SPERMATOGENESIS IN BRANCHIPUS VERNALIS.

PART III.

SECONDARY SPERMATOCYTE, SPERMATID AND SPERMATOZOÖN.

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In two previous papers, Baker and Rosof, Part I, 1927 and Part II, 1928, the general description of the testis and the behavior of the chromatin of the spermatogonia and primary spermatocyte in Branchipus vernalis was described. The outstanding features of the behavior of the chromatin in the spermatogonia are as follows: (1) There are twenty-three chromosomes in the spermatogonia of Branchipus vernalis: (2) There are eleven pairs of homologous chromosomes and one accessory; (3) In the late prophase stage of the spermatogonia, some homologous chromosomes are paired. This pairing was interpreted as being more of a chance occurrence than a regular behavior in the activity of the chromosomes.

The following summary briefly states the changes occurring in the chromatin of the primary spermatocyte:

(1.) The early primary spermatocyte is characterized by a reticulum in which the arrangements of the chromatin is in the form of masses and strands. There is a difference in the number, size, form and relationship of these chromatin aggregates and strands. Some of the chromatin bodies found in different nuclei show marked similarities. The reticulum characteristic of this early stage is affected by the separation of chromosomes from the dense mass of the telophase plate of the last spermatogonial division. This is the early prochromosomal stage;

(2.) The reticulum becomes less pronounced and the chromatin bodies reveal characteristic shapes. The number of chromatin masses is not constant in the various nuclei but in no case does the number of masses exceed twenty-three. This is the late prochromosomal stage;

(3.) The recticulum is less pronounced. In the previous stage, the chromatin aggregates were the more conspicuous elements in the nucleus, whereas, in this stage the strands are

more pronounced. Some of the strands resemble the elongate and ill-defined chromosomes. This characterizes the unraveling stage. These three stages compose the preleptotene stage;

(4.) The chromosomes now undergo a series of changes involving the following processes: (a) Synapsis of homologous chromosomes; (b) Orientation of unlike chromosomes into an end to end association; (c) Equalization in thickness and change in form of chromosomes into definite, uniform and elongate chromosomes. The simultaneous manifestation of these three processes is interfered with by the differences in the relationship of the chromosomes as they emerge from the last spermatogonial telophase. This constitutes the leptotene stage;

(5.) The chromosomes for the most part are well defined bivalent loops which are associated end to end, and are peripherally arranged in respect to the nucleus. This marks the close of the processes which are operating in the pre-leptotene and leptotene stages and marks the initiation of the processes which are involved in the post-leptotene stage. This is the transitional stage.

The post-leptotene stages following the transitional are summarized as follows:

(6.) The chromosomes may or may not form bouquets prior to the following pachytene stage. These two methods of chromosomal transformations may be interrupted by the intervention of synizesis;

(7.) The chromosomes shorten and assume characteristic forms. These chromosomes are scattered throughout the nucleus. This is the pachytene stage;

(8.) The chromosomes elongate and thicken and simulate an ill defined bouquet. Some of the chromosomes are partially coalesced and are less distinct. This constitutes the second orientation;

(9.) Chromosomes become more diffuse and separate, but at the same time the individual chromosomes are again undergoing a process of shortening. This corresponds to the diffuse stage;

(10.) Chromosomes are shortened and contracted and are so intermingled that they somewhat simulate synizesis. This is the second contraction;

(11.) Chromosomes now are no longer diffuse but are separated and again assume characteristic shapes. This is the diakinesis stage;

(12.)   The chromosomes become still further contracted and rounded.   At this time some of the chromosomes are joined to each other.   The nuclear membrane disappears.   This constitutes the late prophase stage in contrast with all previous stages which are parts of the early prophase.

(13.)   Metaphase follows and meiotic division ensues.

In regard to the question of synapse it is inferred that the interval and mode of synapse is dependent upon the arrangement of chromosomes as they emerge from the last spermatongonial division and to the sequence of the processes as indicated in the leptotene stage.   The chromosomes of this species do not synapse in the same condition nor do they synapse at the same time.   If the chromosomes synapse while in the form of filaments, then the mode of synapse is unquestionably parasynaptic.   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.

The present paper is limited chiefly to the description of the observations covering the behavior of chromosomes of the secondary spermatocyte, spermatid and spermatozoön.

THE SECONDARY SPERMATOCYTE.

The secondary spermatocytes are arranged in the testis in cysts as were the spermatocytes of the first order.   In transverse section of the testis the cysts are well defined and are distinctly localized toward or near the immediate vicinity of the lumen of the gland.   The cells composing these cysts are comparable to the cells of the first order only in their shape which is round or oval unless encroached upon by surrounding cells and under such conditions a variety of cell contours may result.   The secondary spermatocytes are somewhat smaller than the primary spermatocytes.

Following the primary spermatocyte division, the nucleus of the secondary spermatocyte reveals the chromatin disposed in a darkly staining and clumped mass in which the individuality of the chromosomes are more or less obscured.   This condition is shown in Figure 1.

The ensuing changes that the chromosomes of the secondary spermatocyte undergo are unlike the changes which occur in the early prophase of the primary spermatocyte.   The behavior

of the chromosomes of the secondary spermatocyte is uncomplicated in that no factors are involved excepting those which operate in connection with mitotic divisions.

Figures 2 and 3 show the chromosomes less intimately related. The individual chromosomes can now be distinguished by their differences mainly in size, and to a lesser extent by differences in their morphological aspects. Some of the chromosomes show connecting strands which are similar to those which connect metaphase chromosomes of the primary spermatocyte. This feature is more pronounced in Figure 2 than in Figure 3. These two figures contain eleven chromosomes each.

Further changes result in the elongation and change in form of the chromosomes so that each chromosome is morphologically dissimilar to the others, and at the same time the connecting strands are less pronounced and for the most part disappear. The chromosomes now are more or less uniformly distributed throughout the nucleus and are somewhat peripherally arranged in relation to the nuclear membrane, (Figure 4.). The full count of chromosomes is not present in Figure 4 since only ten are visible. The chromosomes in Figure 5 are in a later stage and the chromosomes are more elongate. In the lower portion of the nucleus, two strands connecting chromosomes are seen. Some of these nuclei have their central portions somewhat constricted. This cell gives a count of twelve chromosomes.

The next change reveals the chromosomes undergoing contraction. The individuality of the chromosomes at this stage is most pronounced and they exhibit in the main well defined and characteristic contours. It may be emphasized that the chromosomes of this stage are comparable to the spermatogonial chromosomes of a similar stage. Strands connecting chromosomes are no longer visible, (Figures 6, 7 and 8). Figures 6 and 7 show eleven chromosomes while Figure 8 has twelve.

Figures 9, 10 and 11 are polar views of metaphase. These chromosomes are in the form of rounded bodies which may be distinguished from each other by differences in size. Figure 9 shows a central mass of chromosomes which are closely approximated from which extend spindle fibers. There are five chromosomes in the immediate vicinity of this central mass which are apparently lagging behind. No nuclear membrane is present in this or in the succeeding figures of the secondary spermatocyte.

Twelve chromosomes are seen in Figure 10. The arrangement of the chromosomes as seen in this figure is quite character-

istic of secondary spermatocytes of this stage.   Figure 11 shows a mass of chromosomes with one chromosome free.   It is inferred that this is the accessory chromosome.

Figures 12 to 14 are profile views of early metaphase. The chromosomes of Figure 12 show a slight irregularity in their behavior in that three chromosomes are proceeding to their respective poles in advance of the remaining chromosomes.

Figure 13 shows a dense metaphase plate which is so orientated that it reveals an oblique view.   The accessory chromosome is present and is proceeding precociously and undivided to one pole.   Figure 14 presents a similar view, however, the accessory chromosome is divided and occupies the poles of the spindle.

Figure 15 is an anaphase while Figure 16 and 17 are telophases.   The latter figure is of interest in that it shows the beginning of a nuclear membrane.   The contained chromosomes do not show the massed condition which is more in evidence in the previous stages.

THE SPERMATID AND SPERMATOZOÖN.

The spermatids are located around or near the lumen of the testis in a cyst-like arrangement being present throughout the entire extent of the testis but far more numerous in the anterior extremity of the gland.   The arrangement in the cysts is not as typical as was the condition in the preceding stages and the contour of the cyst is not so well defined and is frequently absent.   Spermatids may be found also in the lumen of the testis intermingled with the spermatozoa.

In shape, the spermatids vary from a round or oval to an oblong form and are distinctly smaller in size than the spermatocytes of the second order.

Figure 17 shows a late telophase stage of the secondary spermatocyte and reveals the characteristic massing of the chromosomes.   In some cells this packed condition partially obscures the individual chromosomes, but usually the chromosomes can be distinguished.   Eleven chromosomes are seen in Figure 18.   These chromosomes are rounded and somewhat massed.   Strands connecting chromosomes are visible.   There is little difference in shape of individual chromosomes but a difference in size is more pronounced and with careful scrutiny the individuality of the chromosomes can be detected.   The

area occupied by the chromosomes is marked off from the cytoplasm by a nuclear membrane. On the left, in the immediate proximity of the nuclear membrane are two well defined centrosomes. This condition of divided centrosomes is common in spermatids and spermatozoa. The cytoplasm in this figure as well as in the succeeding figures is not shown. This is the typical condition of the nucleus of a spermatid following the secondary spermatocyte division.

Following the rounded condition of the chromosomes, they assume a more elongate form with characteristic shapes. The strands connecting the chromosomes are more evident, some being more conspicuous than others. Figure 19 shows the chromosomes for the most part in a rounded condition. In the more central portion of this nucleus, an elongate chromosome is seen from which several strands extend to neighboring chromosomes. There are twelve chromosomes in this nucleus.

The chromosomes in Figure 20 show a more pronounced change in form than does the previous figure. A few connecting strands are present and some of the chromosomes are enlarged. Some of these chromosomes appear to be joining with other chromosomes. Although the full number of chromosomes is present in this nucleus a count of only ten chromosomes is obtained.

The condition of the chromosomes in figures previously described indicates the initiation of the changes which results in the final configuration of the spermatozoa. These changes involve the rearrangement and coalescence of chromosomes and results in an apparent diminution of the number of chromosomes in the spermatozoa. Figures 21 to 29 inclusive show the configuration which the chromosomes undergo in the early stages of this coalescence of unlike chromosomes.

It is difficult to obtain an accurate count of chromosomes in Figure 21 on account of the coalescence of some of the chromosomes. However, nine distinct bodies are visible. For the most part, the chromosomes reveal slight differences in shape which is indicative of the fact that the chromosomes have a tendency to change their form, but this change is not so pronounced in most cases as to obscure the recognition of the individual chromosomes. In some cases even though the chromosomes are partially coalesced certain features still remain which assure the identification of the chromosomes

entering into the coalescence.   This figure also shows two well defined centrosomes.

Figure 22 shows eight bodies of chromatin six of which are well defined.   The two chromosomes in the central part of the nucleus are partially coalesced.   Strands connecting chromosomes are evident.   Some of the chromatin bodies of this nucleus resemble the chromosomes of previous stages in the spermatogonia, primary and secondary spermatocytes, as well as, chromatin bodies in other spermatids.   Eight well defined chromatin bodies, some of which are joined by connecting strands are seen in Figure 23.   One centrosome is shown in this figure.   Six chromatin bodies, five of which are well rounded and peripherally arranged are seen in Figure 24. The more central, larger, elongate and irregularly shaped chromatin body which is connected to three of the peripherally arranged chromatin bodies by single thin strands is the result of the coalescence of several chromosomes.   This cell shows one large centrosome.

Figure 25 shows several chromosomes coalescing at the same time, there being only two chromatin bodies which are free. This condition is slightly different from the nucleus just described in that the chromosomes are so orientated as to permit a more rapid union than is indicated in the previous figures. It will be noted that the connecting strands are heavy and that the chromosomes joined by these strands are in close proximity to each other.   One centrosome is present.

Figure 26 shows seven chromatin bodies whose appearance is similar to Figures 22 to 24.   Two centrosomes are seen in Figure 26.   Figure 27 contains seven bodies of chromatin. This figure is different from the preceding ones in that the strands are more numerous.

Figure 28 shows in a pronounced manner the coalescence of chromosomes.   One chromatin body is free.   Three well defined and rounded chromatin bodies are seen in Figure 29, as well as two large irregularly shaped masses of chromatin.   The nuclear membrane is indistinct.

It is evident in the previously described Figures 18 to 29 inclusive that the following processes are involved in the behavior of chromatin: (1) Change in form; (2) Rearrangement; (3) Coalescence.   These three processes operate simultaneously, and continue until the final configuration of the chromatin in the spermatozoa is reached.   The changes in the morphology

of individual chromosomes do not affect all chromosomes equally and as a result the same chromosomes may be subject to a variation of contour. Nevertheless, with careful ob ervation individual chromosomes can be recognized regardless of the variation that they undergo in the various nuclei even though they have changed in contour they retain sufficient characteristics of size and shape to be distinguished.

The rearrangement of chromosomes is indicated by change in relation that individual chromosomes assume to each other and this relation becomes more conspicuous in the ensuing figures. Furthermore, the picture which results from the rearrangement of the chromosomes of any stage indicates that there are several distinct methods involved in the rearrangement of chromosomes prior to the final configuration in the spermatozoön bodies. The same chromosomes may form a different pattern in different nuclei in the same stage depending upon the various relationships assumed by these chromosomes.

The coalescence of chromosomes is revealed by the apparent reduction in the number of chromosomes without an apparent diminution of chromatin content as the spermatids differentiate. During this process of change in the spermatid there is shown a fusion of chromosomes. The rapidity with which chromosomes fuse is for the most part dependent upon the conditions of the chromosomes, and, as well the orientation of the chromosomes. In the early part of this process when only a few of the chromosomes are coalesced, the chromosomes which enter into this fusion retain sufficient characteristics to make them recognizable and furthermore with careful observation the exact method of fusion of chromosomes can be determined.

Figure 30 shows seven masses of chromatin six of which are partially coalesced. Their arrangement is in the form of an incomplete circle and the individual contours of each mass is discernible. The nuclear membrane is absent.

There are six rounded and well defined chromatin bodies in Figure 31. Their disposition is similar to that shown in the preceding figure but they do not show the degree of coalescence that is indicated in the previous figure. Strands are visible which connect the chromosomes, some of which are heavier than others.

Figures 32 and 33 are similar in that each figure presents five chromatin bodies which are connected to each other by varying sized strands. The arrangement of these chromatin

bodies simulates a quadrangular form, the angles of which are occupied by a chromatin body. The other chromatin body partially obscures this configuration. Figure 33 shows the entire spermatid.

Figures 34 to 40 inclusive show the same number and similar orientation of chromatin bodies. The arrangement of the chromatin in these cells simulates an irregular quadrangle with a chromatin body at each angle. These bodies are connected by communicating strands which form the sides of the figure. The patterns presented by these chromatin bodies differ from each other in the following respects: (1) difference in size and form of the chromatin bodies; (2) difference in the length and degree in attenuation of the connecting strands. An additional strand is present in Figure 34 which connects two chromatin bodies. No accessory chromosome is present in Figures 35 to 40.

These figures just described suggest the manner in which the final spermatozoön configurations of chromatin bodies may be achieved. It may be inferred that the connecting strands which are thin and attenuated will disappear while the heavier one will persist and the smaller chromatin bodies will coalesce with the larger bodies. This inference is supported by the succeeding figures.

Figures 41 to 43 show four chromatin bodies which are similar to the figures just described but differ to the extent that the reduction in the number of connecting strands is evident. No accessory chromosome is present. Figure 42 shows the entire cell.

Since the change from a spermatid to a spermatozoön involves a process of metamorphosis, it is impossible to tell just where a line demarcating the differentiation should be drawn. However, the following may be used as a criteria for the identification of a spermatozoön: (1) The cells that contain two or three bodies of chromatin completely or incompletely connected by strands. No accessory chromosome present, (Figures 45 to 62); (2) The cells that contain four bodies of chromatin incompletely connected by strands. No accessory chromosome present, (Figures 41 to 43). The accessory chromosome when present constitutes an additional body in the above mentioned spermatozoa and it can be distinguished by its isolation and shape, (Figures 63 to 71).

Figures 44 to 52 are spermatozoa containing three chromatin bodies. No accessory chromosome is present. Figures 44 and

45 differ from the other figures to the extent that the chromatin bodies are completely connected by strands. One additional strand is present in Figure 44. A complete cell is shown in Figure 44. Figures 46 to 48 are similar stages showing heavy strands which partially obscure the identity of the chromatin masses. Figures 49 to 51 show a similar pattern of chromatin. The chromatin bodies are approximately the same size and shape and the more peripherally placed bodies are connected to the central bodies by a u-shaped strand, Figure 52.

Figures 53 to 62 show the final configuration of the chromatin in the spermatozoa. No accessory chromosome is present in any of these figures. In all these figures there are only two bodies of chromatin. In Figure 53 the bodies are approximately of the same size and the connecting strand is short and thick. Extending from one mass there is a thick extension of chromatin. Figure 54 shows two bodies of chromatin, one being V-shaped and the other elongate. These two chromatin bodies are practically united without the intervention of a connecting strand. The chromatin in Figure 55 is in the form of a crescent. Figures 56 to 58 reveal approximately equally sized masses of chromatin connected by strands which show differences in length, thickness and disposition. Figures 59 and 60 show an elongate mass of chromatin connected by strands to smaller rounded bodies of chromatin. Two unequal sized bodies of chromatin are seen in Figures 61 and 62. In Figure 61, the bodies are connected by a very thin and long strand while the chromatin bodies of Figure 62 are separate. The smaller body of this figure shows an irregular strand extending from it. This figure also shows two well defined centrosomes.

It must not be inferred that the arrangement of the chromosomes in the chromatin bodies is the same in every spermatozoön, as has been previously stated there are several methods by which these various configurations may be produced. The spermatozoa thus far described contain no accessory chromosome.

Figures 63 to 71 are stages of spermatozoa which show, in addition to the ordinary chromatin bodies, an accessory chromosome. Approximately one half of the spermatozoa show the presence of the accessory chromosome. Four masses of chromatin and in addition the accessory chromosome are shown in Figure 63. Figure 64 shows three bodies of chromatin circularly disposed and in addition the accessory chromosome. Three well defined rounded bodies of chromatin which are con-

nected to each other are seen in Figure 65. The accessory chromosome is seen to one side. Figures 66 to 71 show the final chromosomal configuration of spermatozoa that contain the accessory chromosome and it is separate from the main mass of chromatin. By reference to previous figures of mature spermatozoa, (Figures 53 to 62), it is seen that the configuration of the chromatin corresponds in these figures to a similar configuration containing the accessory chromosome, (Figures 66 to 71).

In making a survey of the cysts which contain spermatozoa, it is seen that approximately one half of the contained cells show the distribution of chromatin as indicated by Figures 66 to 71. The shape of the accessory chromosome is quite constant and invariably is isolated from the ordinary mass or masses of chromatin. Approximately, the other one half of the spermatozoa do not reveal an accessory chromosome, (Figures 53 to 62). For this reason, it seems justifiable, therefore, to conclude that in the spermatozoa of Branchipus vernalis sexual dimorphism is clearly demonstrable.

CONCLUSIONS.

1. The secondary spermatocytes are of two types: first, those with eleven autosomes and, second, those with eleven autosomes and one accessory chromosome.

2. The accessory chromosome may divide or proceed undivided to one pole of the cell in the secondary spermatocyte division. In either case the accessory chromosome precedes the autosomes.

3. In the spermatids and spermatozoa the chromosomes undergo a series of changes involving the following processes: (1) change in form of the chromosomes, (2) the rearrangement of the chromosomes, (3) coalescence of chromosomes. These three processes operate simultaneously and continue until the final configuration of the chromatin bodies in the spermatozoa is attained.

4. The arrangement of the chromosomes in the chromatin bodies of the spermatozoa is not the same in every case.

5. The centrosome may or may not be divided in the spermatozoa.

6. Sexual dimorphism is demonstrable in the spermatozoa since approximately one half of the spermatozoa contain the accessory chromosome.

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EXPLANATION OF PLATE I.

(All figures were drawn by aid of the camera lucida at a magnification of 1250 diameters).

Fig. 1. A secondary spermatocyte immediately following the primary spermatocyte division. The chromatin is disposed in a darkly staining mass.

Figs. 2 and 3 are early prophase stages of the secondary spermatocyte showing definite and rounded chromosomes. Each cell contains eleven chromosomes; some of which are connected by intervening strands.

Fig. 4. The beginning of a change in form of the chromosomes is seen in this figure. The connecting strands are less pronounced. This cell does not contain the full complement of chromosomes.

Fig. 5 shows a later prophase stage characterized by the elongation of the chromosomes. Twelve chromosomes are present in this nucleus.

Figs. 6, 7 and 8 show the chromosomes undergoing contraction. These chromosomes are well defined and exhibit characteristic contours. The nuclei in Figures 6 and 7 contain eleven chromosomes, while Figure 8 has twelve.

Figs. 9, 10 and 11 are polar views of metaphase. The nuclear membrane is absent. In Figure 10, twelve chromosomes are seen. Figure 11 shows one free chromosome at the side of the main mass of closely clumped chromosomes and is interpreted as being the accessory.

Fig. 12. A profile view of an early metaphase.

Fig. 13. A profile view of an early metaphase plate oriented obliquely. The accessory chromosome is seen toward the upper pole.

Fig. 14. A profile view of a metaphase similar to the preceding figure. The accessory chromosome is divided and occupies the poles of the spindle.

Fig. 15. Anaphase.

Fig. 16. Telophase. In the lower portion of the figure, eleven chromosomes are seen.

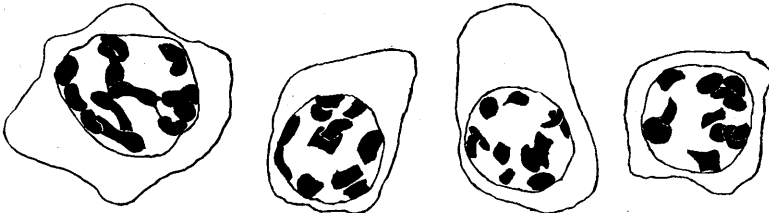Fig. 17. Telophase. Individual chromosomes are evident. Note the beginning of a nuclear membrane.
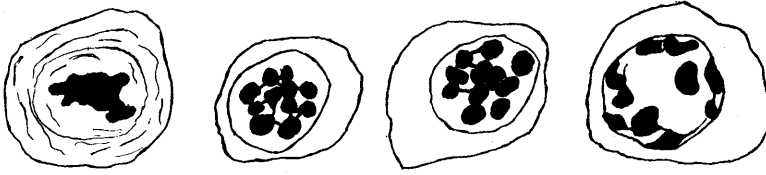
EXPLANATION OF PLATE II.

Fig. 18.   An early spermatid showing eleven rounded chromosomes.   Strands connecting some of the chromosomes are visible.   To the left near the nuclear membrane two centrosomes are present.

Fig. 19.   This spermatid nucleus contains twelve chromosomes, the majority of which present a rounded contour.   The connecting strands are more pronounced.

Fig. 20.   A change in form of the chromosomes is evident.   Note the apparent joining of chromosomes, and decrease in number, although the full complement of chromosomes is present only a count of ten is obtained.   ·

Figs. 21 to 29 show the configurations which the chromosomes undergo in the early stages of coalescence of unlike chromosomes.   It is impossible to make an accurate count of chromosomes in Figure 21, however, nine distinct chromosomes are visible.   Two centrosomes are present.

Figs. 22 shows eight bodies of chromatin, six of which are well defined.   In the central part of the nucleus two chromosomes are partially coalesced.   Strands connecting the chromosomes are pronounced.

Fig. 23.   Eight well defined chromatin bodies, some of which are joined by strands, are seen in this nucleus.   One centrosome which lies close to the nuclear membrane is visible.

Fig. 24 contains six chromatin bodies.   Five of these bodies are round and are peripherally placed.   Note the more central, large and irregularly shaped chromatin body which was formed by the coalescence of several chromosomes.   One large centrosome is present.

Fig. 25 shows the simultaneous coalesence of several chromosomes.   Only two chromatin bodies are free.   One centrosome is visible.

Fig. 26.   Seven chromatin bodies are seen in this nucleus and also two centrosomes.

Fig. 27 contains seven bodies of chromatin.   The connecting strands are more numerous than in the preceding figures.

Fig. 28 shows distinctly the coalescence of the chromosomes.   One chromatin body is free.

Fig. 29.   This nucleus shows five large chromatin masses, three of which are rounded.   The nuclear membrane is becoming less distinct.

Fig. 30 shows seven masses of chromatin, six of which are partially coalesced, arranged in the form of an incomplete circle.

Fig. 31.   Six definite rounded chromatin masses are seen in this nucleus.   The degree of coalescence is not so marked.   The connecting strands are quite conspicuous.

Figs. 32 and 33 are similar in form.   Each of these nuclei present five chromatin bodies connected to each other by varying sized strands.   The entire spermatid is shown in Figure 33.

Figs 34 to 40 inclusive.   These nuclei show the same number and similar disposition of the chromatin whose arrangement simulates an ill-shaped quadrangle with a chromatin body at each angle.   An additional connecting strand is seen in Figure 34.   No accessory chromosome is present in these nuclei.

Figs. 41 and 42 show four chromatin bodies, the disposition of which is similar to the preceding figures.   A reduction in the number of connecting strands is seen.   The accessory chromosome is absent.   The entire cell is shown in Figure 42.

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EXPLANATION OF PLATE III.

Fig. 43.. A spermatozoön containing four chromatin masses. In this stage the accessory chromosome may constitute a fifth body. See Figure 63.

Figs. 44 and 45 are spermatozoa containing three chromatin bodies completely connected by strands. Figure 44 which is a complete cell shows one additional strand. The accessory chromosome is absent.

Figs. 46, 47 and 48 are similar stages of spermatozoa. Heavy strands are visible which connect and partially obscure some of the chromatin masses. The accessory chromosome is absent.

Figs. 49, 50 and 51 are similar in the respect to the number and distribution of the chromatin bodies. Figure 51 is the entire cell.

Fig. 52. The entire cell is shown containing three chromatin bodies of nearly equal size and similar shape connected by U-shaped strands.

Figs. 53 to 62 inclusive show the final configuration of the chromatin in the spermatozoa. Two chromatin bodies are present in these figures and are connected by heavy strands with the exception of Figure 62. This figure contains two centrosomes. The chromatin bodies in Figures 53 and 54 are almost united. The accessory chromosome is absent.

Figs. 63 to 71 are stages of the spermatozoa and show in addition to the ordinary masses of chromatin an accessory chromosome.

Fig. 63 shows four masses of chromatin and an accessory chromosome. The accessory chromosome in the following figures is marked X.

Fig. 64. The entire cell is represented. The chromatin bodies are circularly arranged. The accessory chromosome is seen in the upper part of the figure and is free from the main masses.

Fig. 65. Three rounded chromatin masses are present and are connected to each other. The accessory chromosome is completely isolated.

Figs. 66 to 71 show the final stages of the spermatozoa that contain the accessory chromosomes.

Fig. 66 shows the entire spermatozoön. Two large, irregular and connected chromatin masses are present. The accessory chromosome occupies a position to the right.

Fig. 67. The ordinary chromatin masses are connected by thin strands. The accessory chromosome is seen in the upper part of the figure.

Fig. 68. The accessory chromosome occupies a position above the main mass of chromatin.

Fig. 69. The two ordinary masses of chromatin are connected. The accessory chromosome is seen to the left near a centrosome.

Figs. 70 and 71. The former figure shows two main masses of chromatin with the accessory above and to the right. The latter figure reveals the shape of the entire spermatzoön. The three ordinary chromatin masses are closely approximated, the accessory chromosome occupies an upper position with its convexity pointing downward.

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63   64   65   66   67

68   69   70   71