Studies of Mitosis and Meiosis in Comparison
V. Some experimental observations of mitosis in
***Tradescantia* staminate hairs¹**

By

Yoshinari Kuwada and Takesi Nakamura

Botanical Institute, Kyoto Imperial University

Received July 24, 1941

In the course of experimental observations of mitosis in the staminate hairs of *Tradescantia reflexa*, cases were observed which may suggest that there is a strong tendency in the nucleus in mitosis to undergo changes that are comparable with the phenomenon of the restitution nucleus formation and the phenomena in meiosis which are known as characteristic to some definite stages. These are reported below.

Observation

It seems advisable first to designate various stages in the prophase of the staminate hair mitosis clearly (Fig. 1). We shall begin with the stage in the very beginning of mitosis (*a*). In this stage, *a*, the chromonema structure of the nucleus now presents a "diffuse structure" (KUWADA and NAKAMURA, 1940), and now an "aggregate structure" (KUWADA and NAKAMURA, 1940), but no clear inter-chromosomal space is perceptible. In the next stage (*b*), the inter-chromosomal spaces become visible in places (KUWADA, SINKE and NAKAZAWA, 1939)—the contraction stage. This stage is followed by the spiral stage (*c*), at which the nucleus contains more water and the spiral structure of the chromosomes is now conspicuous. In this stage the chromosomes are distinguishable from one another more clearly, and the general straight arrangement of the chromosomes becomes manifest, if the nucleus is of an elongated shape. In the next stage (*d*), the spirals are drawn out, and the whole structure of the nucleus comes to present a very contorted appearance like an interphasic nucleus. When the spirals have been drawn out, the general straight arrangement of chromosomes is clearer again (if the nucleus is of an elongated shape) as in the spiral stage. This stage (*e*) corresponds with that shown in Text-fig. 2 *e* of BĚLAŘ (1929). We may distinguish between the early and the late *e* stage. In

¹ The cost of this investigation has been defrayed from the Scientific Research Expenditure of the Department of Education.

the late *e*, the chromosomes are thicker than in the early *e*. The late *e* is followed by the final stage (*f*), in which the chromosome split is usually visible in the living state. While in the *e* stage, both early and

Fig. 1. A tip staminate hair cell mounted with water at 2.48 p.m. *a*. At 2.52, *a* stage (interphase). *b*. At 3.25, beginning of *b* stage (contraction stage). *c*. At 3.50, *b* stage. *d*. At 4.07, *c* stage (spiral stage). *e*. At 4.30, *d* stage. *f*. At 5.14, early *e* stage. *g*. At 6.06, late *e* stage. *h*. At 7.00, *f* stage.

late, the chromosomes show some internal structure which may be taken as indicating the new spirals within, in the *f* stage this structure is concealed to a great extent, and the general appearance of the chromosomes is very much like that of those in the metaphase. In this late

prophase the pole caps are developed in both polar regions outside the nucleus, and it is hard to procure in this stage a precise idea as to the presence or absence of the nuclear membrane. It is, thus, very easy to make the error of taking this stage as a pro-metaphase and vice versa. We may call the stages, *b* and *c*, the early, the stages *d* and *e*, the mid, and the *f* stage the late prophase. The *a* stage is probably a preparatory stage.

In the case of staminate hairs, the mitosis may proceed normally in the medium of city water. The above descriptions are based on the results of observation of the living hairs mounted with water.

I. Abbreviated mitosis. By some experimental treatments of the living hair cells, the abbreviated mitosis could be caused, the katechromatic change or the telophasic chromosome dissolution occurring at various stages in the prophase. Below, an example will be given for each case observed.

1. *The case where the katechromatic change began at the b stage (Fig. 2).*

Fig. 2. Medium: water. Mounted: 2.40 p.m. *a*. At 2.53. *b*. At 4.24. *c*. At 4.47. *d*. At 4.59. *e*. At 5.28. *f*. At 6.02. Before 4.33 also, some aggregate structure of the nucleus such as is shown in *a* was perceptible to some extent, but in this period of time (*a* stage) such a remarkable chromosome contraction as shown in *d* and *e* (*b* stage) was never observed.

Hairs were mounted with water¹ at 2.40 p.m., and a tip cell nucleus in the resting stage (interphase) was observed. The nucleus showed the aggregate structure some-

¹ In the present investigation city water was used.

times clearly and sometimes obscurely (the *a* stage). At 4.59 and 5.28, it was evident that the nucleus was entering the *b* stage (contraction stage), the inter-chromosomal spaces being visible very much more clearly in the nucleus. At 5.32, the inter-chromosomal spaces became less conspicuous again, and at 6.02 they were as obscure as when the nucleus was in the resting stage or the *a* stage. Moreover, the chromonema structure of the nucleus was less distinct after 5.32, indicating that the chromonemata had undergone some hydration.

Fig. 3. Medium: water. Mounted: 3.50 p.m. Replaced with 20% alcohol: 4.03; with water: 4.09. *a.* At 4.02, *c* stage. *b.* At 4.16. *c.* At 4.18. *d.* At 4.22.

2. The case where the change began at the *c* stage (Fig. 3).

Mounted with water at 3.50 p.m. At 4.02 the *c* stage (spiral stage) was reached. At 4.03, the water was replaced with 20% alcohol, and at 4.09 the alcohol was replaced again with water (cf. MÜLLER, 1936). At 4.16, a katachromatic change was recognizable, and at 4.22 the nucleus was almost of the structure of the resting nucleus. The observation was continued up to 5.15. The nucleus presented a diffuse structure, but its chromonema structure was distinctly observed as in the resting stage.

In the preparation mounted from beginning with 5% alcohol, too, the same case was observed. But, in no case was this reversal of mitosis at the *c* stage observed, where only water was used as the mounting medium.

3. The case where the change began at the *d* stage (Fig. 4).

Mounted with water at 2.46 p.m. At 2.52 the *c* stage, and at 3.16 the *d* stage, was reached. After that, mitosis did not proceed further, but at 3.44 and at 3.55, the katachromatic change which was going on further and further was conspicuous.

Fig. 4. Medium: water. Mounted: 2.46 p.m. *a.* At 3.02, a transition stage from *c* stage to *d.* *b.* At 3.16, *d* stage. *c.* At 3.44. *d.* At 3.55.

4. *The case where the change began at the early e stage (Fig. 5).*

Mounted with water at 1.32 p.m. At 1.37 the early *d* stage, and at 2.12 the early *e* stage, was reached. After that, the nucleus showed no progress, and at 3.10 the katechromatic change was conspicuous. At 5.00 and 6.10 the nucleus was almost of the structure of the resting nucleus.

Fig. 5. Medium: water. Mounted: 1.32 p.m. *a.* At 2.12, early *e* stage. *b.* At 3.10. *c.* At 5.00. *d.* At 6.10.

5. *The case where the change began at the late e stage (Fig. 6).*

Mounted with 7.5% alcohol at 2.21 p.m. At 2.26 the mitosis was at the *c* stage. At 2.51 the alcohol was replaced with water, when the nucleus was at the *d* stage. The mitosis proceeded normally up to the late *e* stage (at 4.20). At 4.40 the chromosomes came to show their internal spiral structure, and at 5.40 the individual chromosomes became less clearly distinguishable, though the straight arrangement of the chromosomes which was conspicuous at 4.40 and 4.50 was still perceptible to some extent. At 6.05 the nucleus was highly diffuse in structure.

Fig. 6. Medium: 7.5% alcohol. Mounted: 2.21 p.m. Replaced with water: 2.51. a. At 4.20, late *e* stage. b. At 4.50. c. At 5.40. d. At 6.05.

6. *The case where the change began at the f stage (Fig. 7).*

Fig. 7. Medium: water. Mounted: 2.11 p.m. Replaced with 0.5 M sugar solution: 4.30; with water: 4.41. a. At 3.55, *f* stage. b. At 4.54 (after deplasmolysis). c. At 5.30. d. At 5.55.

Mounted with water at 2.11 p.m. At 2.17, the nucleus was at the *d* stage, and at 3.55, at the *f* stage. At 4.30, the medium was replaced with a 0.5 M cane sugar solution to cause plasmolysis. At 4.41 the sugar medium was replaced again with water to cause the deplasmolysis. At 4.54 the chromosomes became visible again, and no remarkable change in the chromosome structure was recognizable up to 5.20. At 5.30 fine spirals were visible in the chromosomes and at 5.55 the distinction between individual chromosomes was less marked. The observation was then discontinued. The next morning the nucleus was found dead, and no mitotic progress was recognizable in the nucleus. The comparison of Fig. 7 with Fig. 6 seems, however, to show that this case probably represents a case of the abbreviated mitosis.

In this case of the mitotic reversal at the *f* stage, neither water nor alcohol alone nor both in combination as medium could cause the untimely katachromasis.

The numbers of cases of the experimentally induced mitotic reversal or katachromatic change observed at different stages of prophase are shown in the accompanying table.

In this table the case of the *f* stage is excluded, since at this stage the chromosomes can undergo the katachromatic change only under the condition

Stage at which the induced katachromasis occurred	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>
Case of water alone used as medium	1	0	5	5
Case of both water and alcohol used in combination as medium	1	3	2	1
Total	2	3	7	6

which causes the katachromasis of chromosomes in metaphase and anaphase, as will be seen later. From this table we see that the induced katachromasis occurs most frequently at the stages, *d* and *e*. According to the verbal information of NAKAZAWA, these cases of the katachromatic change occurring at various stages of prophase can also be observed in the staminate hair cells mounted with liquid paraffin as well as those with a sugar solution of some high concentration. These stages of prophase have been denoted by WADA (1932) as "Rückkehrsphase", who observed this abnormal prophasic katachromasis by puncturing the cell with a micro-needle.

In the present investigation, trials of obtaining similar results from the chromosomes in metaphase and anaphase were made only with negative results. A somewhat ambiguous case we observed may, however, be mentioned (Fig. 8).

Mounted with water at 2.48 p.m. At 2.50, the nucleus was in the *f* stage. At 2.51, the medium was replaced with a 0.5 M cane sugar solution, which caused plasmolysis, and the individual chromosomes became not visible. At 3.28 the medium was replaced with water to cause the deplasmolysis. At 3.47 the chromosomes were visible again, but it was difficult to determine the stage of mitosis. Up to 4.00, the chromosomes were observed to be in a similar condition. At 4.14, the chromosomes showed the internal spiral structure. The determination of the stage was still difficult to make, but there was some indication of the mitosis being at anaphase, the chromosomes appearing divided into two groups, though very obscurely. At this stage the individual chromosomes were distinguishable from one another, but formed a

nucleus of a diffuse structure and of an irregular, elongated shape. Later, at 5.45, the nucleus was found to be of a dumbbell shape, and at 5.53 it was clearly observable that the nucleus was deeply constricted at the middle region, so as to appear at a definite optical section as representing a pair of nuclei of irregular shapes very closely in contact with each other.

Fig. 8. Medium: water. Mounted: 2.45 p.m. Replaced with 0.5 M sugar solution: 2.51; with water: 3.28. *a.* At 4.00. *b.* At 4.14. *c.* At 4.21. *d.* Stained with acetocarmine at 6.00.

The interpretation of this case is somewhat difficult, but it seems likely that at 4.14 the mitosis was at some stage of anaphase. Probably, as an effect of the hypertonic solution, the anaphasic separation of the chromosomes was very incomplete, and the complete separation was possible only in some of the chromosomes. The separated chromosomes formed a nucleus independent from their sisters, but the others formed a common nucleus with the sisters; hence the sum total was the nucleus of a constricted form.

If this interpretation is correct, we may say that according to conditions the katachromatic process may begin at the anaphase also. We were not able to demonstrate this to occur at the metaphase, but some authors have done it (comp. SIGENAGA, 1937). Thus, we may say that the katachromatic process can take place at every stage of prophase, and also at metaphase and anaphase even, and normally in the telophase. It seems, however, important to note that it is very difficult artificially to cause the katachromasis (chromosome dissolution) at the stages where the chromosomes are in a highly dehydrated condition as in the metaphase and anaphase (cf. WADA, 1932). If in these stages, the normal mechanism of chromosome separation exists, the separation process would proceed nor-

mally before the chromosome dissolution process begins. The fact that in the *f* stage of prophase at which the chromosome separation mechanism is developing the artificial performance of katachromasis is as difficult as in the metaphase and anaphase and is possible only when the cell is pre-treated, as in these stages, with such a reagent as that causing plasmolysis, for instance, seems, on the other hand, to show that there may be some antagonistic relation between the mechanism of chromosome separation and the performance of chromosome dissolution, because in this case of prophase there would be a sufficient time for the preparation of the chromosome dissolution before anaphase. We may probably say that the katachromatic process can take place only when the normal mechanism of

Fig. 9. Medium: water. Mounted: 1.35 p.m. *a*. At 1.40, *c* stage. *b*. At 2.52, *e* stage. *c*. At 3.45. *d*. At 4.33. *e*. At 6.07. *f*. At 9.55, the next morning.

chromosome separation ceases to exist, or in other words, that in the higher organisms the chromosome separation and the chromosome dissolution cannot take place hand in hand.

The absence of the chromosome separation mechanism which renders possible the performance of chromosome dissolution at metaphase and anaphase may perhaps be expressed in terms of the physical or physico-chemical changes in the atractoplasm (WADA, 1940). In the case of the nuclear division in a flagellate, *Oxyrrhis* (WILSON, 1925), which may be regarded as one of the cases of intranuclear nuclear division, the chromosomes seem to be in a diffuse condition at metaphase and anaphase. This seems to be due to the physical or physico-chemical properties of the atractoplasm which differ from those of the atractoplasm in the higher organisms.

II. The diffuse stage. In one of the cases of the present investigation, a diffuse stage was observed in the prophase (Fig. 9).

Hairs were mounted with water at 1.35 p.m. A nucleus at the spiral stage was photographed at 1.40 p.m., and this nucleus was watched for further stages. The mitosis proceeded normally up to the *e* stage (at 2.52). At this stage (at 3.27 and 3.45), the spirality became much more clearly visible than normal. About one hour later (at 4.33), the chromosome territories were rendered obscure and the whole nucleus appeared at first sight to be of a diffuse structure like a resting nucleus. At 5.22 p.m., the chromosomes became visible again, and at 6.07 p.m. the nucleus showed the normal appearance of the *e* stage. Thenceforth, the mitosis proceeded normally, and the two daughter nuclei with normal appearance were produced.

In this case, the mitosis underwent a change at the *e* stage toward the abbreviated mitosis, but it recovered from this change before metaphase, hence could accomplish its whole process, the nuclear division. This unusual case of the *e* stage appearing as a diffuse stage is comparable to one of the cases of the "diffuse stage" known in the animal meiosis which arises at a later part of the diplotene as a temporary phenomenon and disappears before the diakinesis.

In the case of the prophasic chromosome dissolution the mitosis may continue its further processes, but in the case of metaphasic or anaphasic chromosome dissolution probably it cannot, because in these cases the change in the atractoplasma which renders the chromosome dissolution possible may bring the mitosis to a close.

In the present instance of diffuse stage, the spiral threads are somewhat indistinct and the general structure of the nucleus (Fig. 9d) resembles that of the resting nucleus of the HS structure, the aggregate structure being perceptible (KUWADA and NAKAMURA, 1940). In the case of the anomaly resulting in the abbreviated mitosis, the change seems to proceed, so far as our observations are concerned, without passing this stage resembling the HS structure. At present, however, we are not in a position to tell whether or not this stage is characteristic of the diffuse stage.

Discussion

In the present investigation, the following results were obtained:—

1) The katachromatic process can take place at various stages in the prophase, beginning in the hydration of the chromosomes.

2) In the late prophase and in the anaphase, it takes place only with difficulty. In these stages, the occurrence of the katachromasis is possible only when first the process of the polar separation of the chromosomes is suspended and the chromosome hydration is caused, both by artificial means. SIGENAGA (1937) has obtained the same results for the metaphase and anaphase¹ chromosomes by application of chemical agents with or without replacement with water.

3) The abnormal chromosome change or the chromosome diffusion in the prophase as a prominent feature of the abbreviated mitosis may be suspended from going further, and the anachromatic process can be recovered to complete the mitosis—the diffuse stage. In this case the mitotic process is suspended during the change, and after recovering from it the further processes of mitosis take place as in the normal case.

From these results we may draw the following conclusions:— The katachromasis which in the normal mitosis occurs only in the telophase can take place at any stage of mitosis, if the chromosomes are brought to a certain condition under which they can be subjected to hydration, provided in the cases of the late prophase, metaphase and anaphase there exists another condition that causes suspension of the process of polar chromosome separation. The katachromasis of untimely occurrence thus abbreviates the normal course of mitosis to various extents. In these abnormal cases of katachromasis the process is irreversible before the nucleus is activated for a new mitosis, as in the normal case of katachromasis in the telophase, and hence they may be regarded as the case of the true katachromasis. In the case of the diffuse stage which we also observed, on the other hand, the hydration occurs as well, but ceases to proceed further. These phenomena, the katachromasis of untimely occurrence and the temporary chromosome dissolution or diffusion during mitosis, seem to have some important significance when the meiosis is compared with the mitosis.

1. **Leptotene stage.** It is generally accepted that in the leptotene stage the chromosome threads are drawn out from the spiralized condition, and the new spiralization which in the mitosis takes place along with the drawing out of the old spirals is postponed to a later stage, hence in the leptotene the chromosome threads are quite free from the spiralized condition. In normal mitosis, there seems to be two and only two methods by which the spirals are more or less drawn out; the first method is one in which the drawing out is accompanied by the new spiralization and which is found in the case of the drawing out from the condensed state in the beginning of prophase, and the second one is that in which it is

¹ Verbal information.

free from the new spiralization and which is found in the case of the telophasic transformation of the chromosomes from their condensed state at metaphase and anaphase. In the former case the drawing out is accompanied by uncoiling at least to a certain extent (KUWADA, 1939), but in the latter it is not (KUWADA and NAKAMURA, 1940). It seems likely that the uncoiling due to drawing-out occurs only when it is associated with internal re-spiralization. In meiosis too, there is a condensation stage preceding the leptotene, which is comparable with the *b* stage in the beginning of mitosis. If the leptotene drawing-out is performed by either one of these methods, and if, in the leptotene, the drawing-out is connected in no measure with re-spiralization, and hence if the method belongs to that of telophasic katachromasis, it becomes incomprehensible how this katachromasis can be reversible within the course of a division. We come then to doubt that the leptotene threads are simple, drawn out threads, but consider them ones drawn out by the first method, hence with a coiled structure as a result of re-spiralization, as are the threads forming the old spirals in the spiral stage in mitosis, and consequently consider the so-called chromomere structure of the leptotene threads as an artifact representing the spiral structure. If the leptotene threads are really drawn out by the first method, the leptotene stage becomes then comparable with the *d* stage in mitosis.

Fig. 10. A pollen mother cell at "aster" stage (a bouquet) of *Trillium Tschonoskii* from the smear acetocarmine preparation, showing the coiled structure of the nuclear threads. Enlarged: $\times 2$.

If, on the other hand, the leptotene drawing-out is associated in no way with re-spiralization, as in the case of the telophasic katachromasis (cf. the prophasic katachromasis in the abbreviated mitosis), and yet if the nuclear division can proceed further, this method of drawing-out must represent a third one which is peculiar to the case of meiosis as a normal process. In this case of the third method too, the chromomere structure of the leptotene threads is still doubted, because in this case the internal re-spiralization as a cause of uncoiling of the external spirals does not occur in

association with drawing out, hence the spirals should remain not uncoiled as in the case of the telophasic katachromasis. We have obtained

some evidence to show that the spiral structure should exist also in the leptotene threads (Fig. 10). In a previous investigation (KUWADA and NAKAMURA, 1939), we have observed that in staminate hair nucleus in interphase the chromomere-like structure of the chromatids of the T-configuration is transformed into the spiral structure. We observed this change in configuration of spiralized nuclear threads also in the beginning of mitosis from the resting nucleus to the spiral stage (KUWADA and NAKAMURA, 1941). If in reality the leptotene drawing-out takes place after the third method, the drawing-out and the new spiralization should occur in meiosis separately in successive order as generally maintained, and the re-spiralization is regarded in this case as being postponed to a later stage. The leptotene is, however, a stage immediately following the "unravelling stage," and therefore, this stage is roughly comparable to the *d* stage in mitosis.

It is a very hard task to answer the question, which alternative is correct, the leptotene method of drawing-out as belonging to the first method or as belonging to the third, but in any case we may probably say that the leptotene stage is comparable with the *d* stage or with a stage about this stage in mitosis.

2. Diffuse stage. The diffuse stage in which the chromosomes become looser in texture is well known as occurring in the animal spermatogenesis and oögenesis, typically in the latter. In the plant kingdom, this stage is of rare occurrence, especially among higher plants, though MATSUURA (1935) has found it in his study of meiosis in *Mitrastemon Yamamotoi*. In this stage, as in the case of the normal katachromasis, the chromosomes undergo changes not only in their texture and contour, but also in their basophily and resistance to the peptic digestion (WILSON, 1925). In the diffuse stage, however, these changes are reversible within the course of the division, and the compact form of the chromosomes are recovered, so that the nuclear division proceeds further normally to complete the meiosis. This reversibility during the division process and this alone can distinguish the diffusion at the diffuse stage clearly from the katachromasis or the telophasic diffusion in which case the chromosomes can regain the condition of being of compact texture only by the anachromasis characteristic of the prophase of nuclear division. The diffusion at the diffuse stage is, therefore, to be regarded as brought forth through a method belonging to the third type of chromosome drawing-out. The prophasic diffusion in mitosis which we observed is also of a temporary nature, and is comparable to the diffusion in the diffuse stage in meiosis. In our case of mitosis, the reversible change took place at the *e* stage. This case of the temporary chromosome change was of extremely rare occurrence, and it has not yet been determined whether or not this change occurs at other stages of prophase too, as in

the case of prophasic katachromasis, but a mere occurrence of this in the *e* stage seems sufficient to show that at least in this stage the chromosomes are liable to this change, as in the diffuse stage of meiosis. It would probably be possible to compare the diffuse stage in meiosis with the *e* stage of mitosis. The diffuse stage is known to occur mostly at the stage between the diplotene and the diakinesis—a later part of the phase before the first metaphase. The *e* stage is a stage just preceding the *f* stage, hence also a later part of the phase before metaphase. It seems highly probable that the two stages approximately correspond with each other. We may probably say that the diffuse stage can occur in meiosis as a normal process, and it can occur in the normal mitosis as well at the corresponding stage, but only under an abnormal condition, hence that in meiosis or a double nuclear division (KUWADA, 1940), the nucleus or the chromosomes are more liable than in the normal mitosis to the change in water relation with which the diffusion seems very intimately connected.

We may remark, in passing, that the prophasic karyosphere which WILSON (1925) distinguishes as the fourth type of the changes in texture and contour of the chromosomes taking place in the stage following the diplotene may belong to the group of phenomena of contraction before the first metaphase one of which is known as the “second contraction”, and shows that in meiosis the nucleus is more liable to the change in water relation than in mitosis not only in the direction toward hydration but also dehydration.

3. Interkinesis. In the resting stage (interphase) the chromosomes are generally of loose structure, and in the metaphase and anaphase they are compact in texture. These two distinct phases in the chromosome cycle may be denoted as the resting and the dividing phase, and the two transition phases between them may be called the anachromatic (prophase) and the katachromatic (telophase) phase. If in the dividing phase the chromosome separation mechanism ceases to exist, the distinction of the metaphase and anaphase will disappear.

While in mitosis the chromosome separation takes place once only, in meiosis it takes place twice successively, so that there is a pause between the two separating divisions—the interkinesis. In the type of interkinesis which is found generally in the polar divisions of animal eggs and in the spermatogenesis in some animals such as *Ascaris*, *Oncopeltus*, etc. (WILSON, 1925), no nuclear vesicle is formed and the chromosomes show no change in texture from metaphase and anaphase, they are very compact as in the preceding and the following metaphase and anaphase. If, here, the chromosome separation process is disregarded, the distinction among metaphase, anaphase and interkinesis will become unnecessary. Through all these stages the chromosomes are, we may say,

in the dividing phase, but the interkinesis is distinguished from the others by the fact that the polar chromosome separation mechanism ceases to exist in this stage.

If the second anaphase is comparable to the mitotic anaphase, the remaining stages in the dividing phase of meiosis, i.e. the first metaphase, the first anaphase, the interkinesis and the second metaphase will become comparable with the metaphase in the dividing phase of mitosis. In the normal type of meiosis the second anaphase is the stage at which the divided halves of chromosomes are separated. This stage corresponds, therefore, to the mitotic anaphase. We may then regard the interkinesis as comparable with a part of mitotic metaphase.

We now know that in the dividing phase also, the katachromatic process can take place, if the chromosome separation is suspended. It is, therefore, a natural conclusion that in the interkinesis a temporary chromosome diffusion or even the katachromasis, both of which begin with the chromosome hydration can take place as a normal process, since in meiosis the chromosomes are shown to be more liable to the change in water-relation at particular stages than in mitosis. In fact, such a change, reminding us of the normal katachromasis, occurs in the interkinesis in the majority of cases, with gradations. The *Tradescantia* type of interkinesis is an extreme one in which the structure of the nucleus very much resembles the structure of the somatic nucleus in the resting stage, and the two extreme types, this case of *Tradescantia* and the case of the polar divisions in the animal eggs, are connected by intermediate types such as are found in *Sagittaria* and *Lilium* (cf. KUWADA, 1939). This series of katachromatic changes in degree suggests that the meiosis which is originally a cycle of mitosis has been driven to a tendency toward two cycles of division (KUWADA, 1940). But even in the case of the extreme type of *Tradescantia* interkinesis, the essential feature of mitosis, that in one mitotic cycle the chromosomes divide once only, is maintained, showing that the meiosis is here still one cycle of mitosis. This fact compels us to question as to whether the katachromasis (in the I-telophase) and the anachromasis (in the II-prophase) as a consequence of the former in the cases of the *Tradescantia* type are true ones or only apparent ones.

According to ROSENBERG (1927), in *Hieracium* the second meiotic division takes place after the first division is suppressed (abbreviated) by the "restitution" phenomenon in prophase, a katachromasis of untimely occurrence. This must show that in this plant the true katachromasis has taken place in the interkinesis, because, if it has not, the anachromasis will not take place for the second division, and in the case of restitution the result will have to be the formation of a monad, as will be inferred from the case of the abbreviated mitosis in the staminate hairs. In the case of *Hiera-*

cium, the chromosomes in the nuclear division following the restitution are long and slender, suggesting that the interkinesis of this plant belongs to the *Lilium* type. Thus it becomes highly probable that the katachromatic process in the case of interkinesis of the *Lilium* type and most probably also of the *Tradescantia* type is the true katachromasis.

According to BARBER (1940), on the other hand, in *Fritillaria* the restitution phenomenon gives rise to the formation of a monad, if it takes place in the diplotene stage, and to the dyad formation, if it occurs after the first division. He has observed other cases of dyad formation which are in effect the formation of the restitution nuclei, but in these cases the chromosome doubling is due to the suppression of the nuclear membrane formation, and not to the restitution.

The interkinesis of *Fritillaria* belongs to the *Sagittaria* type in which the chromosomes undergo little change, and accordingly no remarkable anachromatic process takes place for the second division. In the case of *Fritillaria*, therefore, all the processes of the meiotic mitosis after the restitution was obliterated, and the result was the formation of a monad. Thus, in the case of the *Sagittaria* type of interkinesis, it seems highly probable that the true katachromasis does not take place in the I-telophase but a mere temporary chromosome hydration, if anything takes place. It is expected, that if in such a case as in *Ascaris* or *Oncopeltus* the restitution occurs in the I-prophase, both divisions, the first and the second, will be obliterated.

From the above, it is concluded that interkinesis is originally a part of metaphase and that in the primitive type of meiosis the chromosomes may remain as such and in advanced types they may undergo a reversible hydration or even the true katachromasis. In the latter case, the degree of katachromatic process is different in different organisms, and a katachromasis is followed by the anachromasis of the corresponding degree. A similar difference in the degree of katachromasis is inferred to exist also in the case of somatic mitosis, from the comparison of the nuclear threads with the chromosomes in anaphase in the erythrocyte of *Triton* (cf. KUWADA, 1939), and also from the fact that in the erythrocyte nucleus in *Triturus pyrrhogaster* the structure of the nucleus is made finer by artificial means (SINKE, 1941). The degree of the chromosome diffusion can, therefore, be not an indicator to tell to which case, the case of a mere chromosome hydration or the case of true katachromasis, the diffusion belongs. There may be cases in which the *Sagittaria* type of interkinesis belongs to the case of katachromasis, and the *Tradescantia* type does not. In the latter case the processes of diffusion and its recovery are comparable to those processes in the case of the diffusion in the growth period in the animal oögenesis.

In *Mitrostemon Yamamotoi* in which interkinesis seems to belong

to the *Tradescantia* type, MATSUURA (1935) has observed the occurrence of a giant pollen grain. This monad is characterized by its nucleus which has degenerated. According to the author, the monad was formed because the mother cell became unable to perform the divisions, probably, he considers, in connection with the chromosome diffusion which he observed in the stage following the diplotene. Since in this case the nucleus had degenerated, it is impossible to decide to which case, katachromasis or mere chromosome diffusion, the type of interkinesis of *Mitrastemon* belongs.

It has been reported that in a certain unfavorable condition the interkinesis nucleus is rendered more diffuse in structure than in the normal condition (IWATA, 1940). It is inferred that in the somatic nucleus too, such may be the case, if the nucleus is of such a structure as that found in the erythrocyte nuclei of *Triton* and *Triturus*. In these cases, it is a question whether the abnormal deep katachromasis can be followed by the corresponding anachromasis or not. If not, the result will be the formation of dyads in the case of meiosis.

4. Chromosome diffusion and katachromasis. In mitosis there are three different cases of chromosome dissolution or diffusion from the more or less compact texture. The typical dissolution process takes place in the telophase. The sum total of the changes in this dissolution in telophase is called katachromasis. In this first case the changes cannot be reversible before a new mitosis begins to take place. In the normal mitosis the katachromasis takes place only in the telophase, but in abnormal cases it can do so at any stage of prophase also and if the chromosome separation process is suspended, at metaphase or anaphase even. The second case is found in the early prophase. First the chromosomes in the diffuse state in the resting stage contract so as to be more or less compact (*b* stage), and then the diffusion takes place. The resulting nucleus, or the nucleus at the *d* stage presents a very much similar structure to the resting nucleus, except that the nuclear threads are much thicker than in the latter. In this case the mitotic process goes further, and the diffusion is accompanied by re-spiralization of the chromosome threads, as characteristic features. The diffuse appearance of the nucleus disappears at the *e* stage when the new spirals have grown considerably. The third is found only as an abnormal case. In this case the prophasic chromosomes in the *e* stage are rendered diffuse, but temporarily as in the second case. The chromosomes recover from the diffuse state and re-assume the normal condensed form, and the mitosis proceeds. In this third case, the diffusion occurs free from the re-spiralization, as in the first case of katachromasis, but distinguishes itself from the latter in that mitosis can continue.

In meiosis too, the nuclear division begins with a condensation of

chromosomes of diffuse structure in the resting stage, and this stage is followed by the leptotene stage, a diffuse stage. In this case the division proceeds further, hence the diffusion should belong to either the second case or the third in mitosis. The view of most authors on the time of re-spiralization in meiosis shows that it belongs to the third case. The diffusion in the diffuse stage in the growth period belongs naturally to the third case. In most cases the diffusion occurs also in interkinesis, and in some it does not. In case it occurs, it is regarded as belonging, in the case of the more primitive type of interkinesis, to the third case, and in others, probably in most cases, to the first.

Chromosome diffusion and katachromasis are phenomena which are hardly distinguishable from each other by mere microscopical observation and staining reactions. The distinction depends only on the fact that while the chromosome diffusion is reversible in the course of mitosis, the katachromasis is not. This fact suggests that there is probably no material difference between the two phenomena themselves, but that the difference in presence or absence of the mitotic activity at the time of the chromosome change distinguishes the two phenomena, and this difference appears to be the difference between these phenomena. The katachromasis can, therefore, be regarded as a series of chromosome changes at the completion of which the mitotic activity has disappeared, and the "diffusion" as the same series of the changes that take place in a mitotically active cell. By the katachromasis, therefore, is meant the completion of a nuclear division.

In mitosis the katachromasis follows the separation of the double chromosomes due to chromosome division, but in the reduction division, it follows the separation of those due to homologous pairing in the meiotic case and that of those due to progeny pairing in the somatic case. The katachromasis can, therefore, signify the end of a division cycle, but not necessarily the end of mitosis.

Meiosis is a double cycle of division representing a single cycle of mitosis (KUWADA, 1940), and when an extra katachromasis is introduced, it is divided into two cycles of division one of which is still of a double nature. In the case of the pre-reduction type, the reduction division ends with the first or the extra katachromasis, and in the case of post-reduction type (cf. the case of the unpaired chromosomes in KIHARA's *Triticum* hybrids), the disjunction division (random chromosome distribution in the case of the unpaired chromosomes) being postponed to the second division, it ends with the second katachromasis which originally and in the case of the pre-reduction type signifies the end of mitosis, and the mitotic division is brought to an end by the extra katachromasis. When, therefore, the meiosis is modified into the post-reduction type, the original mitosis is transformed to occupy only the first cycle of the meiotic

divisions. The prolonged cycle of the meiotic mitosis is thus shortened to the normal form of cycle—a reversion (KUWADA, 1940).

If in the case of the post-reduction type, the whole process of reduction division is obliterated, the result will be a mitosis of the single division cycle of the normal form—the reversion type of transformation into mitosis (KUWADA, 1940). The obliteration is possible, because in this case the disjunction process is pushed to the second division which is in this type out of the mitotic cycle, so that a mere elimination of the chromosome pairing would be sufficient for the obliteration, or because even if it is insufficient, an occurrence of restitution after the first division can remove the reduction division. If the second division continues to exist, an extra chromosome division must occur, in order that each division cycle be of a regular type in the absence of the chromosome pairing. The result is then two cycles of mitosis—the progression type of transformation into mitosis.

If the reduction division is obliterated in the case of the pre-reduction type, the result will be again a cycle of mitosis—the reduction type of transformation into mitosis. Here the cycle is complete, but only apparently, however. In this case, the whole process of reduction division (chromosome pairing and disjunction) takes place in the course of the mitotic process, and consequently in order that the reduction division (the first division in this type) becomes obliterated, a restitution phenomenon must occur in addition to the elimination of the chromosome pairing. If in this case the meiosis is one cycle of division, the second division too will be obliterated as in BARBER's case of *Fritillaria*, and the resulting division is an abbreviated mitosis. A complete mitosis can result only when the meiotic cycle has been divided into two division cycles by the occurrence of the extra katachromasis at interkinesis. The resulting mitosis should, therefore, be a mitotic cycle consisting of two division cycles one of which is an abbreviated one.

In the case of the post-reduction type the later part of the reduction division is free from the mitosis which serves as the basic nuclear division of meiosis, the two divisions here being a pair of divisions overlapping rather than a double division. As indicated above, in this partially separated state of the two divisions the reversion or progression to mitosis can occur, and the reduction to mitosis as well, if in the case of the pre-reduction type the first and the second division are made each to represent an independent cycle of division by the occurrence of the extra katachromasis. In the state of meiosis maintaining the original form of the true double division (the pre-reduction type) the elimination of the chromosome pairing results in the semi-heterotype division, and in the original form of one division cycle the obliteration of the disjunction division results in the formation of a monad. No transformation into

mitosis can take place so long as the whole meiosis is of the original form. The shifting in time relation of the two divisions (or the transformation of the pre-reduction type to the post-reduction type) and the occurrence of the extra katachromasis can only afford the condition for the transformation into some regular type of mitosis—essentially a mitosis in the case of reversion, apparently a mitosis but consisting of an abbreviated and an unabbreviated division cycle in the case of reduction and two distinct mitotic cycles in the case of progression. The two phenomena, the shifting in time relation and the occurrence of the extra katachromasis, are thus regarded as steps toward the transformation of the double cycle type of mitosis (the meiosis) into two independent single cycles of mitosis of the normal type.

The double cycle of the meiosis can be transformed into two independent cycles, but never into those two, of which one is a single cycle of the reduction division. The reduction division in meiosis cannot exist as an independent cycle. In this respect, the reduction division of the case of homologous chromosome pairing is clearly distinguished from that of the case of progeny pairing—the somatic reduction division.

The chromosome division is an essential feature of mitosis. In the present state of our knowledge, it seems highly probable that the chromosomes divide at least a cycle prior to the nuclear division at which the divided halves are separated. The chromosome division and the chromosome separation which together constitute a mitosis take place, therefore, in different division cycle, but both occur in each division. If the chromosome separation does not occur, such an abnormal nuclear division is essentially endomitosis. The nuclear division of this type can be repeated with a limitation. If the chromosome division does not occur, this is another abnormal nuclear division, i.e., the somatic reduction division in its essential feature. The division of this type cannot be repeated again, unless it is preceded by endomitosis. The next division will be an abnormal one, because the chromosomes have not divided. The chromosome distribution being irregular, the cell will degenerate. The somatic reduction is, therefore, of a teratological nature. Probably, the occurrence of the somatic reduction division is connected with some abnormal physiological conditions peculiar to the tissues in which it occurs. It is highly probable that the mitotic conditions in the vegetative tissues with particular functions may differ from those in the meristematic tissues where the mitosis is normal.

Meiosis is, on the other hand, a regular physiological phenomenon. Physiologically, each nuclear division must be the mitotic division in which the chromosomes divide, otherwise, the chromosome distribution will become irregular. In the sporogenesis or gametogenesis the chromatin reduction must occur at the same time, however. These requirements

are satisfied by the addition of special type of reduction division to the normal type of mitosis to take place parallel with it in the same cycle. The meiotic reduction division is, therefore, a special division not directly derived from the ordinary mitosis, but of a new type in which the chromosome doubleness by division characteristic to mitosis is replaced by that by pairing, and exists only on the ground of the mitosis in parallel association with it. It cannot exist as an independent division, therefore, while the somatic reduction division which is originally an independent mitosis can exist as an independent division. The companion mitosis or the meiotic mitosis can exist, on the other hand, as an independent division, since it is originally an independent division.

As indicated above, meiosis, or a mitosis of the cycle doubled by the addition of a new type of nuclear division with the chromosome doubleness by pairing instead of by division, can be transformed into two mitotic cycles of the normal type, and through this transformation the cycle of the new type of division can become independent from its companion mitosis. A nuclear division can, therefore, be of a single independent cycle, if in it the chromosomes are made double not by pairing but by their division. The modified types of mitosis of single cycle, the endomitosis, the somatic reduction division, etc. are all characterized by the chromosome doubleness due to division. The chromosome division should thus be the fundamental phenomenon in the nuclear divisions of a single independent cycle, and can occur in the cycle of a nuclear division in which it lacks, to complete the process of the cycle, but can be obliterated from the cycle, if the chromosomes have been previously doubled by division. The meiotic reduction division is, so to speak, a type of nuclear division capable of "progressing" to the normal type of mitosis, while the somatic reduction division is the division reduced from it.

If there is a case in which the meiosis is reduced to one division by suppression of the second division, or in other words, the first division remains regularly to take place ("monokinetic meiosis", LEVAN, 1935), this case is not to show that the meiotic reduction division can be an independent division, but only that this case is comparable to the case of the restitution (untimely katachromasis) in *Fritillaria* observed by BARBER (1940), in which normally the katachromasis is characterized by its occurrence at the second telophase only. In this case of BARBER, the process of the mitosis as a component of meiosis is obliterated after the restitution, but exists before it, and therefore, the part of the process of division before the restitution is a double process as in the case of normal meiosis. In the case of monokinetic meiosis observed by LEVAN, the first division is performed, consequently the result is a dyad, and the whole division presents the form of a complete division, but is not the division of a single process.

5. Comparison of the stages in meiosis with those in mitosis.

Though we still have only meager knowledge about the morphological change occurring in the beginning of the nuclear division (*a* and *b* stages), we may perhaps say that this change probably connected mainly with the change in water-relation in the nucleus is more liable to variation in meiosis than in mitosis (cf. KUWADA, SINKE and NAKAZAWA, 1939). In most cases, however, the early prophase is characterized by the occurrence of the spiral stage (*c* stage) in both mitosis and meiosis (HIRAOKA, 1941*a*). In the next stage (the early part of the *d* stage and the leptotene) the change is similar in both mitosis and meiosis, so far as it concerns only the result of spirals being drawn out. In the next stages in meiosis, however, the amphitene being characterized by the beginning of chromosome pairing and also by "contraction" or "bouquet" (HIRAOKA, 1941*b*), the pachytene by the chromosome pairing being accomplished, the diplotene by the chiasmata formed between the paired chromosomes, and the diakinesis by the spiralization into large spirals, it becomes hardly possible directly to compare both mitosis and meiosis, but from the fact of the "diffuse stage" occurring, in meiosis in the period between the diplotene and the diakinesis, hence probably in the late diplotene, and in mitosis in the late *e*, these two stages, the late diplotene and the late *e*, seem to be comparable. Thus it becomes possible roughly to compare the early diplotene with the early *e* in mitosis and the pachytene, at which SINKE (1934) has observed new spirals, with the later part of the *d*. It seems then likely that the leptotene, the amphitene and the pachytene taken together correspond to the *d* stage in mitosis. Diakinesis which immediately precedes the metaphase may correspond with the *f* stage in mitosis, and the stages from the first metaphase to the second metaphase may be compared to the metaphase as was discussed above, and the second anaphase and telophase to the anaphase and telophase, in the ordinary mitosis respectively. We may probably say that the meiotic peculiarities of primary significance lie in these stages corresponding to the *d* stage. The result of the comparison here attempted is shown in the following diagram.

Meiosis	a	b	c	L	Am	P	D	Dk	M _I	I	M _{II}	A _{II}	T _{II}	R
Mitosis	a	b	c	d			e	f	M			A	T	R

From this comparison, it seems rational to regard the "growth period" as actually corresponding to the prophase of the mitosis, a conclusion which is essentially in accord with the view of FARMER (1905, 1912) on meiosis that the meiosis is a mitosis which does not differ materially from an ordinary mitosis, in which the reduction division (chromosome pairing and disjunction division) is intercalated (cf. KUWADA, 1940). The comparison also postulates that the "third division" which has been observed among lower plants is not part of meiosis (cf. KUWADA, 1940). A discussion on this point will be given in a later paper.

Summary

1. It is demonstrated that mitosis can be abbreviated at any stage of prophase.
2. A diffuse stage is observed in mitosis, which occurs in the mid-prophase.
3. On the basis of these facts, the origin of interkinesis is considered, and a comparison of the stages in meiosis with those in mitosis is attempted. The difference in nature between the somatic and the meiotic reduction division is also considered.

Literature Cited

- Barber, H. N. (1940), The suppression of meiosis and the origin of diplochromosomes. Proc. Roy. Soc. London, S. B, 128.
- Bělař, K. (1929), Beiträge zur Kausalanalyse der Mitose. III. Untersuchungen an den Staubfadenhaarzellen und Blattmeristemzellen von *Tradescantia virginica*. Ztschr. f. Zellf. u. mikr. Anat., 10.
- Farmer, J. B. (1912), Telosynapsis and parasynapsis. Ann. Bot., 26.
- and Moore, J. E. S. (1905), On the meiotic phase (reduction divisions) in animals and plants. Quart. Journ. Micr. Sci., 48.
- Hiraoka, T. (1941a), Studies of mitosis and meiosis in comparison III. Behaviour of chromonemata in the pre-leptotene stage in meiosis. Cytologia, 11.
- (1941b), Studies of mitosis and meiosis in comparison. IV. A contribution to the study of the origin of the "bouquet" and its formation. Cytologia, 11.
- Iwata, J. (1940), Studies on chromosome structure. I. Spiral structure of chromosomes in *Trillium Smalii* MAXIM. Japan. Journ. Bot., 10.
- Kihara, H. (1924), Cytologische und genetische Studien bei wichtigen Getreidearten mit besonderer Rücksicht auf das Verhalten des Chromosomen und die Sterilität in den Bastarden. Mem. Coll. Sci. Kyoto Imp. Univ. S. B, 1.
- Kuwada, Y. (1939), Chromosome structure. A critical review. Cytologia, 10.
- (1940), Studies of mitosis and meiosis in comparison. I. A morphological analysis of meiosis. Cytologia, 11.
- and Nakamura T. (1940), Behaviour of chromonemata in mitosis. IX. On the configurations assumed by the spiralized chromonemata. Cytologia, 10.
- and — (1941), The hydration and dehydration phenomena in mitosis. IV. The chromonemata as natural existence. Cytologia, 12.
- , Sinke, N. and Nakazawa, Z. (1939), The hydration and dehydration phenomena in mitosis. II. A consideration of the spiral stage with the results of experiments and observation. Cytologia, 9.
- Levan, A. (1935), Zytologische Studien an *Allium Schoenoprasum*. Hereditas, 22.
- Matsuura, H. (1935), A karyological investigation of *Mitrostemon Yamamotoi* MAK., with special reference to the so-called "diffuse stage" in meiosis. Journ. Fac. Sci. Hokkaido Imp. Univ. V, 3.
- Müller, W. (1936), Die Wirkung des Alkohols auf die lebende Pflanzenzelle. Protoplasma, 26.
- Rosenberg, O. (1927), Die semiheterotypische Teilung und ihre Bedeutung für die Entstehung verdoppelter Chromosomenzahlen. Hereditas, 8.
- Sigenaga, M. (1937), An experimental study of the abnormal nuclear and cell divisions in living cells. Cytologia, Fujii Jub. Vol.
- Sinke, N. (1934), Spiral structure of chromosomes in meiosis in *Sagittaria Aginashi*. Mem. Coll. Sci. Kyoto Imp. Univ. S. B, 9.
- (1941), The hydration and dehydration phenomena in mitosis. III. Apparent changes in structure of the nucleus. Cytologia, 12.
- Wada, B. (1932), Mikrurgische Untersuchungen lebender Zellen in der Teilung. I. Cytologia, 4.
- (1940), Lebendbeobachtung über die Einwirkungen des Colchicins auf die Mitose, insbesondere über die Frage der Spindelfigur. Cytologia, 11.
- Wilson, E. B. (1925), The cell in development and heredity. 3rd ed. New York.