SPERMATOGENESIS IN BRANCHIPUS VERNALIS.

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PART II.

THE PRIMARY SPERMATOCYTE.

In a previous paper, Baker and Rosof, No. 1, 1927, the general description of the testes and the behavior of the chromatin of the spermatogonia in Branchipus was reported. The salient features of the behavior of chromatin in the spermatogonia may be summarized as follows: (1) There are twenty-three chromosomes in the spermatogonium of Branchipus vernalis; (2) There are eleven pairs of homologous chromosomes and one accessory; (3) In the late prophase of spermatogonia, some homologous chromosomes become paired. This pairing is more of a chance occurrence than a regular behavior in the activity of chromosomes. The present paper is limited chiefly to the description of the observations covering the chromosomal transformations of the spermatocyte of the first order.

Following the formation of the clumped, darkly staining and dense chromosomal mass of the spermatogonial telophase, a series of transformations within the spermatocyte nucleus ensue. These changes involve both karyolymph and chromatin and result in the synapsis of homologous pairs of chromosomes. One of the factors of this behavior is the mutual attraction that synaptic mates have for each other in which they exhibit an affinity greater than that shown to the other chromosomes. This does not imply that the chromosomes which are not homologous have no attraction for each other, for an attraction between unlike chromosomes is evidenced, but this relation is of a different nature from that shown by like chromosomes. The attraction of dissimilar chromosomes for each other is indicated by the end to end association which is assumed by these chromosomes in the early prophase and which has an influence on the subsequent behavior of the chromosomes.
After the last spermatogonial division, the beginning of the changes which occur in the primary spermatocyte is indicated by the diffuse attenuated condition of the chromatin which simulates a disconnected reticulum. The delicate, ill defined attenuated strands, which extend from the irregular masses of chromatin, are intermingled and reveal no apparent definite arrangement. The reticulum that characterizes this early stage is effected by the separation of the chromosomes from the dense mass of the telophase plate of the last spermatogonial division. At this time, no apparent definiteness or order can be detected in the behavior of the chromatin, but with a more detailed study the successive changes in the progression of the formation of definite chromosomes are seen, (Figs. 1 to 16 inclusive).

The relationships of strands and masses of chromatin to each other as well as the rapidity with which homologous chromosomes synapse are dependent upon the arrangement and condition of the chromosome aggregates as the reticulum formation begins.

Figure 1 is a cell taken from a different cyst than are figures 2 to 5 inclusive. This nucleus shows thirteen masses of chromatin which are somewhat peripherally arranged. Two of these chromatin masses (d and e) are well defined while the remaining masses are irregular and diffuse. These are connected by communicating strands. The strands are not definitely oriented and the number of strands extending from the more conspicuous masses of chromatin vary.

Figures 2 to 5 inclusive are taken from the same cyst. Eighteen masses of chromatin are seen in the nucleus shown in figures 2. The arrangement of the masses is similar to the preceding figure but most of the individual masses have a more definite form. At the lower part of this nucleus is a chromatin mass (f) which shows two short heavy parallel strands directed centrally from the mass. Toward the center of the nucleus there is a large conspicuous chromatin mass which is evident in the succeeding Figures 3, 4 and 5 (chromosome a). There are fifteen well defined aggregates of chromatin and eight smaller diffuse masses which give a total of twenty-three in Figure 3. Figure 4 contains seventeen masses of chromatin which in the main do not reveal such a pronounced reticulum as in the preceding Figure 3. Fourteen chromatin masses are counted in Figure 5. These masses present a more filamentous
condition than any of the previous figures. These chromatin masses lie in close proximity to each other and are disposed for the most part to one side of the nucleus. The larger aggregates in this nucleus are similar to those of the previously mentioned figures.

Figure 6 is taken from a different cyst. This nucleus shows twelve masses of chromatin which are connected by relatively thick and dark staining strands. Some of these strands resemble thin, elongate, and ill formed chromosomes. At the lower part of the nucleus a large chromatin mass (g) is visible from which extend parallel projections directed centrally. Toward the center of the nucleus six rounded bodies of chromatin connected by strands are present.

In all the figures thus far cited there is a difference in the number, size, form, and relationship of the chromatin aggregates and strands. Some of these chromatin bodies found in different nuclei show marked similarities. See chromosome (a) in Figure 2 to 5 inclusive. Referring to Figures 1 to 6, it is apparent that some of the chromosomes are similar to the telophase chromosomes of the spermatogonia previously described—in that they are well defined, round bodies which are practically free from connecting strands, (Fig. 1, d and e). The other chromatin bodies present marked degrees of variation. Some have characteristic shapes and are well defined while others are diffuse. Most of the larger masses do not present the filamentous condition which is a common characteristic of the smaller and less defined chromatin aggregates. The strands vary in length and in thickness. Some are thin and attenuated while others are better defined and extend from the chromatin bodies in a more regular manner. The chromatin masses vary in respect to their position in the strand. Some of the chromatin bodies appear as thickenings in the middle portion of the strands while others occupy distal parts.

After the irregular and contorted chromosomal configuration of the stages previously described (Figs. 1 to 6) which correspond to the preleptotene stage, a series of transformations are seen. The changes at this time result in the formation of definite chromosomal filaments which are orientated in a definite manner, (Figs. 7 to 16, inclusive). This series of changes is characterized chiefly by the transformation of chromosome bodies which are not already synapsed into definite bivalent chromosomes by parasynaptic approximation. More-
and some distance therefrom, while its central portions are free. This partially synapsed, loop-shaped chromosome, is joined with the bivalent loop to its right and with the univalent loop to its left by intervening bodies of chromatin.

The characteristic arrangement of the bivalent chromosome loops which have joined at their extremities is seen in Figures 13, 14 and 15. These chromosomes retain their individuality although they are joined end to end. This condition is well revealed in Figure 13, which shows only a few of the total number of chromosomes. The chromosomes of Figures 14 and 15 are for the most part synapsed, and in addition, the bivalent chromosomes are joined end to end. The condition of the chromatin at this time indicates that it is not completely condensed.

It is evident that in the behavior of the chromatin already described, the following processes are involved: (1) The synapsis of homologous chromosomes; (2) The orientation of the chromosomes into an end to end association; (3) The equalization in thickness and change in form of chromosomes into definite, uniform, and elongate chromosomes.

It is inferred that the above processes are influenced by the following factors: (1) The mutual attraction that synaptic mates have for each other; (2) The attraction that unlike chromosomes have for each other which results in the end to end association of unlike chromosomes; (3) The intrinsic chromatin changes which result in the formation of definite, uniform and elongate chromosomes; (4) The differences in relationship of the chromosomes as they emerge from the last spermatogonial telophase.

The first three of the above listed factors tend towards an uniformity in behavior of the chromatin while the fourth factor interferes with the simultaneous manifestation of the previously mentioned processes. By reference to Figures 7 and 8, paper No. 1, Baker and Rosof, 1927, it is seen that in the late spermatogonial prophase there are some homologous chromosomes which are paired while others reveal no close relationship whatsoever. This relationship of chromosomes influences the behavior of chromosomes as they emerge from the last spermatogonial telophase into the prophase of the primary spermatocyte in that they remain in this paired condition during the spermatogonial division and in the early prophase of the primary spermatocyte. This is indicated by the number,
size, form, and relationship of the chromatin aggregates and strands in the different nuclei.

It is inferred that the interval and mode of synapse is dependent upon the above listed processes. The chromosomes in this species do not all synapse in the same condition, nor do they synapse at the same time. Some of the homologous chromosomes become paired in the late spermatogonial prophase, as previously stated, while others give no evidence of synapse until the beginning of bouquet formation. Some of the homologous chromosomes come into close relationship with each other, and synapse during the time when the chromatin is filamentous, while other homologous chromosomes become synapsed during the clumped condition of the chromatin. If the chromosomes synapse while in the form of filaments, then the mode of synapse is unquestionably parasynaptic, Figure 9. There is some variation in respect to the parts of the attenuated homologous chromosomes that first become approximated during synapse. Some become approximated first, at their extremities, while others at varying places along the chromosome. On the other hand, if synapse occurs during the clumped condition of chromatin, it is impossible to determine with certainty whether or not the mode of conjugation is parasynaptic or telosynaptic.

Following the last stage described (Fig. 15), which concludes the leptotene stage, the most pronounced orientation of the chromosome loops occurs. This orientation may or may not result in the formation of a bouquet. In addition to this, the chromosomes which are not already bivalent and equalized in thickness throughout become so by the time this orientation is completed. Cells in this stage reveal the ends of most of the chromosome loops oriented toward the periphery of the nucleus, some being more peripherally oriented than others. This arrangement is accomplished by the shifting of the chromosome loops which are joined end to end.

There are eight well defined bivalent loops which are associated end to end and are peripherally arranged in Figure 16. At the left side of this nucleus in close proximity to the nuclear membrane, there are seen two elongate homologous components of a bivalent chromosome (c). These homologous elements are synapsed only at their upper extremity; the remaining parts are not synapsed but show a parallel arrangement. The lower extremity of the inner most univalent
chromosome of these homologous elements already occupies an end to end relationship with a bivalent chromosome. The upper extremity of these homologous chromosomes as previously stated is synapsed, but in addition, this bivalent extremity also occupies an end to end relationship with two bivalent chromosomes. In the center of the nucleus, at a lower level than the majority of the chromosomes, a rather large and uneven chromosome is seen. This chromosome is thickened in its central portion. At the same level and to the left of this chromosome, there is a long and well defined chromosome (M) which has the form of an incomplete figure eight. Its separated extremities assume an end to end association with other chromosomes. At the upper right portion of the nucleus, two distinct chromatin bodies are seen. These bodies are nodules on the bivalent filaments which extend from them.

This figure is significant in that it practically concludes the processes which have been previously operating and marks a change in the behavior of the chromatin. Previous to this time, the processes involved are: (1) Synapses of homologous chromosomes; (2) The end to end association of dissimilar chromosomes; (3) The equalization in thickness and change in form which result in definite, uniform, bivalent, and elongate chromosomes.

Some chromosomes are seen in this figure which are not as yet completely synapsed, and some that are not equalized in thickness throughout. However, these are in the minority. This Figure as well as Figure 12 also accentuates the fact that the chromosomes do not all synapse in the same condition or at the same time. Furthermore, the general arrangement of the chromosomes in Figure 16 is indicative of the ensuing chromosome behavior.

The figures succeeding the one just described show the character of ensuing chromosomal behavior. At this time the changes which result in the formation of pachytene chromosomes may vary as follows: (1) Chromosomes may undergo complete or partial bouquet formation, and then form pachytene chromosomes or, (2) the pachytene chromosomes may be formed without undergoing a bouquet stage. Either of these two methods may be interrupted by the intervention of the so-called synezeisis stage. This difference in the behavior of chromosomes at this time as just mentioned has lead to considerable confusion in the interpretation of the chromosomal changes in the primary spermatocyte.
Figures 17 to 20 show imperfect bouquets. Ten loops having their ends directed toward a common area at the periphery of the nucleus are seen in Figure 17. Above these loops, two univalent loops whose ends are joined parasynaptically are visible. A comparison of the caliber of these partially synapsed univalent chromosomes with the bivalent loops in this nucleus shows a marked difference, the bivalent loops being much thicker. Furthermore, the general contour and disposition of the univalent loops differ from the bivalent loops in that the unsynapsed chromosomes indicate a serially arranged granular appearance which is not seen in the bivalent chromosomes.

Figure 18 is comparable to Figure 17. The chromosomes (a) of Figure 18 are two bivalent chromosomes which are joined end to end and simulate the partially synapsed univalent loops of Figure 17. However, these two similar configurations of chromosomes are easily discernible on account of their differences in thickness and contour. These two figures mark the last appearance of unsynapsed chromosomes.

A different phase of bouquet formation is seen in Figures 19 and 20. Here all the chromosomes are bivalent but they are not all oriented in the same manner. Both figures show twelve chromosomes, most of which are looped. The chromosomes which are not looped have their enlarged ends either directed centrally or come into contact with some part of a loop of another chromosome. Figure 20 shows clumped and twisted chromosomes disposed toward one side of the nucleus. This may be interpreted as a fixation effect.

The complete formation of a bouquet is seen in Figures 21 and 22. In this stage the chromosomes have their ends oriented toward a restricted area at the periphery of the nucleus. Eleven loops are visible in Figure 21. The full complement of chromosomes is not seen in Figure 22 due to the plane of section.

Figures 23 to 32 show the gradual contraction of chromosomes which results in the formation of pachytene chromosomes. During this process each bivalent chromosome exhibits an individuality of its own, and the changes which each chromosome undergoes can be followed with comparative ease if syneization does not intervene.

The arrangement of chromosomes in Figures 23 to 27 varies considerably. All of these figures reveal chromosomes which are no longer joined to each other, however, the disposition of the chromosomes varies from that of an intermingled grouping
to that of a widely separated condition. The arrangement of the chromosomes in Figure 23 is highly suggestive of a continuity of the chromosomes, but close observation reveals an intermingled grouping of ten distinct chromosomes. Figure 24 shows eleven chromosomes that reveal their free ends oriented toward the periphery of the nucleus. These chromosomes are thicker than those of the bouquet. It may be inferred that this figure is a good illustration of chromosomes which have become disengaged from the bouquet orientation. Figures 25 and 26 show a slight massing of a few chromosomes. A slightly later stage is seen in Figures 27 and 28. These chromosomes are thicker than the previously described chromosomes and resemble more closely the pachytene stage. Twelve well defined chromosomes are present in Figure 28. At the right side of this figure a U-shaped chromosome is seen whose ends are attached to the extremities of a small V-shaped chromosome lying in a different plane. The relationship of these two chromosomes is the last occurrence observed of the process which involves the end to end association of dissimilar chromosomes which was previously described.

The chromosomes of Figures 29 to 32 inclusive are in the pachytene stage. The morphological individuality of each chromosome is marked in these figures. Each chromosome has assumed a characteristic form. This may be seen by reference to Figure 31 in which there are eleven chromosomes. Furthermore, the chromosomes are no longer clumped but are distributed throughout the cell.

Following the pachytene stage the chromosomes become elongated and thickened. The beginning of this process is shown in Figure 33. This condition is well shown in Figure 34. The chromosomes in this nucleus are partially coalesced and are less distinct. Figure 35 reveals the condition succeeding that shown in Figure 34. The chromosomes in the nucleus of Figure 35 are enlarged and diffuse and at the same time, individual chromosomes are again undergoing a process of shortening. The shortened chromosomes which result are still in a diffuse condition as is seen in Figure 36. This second contraction of chromosomes may be confused with synezesis unless the continuity of this process is followed through in close stages. It is difficult to discern the exact morphological characteristics of individual chromosomes at this time. This may be attributed to the difficulty in obtaining good fixation in this stage. These
chromosomes are resolved into the chromosomes of the diakinesis stage by a rearrangement and condensation of the chromatin.

The chromosomes of the diakinesis stage are characterized by their peculiar and constant shapes. Figures 37 and 38 show a gradual transition from the more diffuse condition of Figures 35 and 26 which results from the second contraction to the distinct and characteristic chromosomes of the diakinesis stage shown in Figures 39 to 41.

A typical nucleus in diakinesis is seen in Figure 40. Twelve chromosomes are present in this figure, seven of which are more peripherally arranged than the others. In the upper right portion of this nucleus four small chromosomes are visible. Below these, a large dumbbell shaped chromosome is seen. The remaining chromosomes are median sized. The great differences in size and form of the chromosomes facilitate their identification prior to, and succeeding diakinesis. Certain chromosomes in this stage are suggestive of tetrads, but close observations fail to reveal typical tetrads. Following diakinesis, the chromosomes continue to contract and change in shape until they become more or less rounded—Figures 42 to 46. At this time, the chromosomes are going on the spindle in preparation for the ensuing meiotic division. By reference to Figures 44 to 46, it is seen that the chromosomes become joined to each other by short, darkly staining chromatin strands. These cells give a count of twelve chromosomes. A polar view of the chromosomes on the spindle is seen in Figure 47. The resulting changes which now rapidly ensue are seen in Figures 48 to 54. These include the metaphase, anaphase, and telophase stages.

From an inspection of the above figures illustrating the stages it is seen that the behavior of the X chromosome is variable. In Figure 49 it is undivided and included with the main mass of chromosomes. In Figure 50, the X chromosome is undivided and proceeds towards the pole more rapidly than do the other chromosomes. The X chromosome has divided and occupies the poles of the spindle in Figure 51. In Figure 54, the X chromosome is undivided and lags behind the divided bodies of chromatin.

The dense, darkly staining and compact mass of chromatin which characterizes the synezesis stage has been omitted in the previous description and is seen in Figures 55, 56, and 57. There is a great variation in the type of synezesis exhibited by the various nuclei. Some of the nuclei in this condition show
chromatin loops or strands extending from the main mass, while other nuclei reveal the dark mass without any projecting elements. The loops and strands which project from the main chromatin mass vary considerably. Some are attenuated and irregular while others are thick. As has been previously mentioned the chromosomes may or may not undergo syncezesis. It is our observation that chromosomes may undergo synezesis at any time following the end to end association of bivalent chromosomes, Figure 16 up to the pachytene stage, Figure 29. Without syncezesis the behavior of chromosomes can be followed in an orderly fashion, but by the intervention of syncezesis, it is difficult to follow with certainty the orderly sequence in the behavior of the chromosomes. From the study of this material it is inferred that syncezesis indicates some change in the condition of the chromosomes, which renders the chromosomes susceptible to the action of the fixatives.

The transformations of the chromosomes following the end to end association which is seen in Figure 16 are summarized as follows: (1) Chromosomes may or may not undergo bouquet formation prior to the pachytene stage; (2) These two methods may be interrupted by the intervention of syncezesis; (3) The pachytene stage follows and is characterized by the shortening of chromosomes which exhibits characteristic forms. These chromosomes are scattered throughout the nucleus; (4) Following the pachytene stage, the chromosomes elongate and thicken; (5) This stage is succeeded by a thicker, shorter and more diffuse type of chromosome; (6) Following this, the chromosomes undergo a second contraction and retain for a time their diffuse condition; (7) This is succeeded by the diakinesis stage. The chromosomes of this stage are no longer diffuse but again assume a characteristic shape; (8) The ensuing phase of development reveals the chromosomes in a contracted or rounded form. At this time, some of the chromosomes are joined to each other while going on the spindle preparatory to division; (9) Meiotic division now ensues.

The chromosomal transformations in Branchipus vernalis as described in this paper compare favorably with some exceptions to the table based on Winewarter’s terminology as given in Figure 262 in “The Cell in Development and Heredity,” by Wilson 1925, (third edition). A general consideration of maturation in this species will be reserved for the last of this series of papers.
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EXPLANATION OF PLATES.

PLATE I.

All Figures were drawn by aid of the camera lucida. Magnification of plate figures 1250 diameters. Comparisons are made as far as it is possible with the diagram, based on Winewarter’s terminology in Wilson, “The Cell in Development and Heredity,” Figure 262, (third edition). Figures 1 to 6 inclusive represent the preleptotene stage.

Fig. 1. This nucleus shows 13 masses of chromatin which are for the most part connected by communicating strands. The arrangement of the strands is indefinite and the number of strands extending from the more conspicuous masses varies. The reticulum, characteristic of this early stage is effected by the separation of the chromosomes from the dense mass of the telophase plate of the last spermatogonial division. This figure corresponds favorably to the resting stage (Wilson).

Figs. 2 to 5. These cells are from the same cyst. The number of chromatin masses is not constant in the various nuclei, but in no case do the masses exceed 23. The chromosome (a) is similar in form in these nuclei. The arrangement of the masses is similar to Fig. 1, but most of the masses have a definite and characteristic form. These figures correspond practically to the prochromosome stage.

Fig. 6. This nucleus shows 12 chromatin masses. Some of the chromatin strands resemble thin, elongate and ill defined chromosomes. The apparent reticulum is less pronounced. The figure is comparable to the unraveling stage.

Figs. 7 to 16. These nuclei reveal the most pronounced manifestation of the following processes: (a) Synapses of homologous chromosomes; (b) The orientation of dissimilar chromosomes into an end to end association; (c) The equalization in thickness and change in form of chromosomes into definite uniform and elongate chromosomes. These figures correspond to the leptotene stage.

Figs. 7 and 8, show only a few of the total masses of chromatin. The thick and even chromatin filaments which are joined to each other are bivalent chromosomes. The other masses and strands are not so conspicuous.

Fig. 9. The chromatin in this nucleus shows a uniform behavior. In the lower portion of the nucleus, the univalent chromosomes are pairing side by side. In the upper left portion, there is a well defined bivalent chromosome (h).

Fig. 10. At the left, in this nucleus, one bivalent chromosome (k) is seen which has formed a loop. Beneath this loop there are two homologous chromosomes which are attached at their extremities and form a somewhat oval figure.

Fig. 11. This nucleus shows two large, diffuse and irregular masses from which protrude several filaments. Two of these filaments are well defined. This nucleus shows evidence of poor fixation.

Fig. 12 shows 13 bodies of chromatin. Chromosome B of this figure is one which is synapsed at its extremities and some distance therefrom, while its central portions are free. This partially synapsed, loop-shaped chromosome is joined to a bivalent loop to the right and to a univalent loop to the left by intervening bodies of chromatin.

Figs. 13, 14, 15. The characteristic arrangement of bivalent chromosomes which have conjugated at their extremities is seen in these figures. The chromosomes retain their individuality although they are joined end to end. The condition of the chromatin in this stage indicates a diffuse state.

Fig. 16. This nucleus shows eight well defined bivalent loops which are associated end to end and are peripherally arranged. Chromosomes C shows two elongate and homologous components of a bivalent chromosome. These homologous elements are synapsed only at their upper extremity. The remaining parts are not synapsed but show a parallel arrangement. This figure is significant in that it marks a change in the behavior of chromatin, and may be interpreted as a transition stage between leptotene and post leptotene stages.
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PLATE I

1. 2. 3. 4.
5. 6. 7. 8.
9. 10. 11. 12.
13. 14. 15. 16.
PLATE II.

Figs. 17 to 28 inclusive show the character of the chromosome behavior prior to the pachytene stage without the intervention of syn緣esis. Fig. 17. Above the loops, two univalent chromosomes are seen, the ends of which are joined parasynaptically.

Fig. 18. Chromosome A of this figure represents two bivalent chromosomes which are joined end to end and simulate the partially synapsed univalent loops of Fig. 17. It is inferred that Figures 17 and 18 may be stages prior to complete bouquet formation or that they may form pachytene chromosomes without undergoing bouquet formation.

Fig. 19 and 20. These nuclei show a different type of bouquet formation. Here, the chromosomes are bivalent but they are not all arranged in the same manner. Both figures show 12 chromosomes most of which are looped. The chromosomes which are not looped have their enlarged ends either directed centrally or come into contact with some part of a loop of another chromosome. The following interpretations may be placed on these two figures. (a) They may be stages just prior to complete bouquet formation. (b) They may immediately succeed bouquet formation. (c) They may form pachytene chromosomes without forming complete bouquets. (d) These stages may mark the intervention of synèmeisis.

Figs. 21 and 22 show complete and typical bouquets. Eleven bivalent loops are visible in Fig. 21, and only 8 loops visible in Fig. 22. In no case are there more than 12 loops.

Figs. 23 to 29. These nuclei show chromosomes following the conditions mentioned above and just prior to the pachytene stage.

Fig. 23. The arrangement of chromosomes is highly suggestive of their continuity. Ten separate chromosomes are intermingled which suggests this continuous condition.

Fig. 24. This nucleus shows 11 chromosomes that reveal their free ends oriented toward the periphery of the nucleus. It may be inferred that this figure is a good illustration of chromosomes that have become disengaged from the bouquet orientation.

Figs. 25 and 26. These nuclei show a slight massing of a few chromosomes.

Figs. 27 and 28. These nuclei are in a later stage than any of the previous figures. These chromosomes are thicker than the previously described chromosomes and resemble more closely the pachytene stage.

Fig. 28 shows 12 well defined chromosomes. At the right side of this figure a U-shaped chromosome is seen whose ends are attached to the extremities of a small V-shaped chromosome.

Fig. 29 to 32 inclusive show nuclei in the pachytene stage. The total number of chromosomes is not present in these nuclei due to the plane of the section. However, a count of chromosomes show that in no case does the chromosome number exceed twelve. The morphological individuality of the chromosomes in these nuclei is marked. Each chromosome has a characteristic form. The chromosomes are no longer clumped but are distributed throughout the cell.
PLATE III.

Fig. 33. This nucleus contains 12 chromosomes, some of which are elongating and thickening.

Fig. 34. The chromosomes in this nucleus are elongated and thickened. Some of the chromosomes are partially coalesced and are less distinct.

Fig. 35. The chromosomes in this stage are thickened, enlarged and diffuse, but at the same time the individual chromosomes are again undergoing a process of shortening. This corresponds to the diffuse stage.

Fig. 36. The chromosomes in this nucleus are shortened and are still in a diffuse condition. This second contraction of chromosomes may be confused with synexesis unless close stages are followed. This corresponds to the second contraction stage.

Figs. 37 and 38. These two nuclei show a gradual transition from the more diffuse condition resulting from the second contraction to the distinct and characteristic chromosomes of the diakinesis stage.

Figs. 39 to 41. These nuclei show characteristic chromosomes of the diakinesis stage.

Figs. 42 to 46 show the condition of chromosomes following diakinesis. The chromosomes at this time continue to contract and change in shape until they become more or less rounded. In Figures 42 and 43 the nuclear membrane is present. The nuclear membrane has disappeared in Figures 44, 45, 46. The chromosomes of these nuclei become joined to each other by short and darkly staining chromatin strands. These cells give a count of 12 chromosomes. In some of these figures, the centrosomes are visible.

Fig. 47. This figure is a polar view of the chromosomes on the spindle. In this figure as well as in the succeeding figures the accessory chromosome is indicated by the letter X.

Fig. 48. A profile view of a spindle.

Fig. 49. Metaphase.
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PLATE III

33. 34. 35. 36.
37. 38. 39. 40.
41. 42. 43. 44.
45. 46. 47. 48. 49.
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Plate IV

Fig. 50. A metaphase showing the undivided accessory chromosome proceeding in advance of the main mass of chromatin.

Fig. 51. The divided X chromosome occupies the poles of the spindle.

Fig. 52. Early anaphase. The X chromosome is undivided and is proceeding toward the pole with the main mass of chromatin.

Fig. 53. Late anaphase.

Fig. 54. Telophase showing the X chromosome undivided and lagging behind the divided bodies of chromatin.

Figs. 55, 56, and 57. These nuclei are in synezesis. These figures were placed at the end of the series because without synezesis the behavior of chromatin can be followed in an orderly fashion, but with the intervention of synezesis it is difficult to follow with certainty the orderly sequence. The chromosomes may undergo synezesis at any time following the end to end association of bivalent chromosomes up to the pachytene stage. The chromatin in these figures is disposed to one side of the nucleus and is arranged in a dense, darkly staining and compact mass.

Fig. 55. This nucleus shows two filaments which extend from the synezesis mass.

Fig. 56. Several irregular strands extend from the synezesis mass in this nucleus. These synezesis masses resemble closely contracted bouquets.

Fig. 57. Three distinct loops and two pronounced and elongate chromosomes are seen extending from the synezesis mass.

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