitosis, I.)

Page 135, line 9 from above, read *definite* instead of *difinite*.

„ 136, „ 15 „ below, „ *These spirals* „ „ *Both these spirals*.



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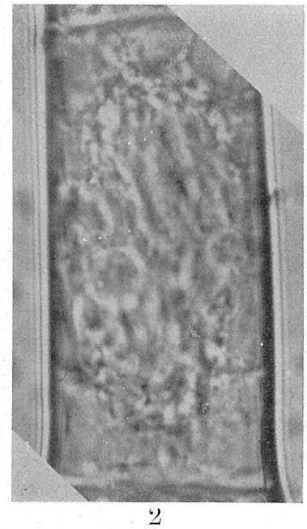

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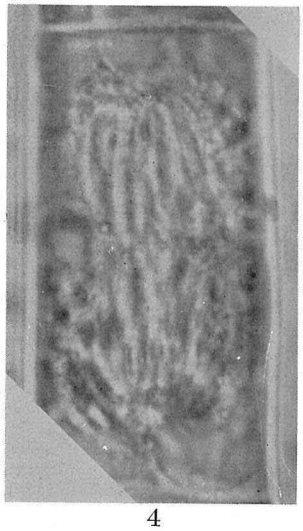

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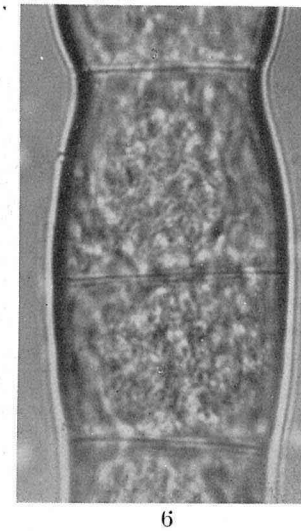
5



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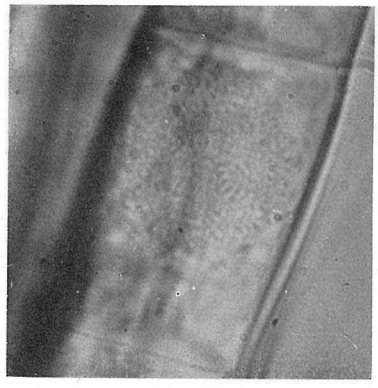

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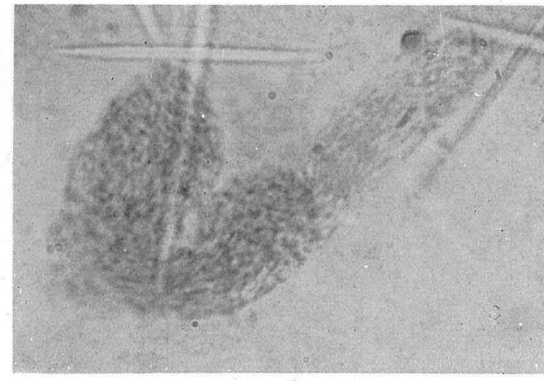

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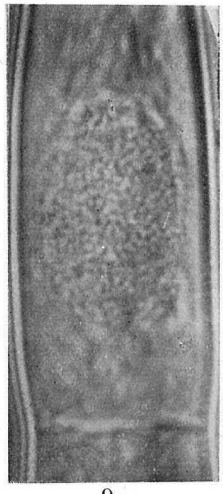

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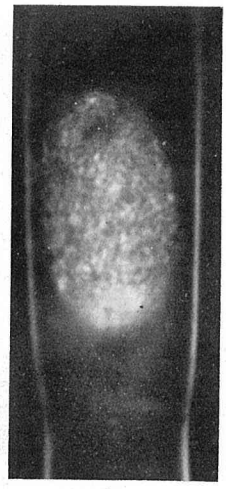

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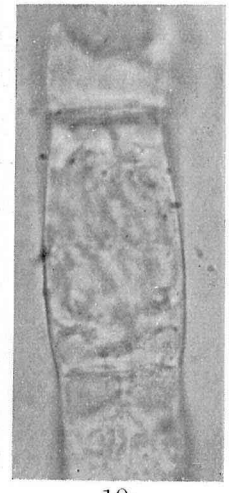

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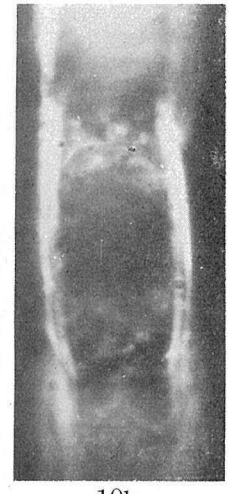

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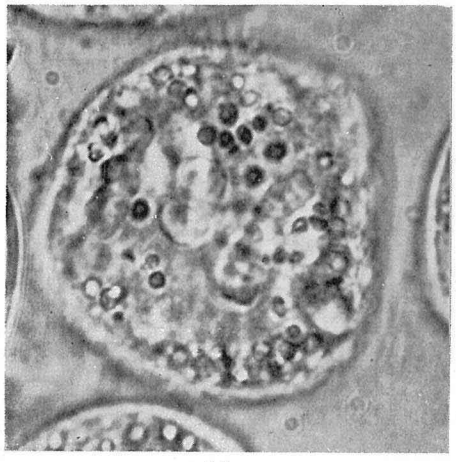

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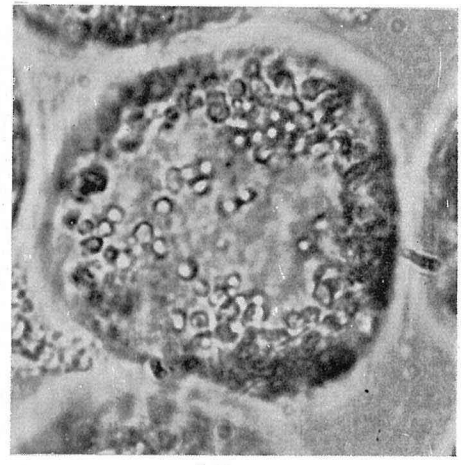

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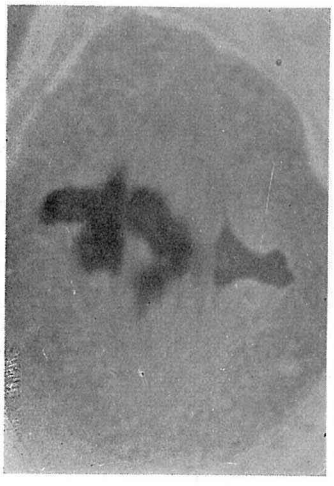

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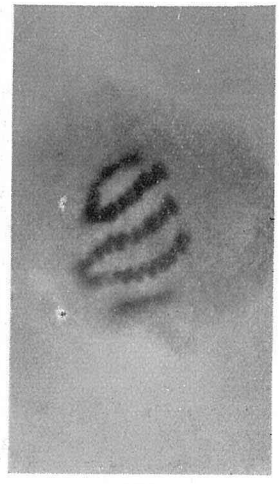

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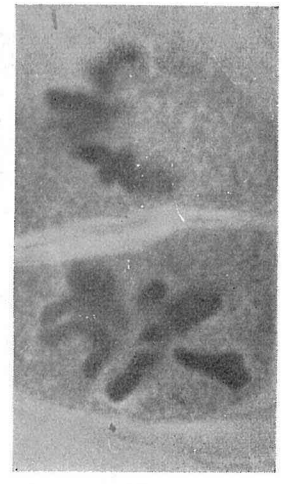

12c