SPERMATOGENESIS IN BRANCHIPUS VERNALIS.

PART I.

THE TESTIS AND SPERMATOGONIAL DIVISIONS.

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Maturation in Branchipus has not apparently been studied since Fries' work on oogenesis in 1910. Concerning either the general or the detailed processes of maturation that should be basic for certain embryological studies, Branchipus furnishes excellent laboratory material for the following reasons:

(1) It is easily available and has a rather wide distribution.

(2) The mitotic figures illustrating the various stages of maturation are extremely numerous.

The important researches and theoretical postulates produced during the last quarter of the 19th century in the field of Cytology, initiated a series of investigations which resulted in the apparent demonstration of the genetic continuity of chromosomes and their role in heredity.

The discovery of reduction in animals by Van Beneden (1883), and reduction in plants by Strassbouger (1888), coupled with Roux' (1883), statement that chromatin has qualitative differences and that these different qualities are arranged in linear order in the chromosomes, together with Weismans (1887), speculative but fruitful analysis of reduction served as a beginning for the efforts that cytologists have made in the study of reduction.

In the early part of the 20th century the individuality and relationships of chromosomes to heredity was placed on a firmer basis by (Sutton, Montgomery, Wilson, McClung, Welling, Morgan, their students and others). An enormous amount of work has been done, and much as been accomplished toward the first solution of the problem and the main features of the steps involved in reduction of chromosomes has been established. Yet, however, there is some disagreement among cytologists as to the exact method and mechanism of reduction.

This divergence of opinion is greatest in the interpretation of the changes that occur in the chromatin during the early prophase of the meiotic division, for it is at this time that chromosomes conjugate to form bivalent chromosomes. That this conjugation of chromosomes is brought about by a synapsis of homologous pairs of chromosomes is a fact unquestioned by most cytologists today. However, the mechanism and mode of synapse is not too clearly described in any of the evidences produced to prove synapsis of chromosomes. The two views regarding the nature of synapse are, conjugation of chromosomes side by side (parasynapsis) and end to end conjugation (telosynapsis).

The theory of parasynapsis predominates at the present time, although those workers holding to the telosynaptic viewpoint are many in number. This difference in interpretation is due to the difficulty of following chromosome behavior in the early prophase stages preceding the synaptic period, for at this time the chromatin masses are very indistinct in most forms.

It is the purpose of this series of papers to give observations covering the spermatogensis of Branchipus vernalis with special emphasis on the mode of synapse, bouquet formation, chromosomal transformations and formation of spermatozoan chromosome figures.

The material on which this paper No. 1, Spermatogonia, and ensuing articles, No. 2, Primary Spermatocyte, and No. 3, Secondary Spermatocyte, Spermatid and Spermatazoa, are based, was collected from ponds during March and early April, and was identified as Branchipus vernalis. This material was fixed in absolute alcohol seven parts, and glacial acetic acid two parts from three and a half to four hours; then washed in four or five changes of absolute alcohol twenty four hours. Only part of the specimen containing the testes was imbedded. Sections were cut five to seven microns in thickness and stained with Heidenhain's haematoxylin. Prior to fixation it is well to keep the specimen in water from twenty-four to thirty-six hours in order that the digestive tract may be freed from silicon.

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The Spermatogenesis of Branchipus.

A series of longitudinal sections reveal the testes as paired tublar structures on each side of the digestive tract. They extend from the most caudal portion of the abdomen anteriorly to the thoracic region. A study of a longitudinal section shows that a division of the testis into two parts can be made. (1) The anterior one-fourth or glandular portion and, (2), the posterior three-fourths or germinal portion. Histologically, these two regions are markedly different. The former is characterized by tall, darkly staining columnar cells and the latter by flattened or compressed cells, which form the wall of the tubule. From the ventral side of the testis at the anterior end, the gonoduct is attached. This duct which is glandular in structure, courses ventrally and caudally terminating in the copulatory organ.

The glandular part of the testis secretes a mucoid-like substance which has a vacuolated appearance and stains quite readily. This substance fills the lumen and in it spermatids and spermatoza are found imbedded in great numbers. The secretion is not confined altogether to the anterior region of the testis but is more abundant there and stains more deeply at that particular region. The germinal part of the testis is concerned in the process of transformation of the basal cells or spermatogonia into the mature free ameboid spermatozoa which are found imbedded in the mucoid material in the lumen of the tubule. The more immature cells are located along the basal part of the tubule and are for the most part spermatogonia. These cells divide and differentiate into more mature forms as they pass from the periphery toward the lumen of the tubule.

A typical cross section of the germinal part of the testicular tubules reveals that each tubule is composed of cells arranged in cysts, (Fig. 14). These cysts vary considerably in number, appearance, location and in the number of cells contained within them. Each of these cysts contain cells which are apparently in the same stage of development. The cysts located more basally contain spermatogonia while those cysts which are nearer the lumen of the tubules contain more differentiated types of cells; some of the cysts contain spermatocytes of the second order, while others contain spermatids or spermatozoa. The cysts containing spermatogonia are large and are the least apparent type. In some cases they are hardly

recognizable as cysts, but usually they are distinguishable. The cysts containing the more differentiated succeeding cellular generations are well defined, smaller, and contain fewer cells.

The inner wall of the tubule lining the lumen is apparently a definite membrane which ruptures at the time of the discharge of the spermatid and spermatozoa into the lumen, leaving an irregular torn area which soon resumes its former condition by the placing of another cyst in the position occupied by the discharged cells.

The lumen of the tubule contains the spermatids and the ameboid spermatozoa which are imbedded in the mucoid-like substance previously mentioned. It is apparent that some of these cells have migrated or have been carried into their present position from a position farther up the tubule.

Superimposed upon the cysts located at the more basal portion of the testicular tubules are a number of very large cells unevenly distributed through the entire germinal portion of the testes. These are Giant cells which are from two to four times the diameter of the ordinary spermatogonia. The cells take a darker stain than do the surrounding ones, and they vary in shape from that of an imperfect sphere to that of an elongated oval form. There is no definite nucleus within these cells, but instead the chromatin is aggregated into several large irregular clumps and many small granules. The larger masses of chromatin have no definite relationship to each other, but are scattered throughout the cells in an irregular manner. Occasionally tripolar spindles are found in these cells. The significance of these cells is uncertain but it is surmised that they are degenerate sex cells. The inference that these cells are degenerate sex cells rather than nurse cells is drawn from the fact that the chromatin is undergoing degeneration and that the cells have no definite relationship to the developing spermatids and spermatozoa.

The examination of a series of sections through the germinal portion of the testis shows an apparent periodity of function similar to that of the testes of higher forms. This is evidenced by the differences in the cellular content of the germinal portion of the tubules of the testes. As the sections are read consecutively, from one extremity to the other it is noticed that as the more differentiated cells increase in number that the spermatogonia show a corresponding decrease. There are typical regional areas where there is an increase in cellular proliferation.

These regions are separated by portions of the tubules in which there is marked quiescence. The entire picture of this type of tube simulates a wave-like character in which the crest of the wave is the thicker portions of the tubule containing a large number of dividing spermatogonia and primary spermatocytes. There are relatively few mature spermatozoa in this part. The more inactive portions of the tubules between these crests are thin walled containing few spermatogonia and practically no cellular division. The lumen of this part of the tubule is usually packed with spermatozoa.

SPERMATOGONIA.

The spermatogonia of Branchipus are located along the periphery of the germinal portion of the tubule throughout its entire extent. The cysts containing the spermatogonia are most apparent and best defined in the regions of more active proliferations. In more inactive regions of the tubule the spermatogonial cysts are not discernible. Instead the cells are arranged in ill defined rows which vary in number from that of a single row to several rows. In the region of activity the cysts are usually large and ill defined but they are nevertheless discernible as cysts. This condition is due to the large number of cells contained within the cysts, the large size of the cysts and the distortion of the boundary of the cysts by the impingement exerted upon them by neighboring cysts which contain cells actively differentiating. The smaller and better defined spermatogonial cysts contain fewer cells which not being closely packed, permits of better inspection of the contained cells since the cells are separated and their contours not distorted by neighboring cells. On the other hand the large and crowed cysts contain cells which are closely packed together and it is only with difficulty that individual cells are recognizable. These cysts are more numerous.

A good criterion for the identification of spermatogonia aside from what has been previously stated is their staining properties. The spermatogonia stain readily and as a result they are darker than the other cellular contents of the testis. The cells other than the spermatogonia and Giant cells have a clear background, the cytoplasm and nucleus appear almost colorless except for the elements that are stained black by the hematoxylin. The spermatogonial cytoplasm and nucleus possess a grey background when colored with this stain. This

greyness of background is quite characteristic in all spermatogonial stages, whether the cells be in resting condition or actively dividing.

The nucleus which occupies almost the entire cell is surrounded by a very narrow rim of grey protoplasm. If the cells are closely crowed then the cytoplasm being compressed appears small in amount. The shape of the nucleus is spherical but at times it may reveal slight variations. The size of the nucleus is not constant. During the resting condition and early prophase, the nucleus is small, being a little larger than one-half the size of a primary spermatocyte. However, the distinction between the spermatogonia and primary spermatocytes are easily made since the nucleus of the former is smaller during early stages and has a different chromatin arrangement in later stages. In addition to this, there is a marked contrast in staining reaction which has been previously stated.

The following description of the behavior of the chromatin in the nucleus of the spermatogonium in Branchipus is based upon the study in the transformation of the chromatin in a large series of sections.

A typical spermatogonium in the resting stage reveals the chromatin rather evenly scattered throughout the nucleus with the exception of two or more larger, irregular aggregates. The other chromatin particles vary in size from almost indistinct granules to masses as prominent as the large ones. The chromatin varies in staining properties, some of the granules staining more faintly than others. The nuclear wall is very evident. The cell as well as the contained nucleus is small and somewhat spherical in contour. The size of the resting cell, (Fig. 1) is smaller than any of the ensuing stages. There are only a comparatively few cells in this stage. The chromatin of the nuclei of most of the cells is in the form of different sized aggregates connected with each other by strands exhibiting various degrees of attenuation as the following description reveals.

Gradual changes are seen in the nuclei during the early prophase which results in the formation of definite chromosomes, (Figs. 2, 3, 4, and 5). The first change that is noted is a beginning of a reticulum. This recticulum becomes apparent when the chromatin condenses in certain areas resulting in the formation of larger and fewer aggregates, (Figs. 2 and 3.) The strands which form this reticulum vary in size and form and

are oriented in an indefinite manner. Sometimes, they are long and attenuated— or they may be short and heavy. The chromatin aggregates also show these indefinite characteristics. It is apparent by the inspection of Figures 2, 3, and 4, that the size of the nucleus has increased, and the nuclear membrane is very distinct.

A further change of the chromatin mass continues. The strands of the reticulum contract and separate in certain regions, gradually condensing in an irregular fashion, and form indefinite chromosomes, (Fig. 5). This is the condition of the cells in the majority of the spermatogonial cysts. So numerous are the cells in these cysts that it is only with difficulty that individual cell boundaries are discernible.

The indefinite chromosomes of this phase of development now change in shape and form definitive chromosomes, (Fig. 6). In reference to this figure the remaining attenuated chromatin strands have entirely disappeared. These definite chromosomes are formed by the chromatin becoming equalized in thickness throughout. At this time, homologous chromosomes cannot be identified due to their similarity and their crowded condition. The nuclear membrane disappears and at the same time there is a decrease in the staining property of the cytoplasm resulting in an almost clear background for the late prophase.

By further contraction the individual chromosomes assume their characteristic size and shape, (Figs. 7 and 8). It is quite usual in this stage of development to find individual chromosomes rather equally distributed throughout the cytoplasm of the cell. On account of this scattered condition and the clearness of cytoplasm, these cells are particularly favorable for determining chromosome counts, and relations of individual chromosomes, (Figs. 7, 8, and 9).

There are twenty-three chromosomes in the spermatogonium of Branchipus. There is little difficulty in distinguishing homologous pairs, and the accessory chromosome. Figure 13 shows individual chromosomes taken from the cell illustrated in Fig. 7. By reference to Fig. 13, the chromosomes which are arranged according to their similarity, the differences between the unlike chromosomes can be observed. Arranging chromosomes in this manner enables one to detect homologous chromosomes. There are eleven pairs of chromosomes and one chromosome which has no mate (chromosomes of Figs. 7, 8, and 13).

In Figs. 7 and 8, there are some homologous chromosomes which are already paired. Chromosomes D, E, and F, are paired while chromosomes L are in close relationship with each other, (Fig. 7). In Fig. 8, this same condition is observable in three pairs of chromosomes although the chromosomes which revealed a paired condition in Fig. 7, are not all paired in Fig. 8. The only chromosomes paired in both cells are chromosomes F. The fact that the same pairs of homologous chromosomes are not found associated together in every case indicates that this is more of a chance occurrence than the regular procedure in the activity of chromosomes. A significant fact of this association is that the chromosomes involved in individual pairings have the same morphological characteristics.

Following the stage just described, the chromosomes continue to contract and change in shape until they become more or less rounded. At this time, the chromosomes are going on the spindle in preparation of the ensuing division. Fig. 9, shows a polar view of the chromosomes on the spindle. (In this figure there are three enlarged and poorly fixed chromosomes). Chromosomes in the metaphase stage arranged on the spindle are seen in Fig. 10. Here separate chromosomes are observable, but no distinguishable characteristics mark individual chromosomes.

A slightly later stage is represented in Fig. 11. The chromosomes of this particular stage are proceeding to their respective poles. In reference to this figure, two chromosomes which are separated from the main mass of chromosomes are seen. These are the divided accessory chromosomes. The accessory chromosome is not always detached from the main mass of chromatin, for frequently, cells can be found showing no isolated, dividing X chromosome. As the chromosomes near their respective poles, individual chromosomes are no longer distinguishable, but instead they are crowed into a dense mass whose concavity is directed centrally.

Not many anaphase and telaphase figures are found. This indicates that the process of division progresses with rapidity in these particular stages. The daughter cells of the spermatogonial division either differentiate into a primary spermatocyte or form other spermatogonia.

CONCLUSION.

(1) The testes of Branchipus is divided into two parts:
   1. the anterior one fourth or glandular portion, and,
   2. the posterior three fourths or germinal portion.

(2) The germinal cells are arranged in cysts.

(3) There is a periodicity of function of the testicular tubules as is evidenced by the wave-like areas of cellular proliferation and quiescence.

(4) There are twenty-three chromosomes in the spermatogonium of Branchipus vernalis.

(5) There is one accessory and eleven pairs of homologous chromosomes.

(6) In the late prophase some homologous chromosomes become paired. This pairing is more of a chance occurrence rather than the regular behavior in the activity of chromosomes.

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EXPLANATION OF PLATE I.

These Figures were made by aid of camera lucida at a magnification of 1250×.

Fig. 1. A resting spermatogonial cell.

Fig. 2. An early prophase stage showing the beginning of a reticulum.

Fig. 3. A less apparent reticulum with the chromatin condensed in areas of the reticulum.

Fig. 4. Shows the strands of reticulum thickened and separated in some regions. The large mass located centrally represents several condensations of chromatin closely approximated.

Fig. 5. Shows the formation of indefinite and irregular chromosomes.

Fig. 6. Late prophase; the nuclear membrane has disappeared and definitive chromosomes are present.

Fig. 7. The well defined chromosomes show their characteristic size and shape. Homologous pairs are distinguishable and a chromosome count is easily made. Letters E, D, and F, refer to the paired chromosomes. L indicates two homologous chromosomes closely approximated but not paired, X is the accessory chromosome. (See Figure 13).

Fig. 8. Another cell similar to Figure 7. F indicates homologous chromosomes which are paired. The same chromosomes are paired in Fig. 7. X is the accessory chromosome.

Fig. 9. A polar view of 23 separate chromosomes.

Fig. 10. Early metaphase stage.

Fig. 11. Anaphase stage showing the X chromosome divided and separated from the main chromatin masses.

Fig. 12. Shows chromosomes approaching their respective poles.

Fig. 13. Shows chromosomes of Fig. 7, arranged according to morphological similiarities. The legend of Figure 7 is the same as that of Figure 13.

Fig. 1

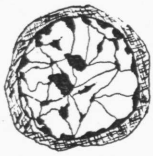

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

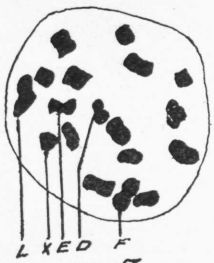

Fig. 7.

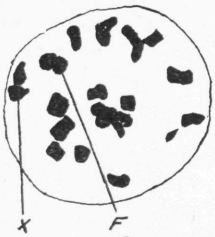

Fig. 8

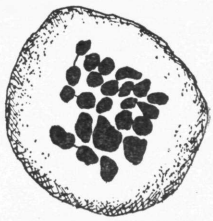

Fig. 9.

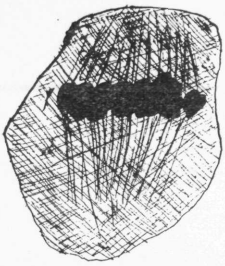

Fig. 10.

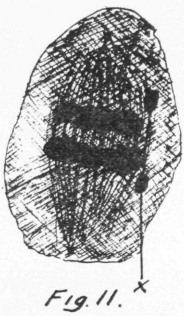

Fig. 11.

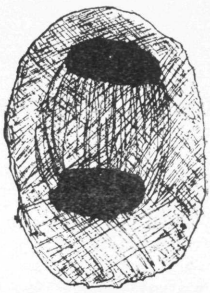

Fig. 12.

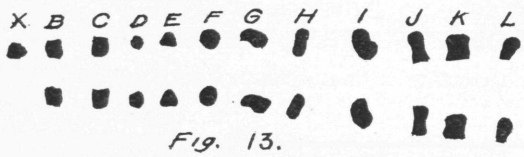

Fig. 13.

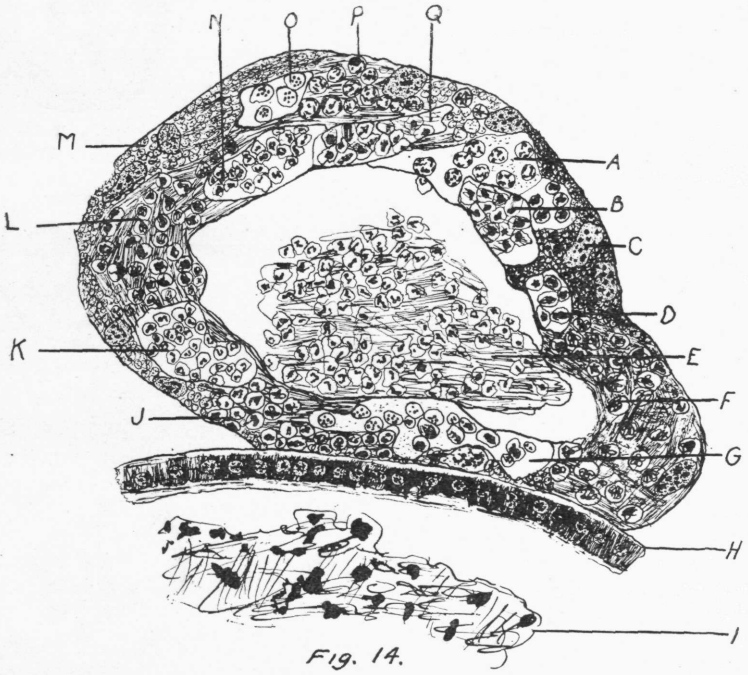

Fig. 14. A camera lucida drawing of a cross section through the germinal portion of a testicular tube × 250, reduced to 125.

A. A cyst of primary spermatocytes in diaknesis.
B. A cyst of dividing primary spermatocytes.
C. A giant cell.
D. Cyst of dividing primary spermatocytes.
E. Spermatids and spermatozoa imbedded in the mucoid substance in the lumen.
F. Primary spermatocytes in bouquet stage.
G. Stages of primary spermatocyte similar to B and D.
H. Wall of intestine.
I. Intestinal contents.
J. Primary spermatocytes in synizesis.
K. A cyst containing a few spermatids and numerous spermatozoa.
L. A cyst of primary spermatocytes emerging from synizesis.
M. Resting spermatogonia.
N. A cyst containing few spermatozoa and numerous spermatids.
O. Primary spermatocytes showing chromosomes going on the spindle.
P. Primary spermatocytes previous to diaknesis.
Q. Dividing secondary spermatocytes.