# THE SPERMATOGENESIS OF SCELOPORUS UNDULATUS

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# THE SPERMATOGENESIS OF SCELOPORUS UNDULATUS.

(with four plates)

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# THE SPERMATOGENESIS OF SCELOPORUS UNDULATUS

## Introduction

During the last decade, the attention of cytologists has been focussed upon the phenomena incident to the indirect division of cells and upon the processes to be observed in the maturation of the germ cells. Of the two phases, maturation easily can be considered as the more interesting and important. The primary work on maturation, or the quantitative reduction of the chromatin of the nucleus in the germ cells to one-half, a phenomenon peculiar to the germ cells, was done to trace the distribution of the chromosomes to the daughter cells in both male and female germ cells. But in this earlier work it was noted by several observers that there was a difference in chromosome number between the male and female somatic cells. Further work on the germ cells showed that this accessory, or extra chromosome, behaved in an unusual fashion during the maturation divisions of the spermatogonial and oogonial cells to form spermatozoa and ova. In most forms, it was found to be morphologically and physiologically different from any of the ordinary chromosomes, so that its conduct could be accurately studied during the divisions of the cell. On this peculiar chromosome, the accessory, hinges much

of the best modern cytological work.

As long ago as 1891, Henking noticed that in the male germ cells of Pyrrhocoris a single large chromosome lagged on the spindle and passed undivided to one of the poles during one of the maturation divisions. Half of the resulting spermatozoa from the original spermatogonium were thus seen to be morphologically different from the other half in possessing one more chromosome. In the case of the male, then, there is dimorphism in the spermatozoa; that is, the male has two kinds of germ cells. Henking did not observe the oogenesis of his species with a view to finding out if there was a corresponding dimorphism in the eggs of Pyrrhocoris. Following Henking in 1899, Paulmier, working in Wilson's laboratory, found the same dimorphism in the case of Anasa. Protenor was investigated by Montgomery ('01) and confirmation of the condition discovered by Henking again resulted. Sinéty ('01) found dimorphism in the case of certain of the Phasmidae. McClung ('02) and Sutton ('02) described an eccentric chromosome in Orthopteran insects which behaved like that in Pyrrhocoris. To McClung goes the credit for putting forth a plausible theory to account for the conditions as seen in the insects studied.

McClung suggested that the accessory chromosome might be a sex determining element. He thought that the chromosome was a specific male determinant so that

eggs fertilized by spermatozoa containing the chromosome would be male individuals and that those fertilized by spermatozoa not containing the element would be females. McClung argued his case with no knowledge of the female condition. Subsequent work proved his assumption concerning the accessory to be the exact opposite of the truth. The accessory chromosome was proved to be a specific female determinant and not a male determinant. The true status of the eccentric element was determined in work by Wilson and Stevens in 1905 and 1906.

Wilson and Stevens in independent researches upon the Hemiptera and the Coleoptera worked out the female condition as well as the male. Two chromosomes of the accessory type were found in the female in addition to the ordinary chromosomes. Although these accessory chromosomes did not behave in an eccentric manner in the female, several clear cases showed their morphological difference from the ordinary chromosomes. In Protenor, for example, the spermatogonial group shows the accessory as the largest of the chromosomes. The oogonial group demonstrates clearly two of these large structures which behave like ordinary chromosomes and give matured eggs all of which contain the accessory chromosome. The eggs are thus seen to be all of one kind, or "homogametic", while the spermatozoa are of two kinds, or "digametic". Using the symbol X to denote the accessory, the female

somatic condition with respect to the accessory is XX, while that of the male is X. Maturation of the oogonium gives gametes which all contain a single X element. This conclusion concerning the maturation of the egg was verified by Morrill ('10) on several genera of the Hemiptera. Maturation of the spermatogonium gives two types of spermatozoa, half of which contain the accessory. We may conclude, therefore, that zygotes formed by the union of a spermatozoon containing the accessory with any egg will restore the female XX condition again and that the specific male zygote will be formed from the union of a spermatozoon lacking the accessory with any female gamete.

Variations from this simple mechanism of sex determination are described by Wilson. Wilson finds that in insects of the Lygaeus type there is associated with the accessory in the male cells another element which he calls the Y-chromosome. The Y-chromosome segregates out during the maturation of the male germ cells and appears in half of the spermatozoa while the other half contain the true accessory, X, which is a female determinant. The Y then, in this case, is confined to the male and never appears in the female. The following formulae, based on Wilson, express the production of sex as conceived by that worker:

I. Protenor type

a. EggX plus SpermX = XX(female)

b. EggX plus Sperm = X (male)

a. EggX plus SpermX = XX(female)

II. Lygaeus type

b. EggX plus SpermY = XY (male)

Intergrading forms between the two described in the formulae have been found. They may be explained from an evolutionary standpoint as cases in which the Y is gradually disappearing and is leaving the X condition as the end product. In several papers, Wilson has also expressed the belief that there may be animals in which there is no visible sex-determining chromosome, thus expressing perhaps a further evolution from the type containing only a simple accessory. These animals would then have a physiological factor for sex, not, of course, being visibly expressed in any one chromosome. No cases of such relations have yet been discovered. Wilson's study of Nezara at first convinced him that there was no accessory in this insect. Subsequent observation caused him to change his mind and to announce that in this insect the X- and Y- elements are equal in size.

Variations in the morphology of the accessory are numerous. It may be a multiple element unaccompanied by a Y as in the case of Ascaris, which has a pentad accessory (Edwards). Another condition is that of multiple X components. The case of Acholla multispinosa (Payne) is interesting in this connection. There are three small Y-components and two large X-components. All of these cases of variation can be reduced to the formulae

given above so that the theory of sex production by a chromatic element does not suffer because of them.

In all of the insect forms which were described during the earlier work on the germ cells, the female was found to be "homogametic", that is, all of the eggs are alike. The opposite speculation, that there might be forms in which the male is "homogametic", has been put forward by some biologists. So far, Baltzer ('09), working on Sphaerechinus and Echinus, is the only one who has produced evidence showing a female digametic condition. One can easily see that Wilson's sex formulae can be applied to Baltzer's findings. Additional strong evidence for the chromosome theory of sex determination has been furnished in recent years by the work of Morgan ('09), von Baehr ('09) and Stevens ('09) on the Phylloxerans and Aphids. In these forms, fertilized eggs produce females, the spermatozoa receiving no accessory element, degenerating without becoming functional. It is thus shown without doubt that the XX condition is female because in these forms only spermatozoa containing the accessory element are found.

For a long time cytological research was confined to invertebrate forms, but in recent years the attention of several workers has been turned toward vertebrate animals. In most of these cases, the female condition is as yet unknown. Hence, reasoning by analogy with

the invertebrates has been employed to explain sex production. In this connection, it is well to say that some progress is being made toward finding the chromosome condition in the female by the cytological study of embryos. Wodsedalek ('13) reports the female somatic number in the pig from embryos. It justifies the theoretical considerations laid down in Wilson's formulae already quoted, for there is one more chromosome in the female.

Guyer is the pioneer in the field of the vertebrate chromosomes. In four papers published in rapid succession, he reports the accessory chromosome in the guinea fowl ('09a), the chicken ('09b), the rat ('10), and in man ('10). In the last case, the accessory was found to be a double element similar to that described by Wilson for Syromastes. Contemporaneously with Guyer, Newman and Patterson ('10) discovered the accessory chromosome in the armadillo. Stevens ('11) for the guinea pig, King ('12) for Necturus, and Jordan ('11) for the opossum also demonstrated the accessory elements. Wodsedalek ('13) extended the accessory chromosome discoveries to the pig when he obsefved a double X-chromosome element in that animal. Jordan ('13) at first disagreed with Wodsedalek on the pig, as his material seemed to show no accessory chromosome. Recently, however, Jordan has verified Wodsedalek's findings, so the results of the latter worker with respect to the accessory are undoubtedly

correct. A recent paper on the subject of sex determination is that of Boring and Pearl ('14) on the chicken. In the chicken, a peculiar condition of affairs exists. The breeding results are not in keeping with the cytology as worked out by Guyer. The research of Boring and Pearl was undertaken to test the results of Guyer on this subject. The conclusions reached in this work are of such a nature that some doubt may be cast on Guyer's findings. Probably more work will be done on this form, however, to clear up the confusion now existing. Wodsedalek ('14) has recently described an accessory element in the germ cells of the horse, a further extension to the field of the vertebrates of the chromosome theory of sex determination.

As far as I know, no complete results have been published on any reptile. Jordan ('14) has made a preliminary report in which he states that the accessory is present in one of the turtles, but other than this, no work has been undertaken to ascertain whether the reptiles conform to the chromosome scheme observed in other animals.

The present research was undertaken in the spring of 1913 at the suggestion of Dr. George Lefevre and is an attempt at tracing the behavior of the chromosomes in the common fence swift, Sceloporus undulatus.

## Material and Methods.

The best material for this study was obtained from two adult males which were taken in June. Many other lizards were captured but none of them approached the first two in number of mitoses. Summer and fall individuals were collected, but nearly all showed resting testes. Young, immature specimens showed only sparse spermatogonial divisions. The testes were dissected rapidly from the body after the animals had been killed by decapitation. In all cases, the fixatives were used immediately and, in some instances, they were heated to body temperature. In all except the first two good specimens, fixation was excellent at the periphery of the testis. Flemming's fluid gave good fixation for the entire testis in the case of the best specimens. Bouin's fluid was found to be very satisfactory also. It was used primarily for the smear preparations. These preparations were fixed in Bouin's fluid and stained in iron haemotoxylin with some success. Three of the stains used throughout the work which gave good results on sections were, iron haematoxylin, safranin and gentian violet. The provisional fixative and stain, aceto-carmine, was tried on material with few mitoses, so good results were not obtained. Sections of the ovaries and testes were made at 6 micra and at 8 micra. The ovaries were fixed

in Bouin's fluid and stained in the ordinary way with iron haematoxylin with good results. Safranin and light green gave brilliant pictures. The latter stain was used as a differential coloring for certain bodies in the egg cytoplasm about whose chromatic nature there was no doubt. The preparations of ovaries were made with the hope of obtaining follicle cell mitoses, but in these cells crowding and indistinctness made chromosome counts impossible.

## Spermatogonia.

The primary spermatogonium, which may be studied to advantage in the testes of immature individuals, is a small cell with rather indefinite boundaries. The nucleus, a relatively large structure, contains two chromatin nucleoli (Fig. 1). These nucleoli are apparent in the spermatogonia and in the growth stages of the primary spermatocytes to synapsis, but at the latter stage they disappear entirely until the resting stage of the second spermatocyte (Figs. 1-12). The nucleoli perhaps give rise to chromosomes, although there is no other evidence than that of their disappearance at synapsis. The secondary spermatogonia are abundant in testes with many spermatozoa. They are characterized by increased size of both nucleus and cytoplasm. At the end of nuclear growth stages, the chromatin forms into a thick spireme thread (Fig. 3).

Segmentation of the spireme for chromosome formation then follows. The segments are at first long and somewhat diffuse. Condensation shortens the pieces into definite chromosomes during the prophases (Fig. 4).

Spermatogonial metaphase exhibits a characteristic distribution of the twenty-two chromosomes (Figs. 5, 6, and 7). One characteristic feature is the fact that the plate always shows the chromosomes in constant position. Ten large U-shaped, or rod-shaped, elements are usually found at the periphery of the group. These chromosomes find a parallel in a similar condition discovered in certain Orthoptera by McClung. In the Orthoptera mentioned, the large chromosomes of the group are also peripherally placed. Inside of the ring of ten large elements are twelve smaller and more rounded chromosomes. Eight of the twelve are minute pieces of chromatin while the remaining four are somewhat larger. The synaptic mates can be assumed from this grouping by a consideration of relative positions. The ten large elements on the margin undoubtedly give rise to the five tetrads which manifest themselves at first spermatocyte metaphase. The twelve smaller chromosomes can be paired off two by two to give the four microsomes and the two intermediates of the first division. Figure 7 shows a possible grouping of the elements. In this figure, only the grouping of the inner twelve is considered. Synaptic mates of the peripheral chromosomes can

be easily assumed from relative positions.

Metaphase of the spermatogonia is followed by an equational splitting of all elements so that each daughter cell receives twenty-two chromosomes. Wodsedalek ('14) notes that there is occasional lagging of the accessory in the spermatogonial phases of horse spermatogenesis. Anaphases of the spermatogonia in the lizard sometimes show lagging chromosomes, but the cases were not numerous enough to permit conclusions to be drawn. One of these telophases is pictured in Figure 8. Several chromosomes have not yet joined the polar masses in this case.

It will be noted that there is no unpaired element. Because of this fact, I have not been able to identify a sex chromosome at all. An X and Y condition may be present, paralleling Nezara where the X and Y are equal in size. But there is no evidence for supposing that this is a case like Nezara. More will be said in this connection in the discussion at the end of the paper.

## Primary Spermatocytes.

### 1. Growth Period.

At the end of the last spermatogonial division, a short period of rest ensues. The chromatin of the nucleus is scattered and a pair of chromatin nucleoli can be clearly seen (Fig. 9). Following this period of

rest, an extended series of growth stages is the order. The chromatin begins to assemble in definite thread-like leptitene masses leaving large clear spaces here and there in the nucleus (Fig. 10). The next step is a collection and a massing of the chromatin threads in the middle of the nucleus (Fig. 11). The whole mass stains deeply. Closely following the stage just described, the thread-like tangle migrates to the side of the nucleus. This is probably the synezesis stage, but, as the mass stains heavily, it is impossible to determine the nature of the process (Fig. 12). The amphitene nucleus which is resolved out of this thread-like mass is evident as a long and thin spireme-like thread which soon condenses into the thicker pachytene stage (Figs. 13-15). During the early amphitene stage, the twisting of the leptitene threads to form this double thread was observed in several cases. The pachytene thread was usually very heavily stained, so details of the structure were not obtained. Chromosome formation takes place when the pachytene nucleus segments into the first division elements.

## 2. Prophase.

Figures 15 and 16 give an early stage in the formation of the chromosomes. The crossing of the homologous chromosomes during the early stages, as described by Janssens in his work on these phases, can be seen very

clearly during the stage figured in 16. Condensation of these diffuse elements is, of course, necessary for the formation of the chromosome units as seen in the later stages. This shortening forms the next step in the prophase series (Figs. 17 and 18). Figures 17 and 18 show the formation of the large tetfads seen in first spermatocyte metaphase very well. These chromosomes are very large and conspicuous during the prophases and are, of course, formed by the synapsis of the ten large chromosomes of the spermatogonial group. A more extended explanation of the mechanism of formation is given in another section of the paper.

After the chromosomes have become definitely formed, migration toward the equatorial region of the cell commences. Side views of early equatorial plates show generally one or more of the large tetrads lagging in their migration to the spindle. I am of the opinion that the bulk of these elements is an important factor in retarding their migration toward the center. In all cases of lagging observed, the largest chromosome was concerned (Fig. 19). There are exceptions to this condition, however, as can be seen in Fig. 20. Eleven chromosomes can be counted very easily in many of the prophases at this stage.

A peculiar behavior of the small chromosomes is evident throughout prophase. During early prophase, two dumb-bell elements are seen very constantly (Figs. 19,

20, 21). Metaphase of the first division shows four of these little bodies. On the other hand, spermatogonial metaphase demonstrates eight. The idea has occurred to me that during synapsis these small units condense into two. The prophase stages then see a precocious division of these quadrivalent structures which is completed before the definitive metaphase plate is formed. Consequently, four appear on the metaphase plate of the first spermatocyte. This phenomenon would seem to indicate a double coupling of these chromosomes during synapsis. Particular attention was bestowed upon the other chromosomes to see if such a condition were present in them, but no evidence of double coupling was observed in any of them. It was suggested to me in this connection that some forms may exist having chromosomes which fused in fours during synapsis and suffered disjunction into pairs during prophase. The fence swift seems to be a case in which this is, at least, partially true. Sufficient evidence is not at hand, however, for a serious consideration of this hypothesis. A further treatment of this point will be made when more material is secured.

### 3. Metaphase.

Side views of metaphase plates show the typical tetrad chromosome shapes for the larger chromosomes (Fig. 21). As was said before, early metaphases often

show one or more of the large chromosomes lagging. Their form and staining reactions can be easily noted under these conditions. Ordinarily, these large chromosomes do not stain uniformly. Vacuoles are often present also. Figure 22 is a diagram of a typical large tetrad as seen in a lagging case. Side and top views are given. It will be noted that entire fusion of the constituent parts of the cross has not occurred yet.

Polar views of the first spermatocyte metaphases are very numerous in my preparations (Figs. 24, 25, 26). The number of chromosomes is eleven. Size differences are pronounced and constant. Five of the chromosomes are large tetrads, one of which is larger than any of the others in this group. If one notes again Fig. 5, a spermatogonial group, the chromosomes forming the largest element can be picked out (a). The probable synaptic combinations for the other tetrads can also be inferred from this figure and from figure 7. Next in size to this group of five tetrads is a smaller somewhat rounded chromosome which is undoubtedly the product of the linkage of the two largest pieces of chromatin in the inner complex of the somatic group (Fig. 7). An oval element follows in size the intermediate rounded chromosome just described. The four smaller chromosomes are of about the same size and shape (Fig. 24). It is thus seen that there are marked size differences in the primary chromosomes as well as in

the spermatogonial univalents.

4. Anaphase.

Anaphases of the first division do not show a lagging chromosome of any kind. All chromosomes behave alike, the division of all units being equal at this time. Figures 27 and 28 are anaphases illustrating this condition. Variations and irregularities occur in this anaphase process, but the essential end product is an equal splitting of the chromosomes so that eleven are sent to each of the daughter cells.

A number of good polar views of these anaphase groups were obtained. Figures 31 and 34 illustrate the stage. Both show the chromosomes as about one-half the size of the metaphase chromosomes in the primary spermatocytes. Figures 32 and 33 illustrate a case where both anaphase groups are to be seen in the same cell. The distribution of the chromosomes can be clearly seen to be that of eleven to each of the daughter cells.

The telophase of the first spermatocyte division division shows a massing of the chromatin and a gradual loss of chromosome identity. Secondary synapsis occurs in the lizard. It is my opinion that the reduced number of chromosomes which appears in secondary metaphase is the product of this synapsis during telophase. Figures 35 and 36 show stages where the reduced number of chromosomes is the case.

After synapsis, the chromatin mass breaks up and scatters throughout the nucleus in preparation for the resting stages of the secondary spermatocyte.

## Secondary Spermatocytes.

Uncertainty surrounds my results on the secondary spermatocyte stages. In the material, secondaries were not found in sufficient numbers to justify definite conclusions. More work will be done on these stages when the breeding season arrives.

The work on the secondaries has been rendered difficult by an apparent condition of trimorphism. There are three kinds of secondaries if considered from the standpoint of chromosomes. The first type contains seven chromosomes; the others six and five respectively. Figures 38, 39, 40, and 41 are cases where seven chromosomes appear at metaphase of the second division. There has evidently been a second synapsis of some kind during the telophase stage of the first division, but the nature of the process is obscure. Figures 42 and 43 show metaphases with five chromosomes. The chromosomes here (especially in Fig. 42) are of the same size as the tetrads of the primary prophases. Figures 44, 45, 46 and 47 are cells with six chromosomes. Figures 46 and 47 demonstrate chromosomes which are noticeably smaller than the primary elements. The distribution of the chromosomes in the anaphases of the second

division is not clear. However, the division is probably equational. Secondary telophases have massed chromatin at the poles which makes study almost impossible (Fig. 47). In figure 47, there is a small lagging element, but such a condition is not the rule for these stages.

## Spermiogenesis.

The general course of the spermiogenesis can be worked out in the material. After the spermatids have completed their series of changes, the chromosomes lose their identity and the cell goes into a stage of rest (Fig. 47). The brief resting stage, in which the chromatin does not hold the stain well, is followed by a period of chromatin change. The chromatin of the nucleus changes its staining reaction so that extraction is more difficult. The nucleus appears densely granular (Fig. 49). Condensation of the nucleus then commences until it finally appears as a heavily staining sphere. The next step is an elongation of the nucleus and a corresponding change in the cytoplasm surrounding it (Figs. 52-53). Elongation of the nucleus continues and a clear space begins to show around the developing spermatozoon. The perforatorium is evident as a small knob at the narrow end of the cell (Fig. 54). The tail, which is cytoplasmic, is probably starting at this time from the cytoplasmic region near the larger end of the nucleus, but my technique was not fine enough to detect

it during these early stages. Figure 55 shows a continuation of the lengthening process. The perforatorium and the clear area are definitely marked. The clear area probably signifies a digestion of the cytoplasm for the nourishment of the growing spermatozoon. The digestion is carried on into the next stage where the mass of the cytoplasm is seen clinging to the base of the growing spermatozoon much diminished in volume. The latter stage (Fig. 56) shows a well developed perforatorium, the tail is quite thick and not very long, and the cytoplasm in the process of digestion can be seen clinging to the cell. This residual cytoplasm is not all used. Some of it is lost when the spermatozoon grows entirely out of the spermatid. Masses of residual cytoplasm may be seen in favorable preparations after the adult spermatozoa have oriented themselves to their new relations with the Sertoli cells. A heavy clump of material is seen below the cytoplasmic mass. This structure has not been worked out carefully, but a little evidence points toward its being the structure from which the tail is arising. The tail is in relation to it as one may see in the figure of the stage, so that it is entirely possible that we have here the substance from which the tail is formed. A middle piece was not seen. Ballowitz figures a spiral middle piece extending about one-half the length of the flagellum in the spermatozoon of the snake. In the case of the lizard, there is

none present and ~~that~~ the tail emerges from the cytoplasm in the region of the broad end of the spermatozoon. A ground for the belief that there is no middle piece rests in the observation that there is no apparent difference in the thickness of the tail in various regions. Centrosomes were not observed in any stages of this study, so their presence in the spermatozoa was not expected.

The adult spermatozoon is an elongate flagellate cell having a long perforatorium at its narrow end. The tail is quite long in proportion to the length of the head. All of these adult spermatozoa were studied in smear preparations made under the technique already described.

Measurement of Adult Spermatozoa.

Dimorphism with respect to actual size of adult spermatozoa has been demonstrated by several observers in forms having a definite accessory chromosome. Zeleny and Faust ('15) have published comprehensive results from thirty-three sets of measurements of spermatozoa, from widely different species. Dimorphism of the spermatids had been previously established in each of these species. Zeleny and Faust successfully connected adult dimorphism in size with spermatid differences in chromatin content. Their work shows a definite bi-modal curve for each of the species examined. Two kinds of adult spermatozoa in the pig

are demonstrated by Wodsedalek after the measurement of four hundred individuals. All of these results clearly show that in the forms considered, two kinds of spermatozoa, differing in length, exist. The assumption made from these facts is that the longer spermatozoa contain the accessory chromosome.

Measurements were made upon the spermatozoa of Sceloporus to determine if such a visible dimorphism exists in this reptile. Smears of adult spermatozoa were prepared, fixed in Bouin and stained with Iron Haematoxylin. The actual measurement of the specimens was accomplished with a Stufen micrometer ocular, No. 2, and a Zeiss 2mm. objective. The observations were all made with the same eye. Only the deeply stained chromatic part of the cell was considered. As the mean was quickly discovered, only 185 individuals were measured. This number may not be enough from which to draw conclusions, but, as 141 individuals of the total number lie at the mode of the curve, it seems safe for me to assume that a larger total would show the same relative per cent. The curve as finally completed shows only one mode. This mean is of spermatozoa ranging from six to six and one-half micra in length. A few were found at the extremes of five and ten micra. (Plate IV).

The results as obtained show that there is no

marked dimorphism in the spermatozoa, If two modes exist for the spermatozoa they must both lie between six and six and one-half micra. The means at my command forbade any closer measurement than one-half micron so at present I am unable to decide the point decisively. Assuming, however, that there is no second mode, it would seem that the spermatozoa can not be thrown into two classes with respect to adult length. The results as gotten are in keeping with the idea that there may be only one class of spermatozoa in this animal. A clearing-up of the problem of the condition found in the secondaries will do much to explain the facts ascertained about adult spermatozoa.

## Sertoli Cells.

As these cells are quite distinctive and characteristic, a description of them may be inserted at this place. They are more or less irregular in shape, conforming, in general, to the shapes of the germ cells around them. The nucleus, which is pictured in Figures 82 and 83, is very well seen but the cytoplasm is small in amount and is distributed between the germ cells so that it can not be made out with any degree of ease. The nucleus is characterized by a large indifferently staining plasmasome. Indenting it on either side are seen usually two small chromatic bodies which hold safranin and haematoxylin tenaciously. The rest of the nucleus takes the stain very

well, the granules of chromatin being finely distributed throughout. The little nucleoli described above are not always found in apposition to the plasmasome. In some cases, they may be seen at some distance from the plasmasome. These latter cases were not numerous, however.

Miss Stevens describes Sertoli cells in the guinea pig which are essentially like the interstitial cells discussed above. I have no doubt but that these cells in the lizard are of the same type. The spermatozoa, however, evidently establish nutritive relations with them after the spermatid cytoplasm has been almost entirely used.

Chromatoid Body.

It was with some interest that I noted in the spermatogenesis of the lizard a chromatoid body similar to that described by several workers. In the lizard, a definite cytoplasmic body with chromatin staining reactions can be seen as early as the resting phases of the secondary spermatogonia (Fig. 2). In metaphases of the spermatogonia it may be seen outside the ring of large chromosomes (Fig. 60). During divisions of the spermatogonia, it is inertly carried over into the cytoplasm of one of the daughter cells. Growth period of the primary spermatocytes shows the chromatoid body in about one-half of the cells. In the early growth stages, it is surround-

ed by a heavily granular portion of the cytoplasm which resembles the idiozome of other forms. Figure 61 is a prophase of the first division; figure 62, a metaphase; figure 63, an anaphase; and figures 64 and 65 telophases. All show the chromatoid body lying in the cytoplasm of the cell. The telophases clearly demonstrate that it goes entire to one of the daughter cells. Figure 66 is a metaphase of the second division. The chromatoid structure is again present. Telophase again sends the body, somewhat diminished in size, to one of the spermatids. The whole process is one of a simple carrying-over of an inert body from one stage to the next. The chromatoid body is not in relation to the spindle fibers in most cases, but lies in the cytoplasm and is constricted off with that part of the cell during the mitosis. After reaching the spermatids, the body gradually disappears. Stages in the disappearance are shown in Figures 47-53.

## The Ovary.

An attempt was made to ascertain the female somatic number of chromosomes from the follicle cells of the ovary. In all of my ovarian material, the chromosomes of the follicle cells were so crowded that an accurate count was impossible. Incidentally in this work, my attention was attracted to a phenomenon which was rather

common in the developing eggs. There seems to be a definite ingestion of follicle cells by the developing ova. Large eggs showed entire cells in their cytoplasm (Figs. 69-74). Other eggs showed different stages in the breaking down of these ingested ova. Figure 68 pictures an egg in which there are no less than four smaller neighbors. Figures 70, 71 and 73 are similar stages with only the nucleoli of the ingested cells undigested. The process of digestion seems to be one of gradual fluidification with no fragmentation. The nucleolus apparently is more resistant than the rest of the cell, so that it remains until last in the midst of the clear digestive area. It then dissolves slowly and uniformly.

A yolk nucleus is present in the cytoplasm of the egg. It is nearly always in relation to the ingested cells, a fact which would seem to indicate that it has s something to do with the digestion process. Although one is the usual number of these yolk nuclei observed in a single cell, two or more have been seen as exceptions to the rule (Fig. 71). The method of yolk elaboration is seen in figure 73. This figure demonstrates the fragmenting distribution of the yolk from the yolk nucleus. The particles of yolk seem to be shot off from this central nucleus.

Haematoxylin and the safranin-light green combination were used on the ovary sections. The latter gave

a good contrast in which the yolk nucleus and the cytoplasm were green and the nuclear materials, including the ingested nuclei, were red.

Explanation of the Formation of Tetrads.

The characteristic large tetrads of the primary spermatocyte stages are formed in the following manner: The pachytene thread, a short thick strand, is resolved out of the synezesis mass of threads. I have not been able to see a rope-like structure for this thread but the conditions seen in the segments resulting from it seem to indicate that the pachytene coil is rope-like. The thread soon breaks into segments, the largest of which gives rise to the five tetrads.

Each segment consists of two strands, or threads, in relation to each other like the strands of a rope. The ends of the strands at the extremities of the segment swing apart in a manner which suggests unwinding. The divergence of the ends continues until finally the strands cross each other only at one place. That they are not completely fused at that place can be easily seen in the preparations. They are possibly merely stuck together at that point. It seems reasonable to assume, however, that partial fusion may also take place at the crossing-place. The next stage in the process is a condensation of the arms of the cross which resulted from the preceding series of changes. The

condensation is toward the center. The adult tetrad shows no trace of the crossing of the components, evidently indicating that entire fusion has occurred. Figure 75 is a diagrammatic illustration of the method of tetrad formation.

During synapsis, homologous chromosomes are paired. It is also very evident that para-synapsis is the case for the lizard if we may judge from the conditions seen in prophases. The question which then arises is, "Can we assume that there is complete fusion of both in a given pair along their entire length?" Janssens has brought forward evidence to show that the chromosomes do not fuse along their length but that fusion and consequent exchange of chromatin takes place only at certain nodes, or crossing places of the homologues. At disjunction, then, the original synaptic chromosomes are quite different in constitution. Besides the fusion at the nodes, there is entire exchange of the parts of the two chromosomes between the nodes, so that the original chromosome exchanges some of its chromomeres with its mate.

In the present instance, the component chromosomes after synapsis are twisted about one another in the manner previously described. Does fusion and transfer of the chromomeres take place along the entire length of the contiguous surfaces? Evidently not, for when the strands

swing apart forming a cross, one of the strands can be seen crossing the other. Fusion at this place does not occur until condensation begins. The material would seem to show that there are not several nodes but only one in the lizard.

The above explanation of the formation of the tetrads is different from that given for insects, in that there is no evidence of a longitudinal splitting in the pachytene segments. The form seems to show a process which looks like the unravelling of a rope.

## Discussion.

The phenomenon of double synapsis in the case of the smallest chromosomes during the synezesis period, is in my mind one of considerable importance. Evidence was sought for a consideration of the whole chromosome group from the standpoint of a synapsis in fours, but the condition could not be proved. Only the small chromosomes show this grouping during synezesis. It is very possible that other forms may show double pairing for the whole group. If the latter case is demonstrated in any one of the reptiles, the conclusion to be reached from the study of the lizard is that we have here a transition stage to the condition of complete double pairing. To carry the hypothesis further, two of the chromosomes are of paternal origin and two are of maternal derivation. This is

perhaps the case for the small chromosomes. But the larger elements evidently do not have this mixing at primary synapsis. It will be recalled that the view was expressed that perhaps germinal mixing of the tetrads was not great during synezesis. The fact that there seemed to be incomplete fusion of the elements in the tetrad stages strenghthens the idea that mixing occurs at the time of second synapsis. Primary synapsis is, then, a non-important stage for the tetrads.

Guyer ('09) puts forward the following speculation to account for secondary synapsis in the guinea fowl, "The idea suggests itself that in this secondary reduction in numbers we have an exemplification of a tendency toward a diminution in the number of chromosomes, due possibly to more closely-knit correlations in the germinal substances." Closely-knit correlations may, it is possible, be responsible for the double conjugation which was observed in the lizard during synezesis and for the second reduction in numbers in the secondaries. This is only a guess, however, as there is no way of proving it at present.

Throughout the entire course of the spermatogenesis, a definite cytoplasmic body, similar to that described by Wilson for Pentatoma , was observed. Wilson

finds that this cytoplasmic body, which simulates a chromosome, arises from an enlarging granule in the cytoplasm of the primary spermatocytes. Previous to the formation of the definitive cytoplasmic body in Pentatoma, several granules in clear spaces appear in the cytoplasm. All but one of these disappear. The survivor then enlarges to form what Wilson calls the "chromatoid body." The chief characteristic of the structure is the fact that it behaves like chromatin. Furthermore, it behaves like a typical accessory chromosome. The warning is given by this monograph that all lagging bodies should be thoroughly studied before the function of accessory chromosome is assigned to them

Other instances of the presence of cytoplasmic bodies with peculiar behavior are recorded by King ('07) for Bufo and by Benda ('91) for a mammal. Benda found a "chromatoid Nebenkorper" in the primary spermatocytes which was not in relation either to the centrosome or the attraction sphere. Benda traces this body to the spermatids where it disappears, but ventures no explanation for its presence. The origin of this body is discussed by him and the conclusion is reached that it is thrown out of the nucleus.

Miss King finds a similar structure in Bufo which she considers a condensed cytoplasmic body. The body divides at every mitosis until finally each sper-

matid receives a part of the original structure. The function assigned by Miss King is that of acroblast, or portion of the spermatid which gives rise to the acrosome. More recently, other vertebrates have shown a definite chromatoid body. Wodsedalek, for example, points out a chromatoid body in both the pig and the horse. In these cases, it is a structure which disappears in the spermatids.

The chromatoid body in the lizard is seen to be somewhat inert during the prophases. It does not go to the metaphase plate with the other chromosomes but remains in the cytoplasm while the spindle is forming. When the cell divides, the chromatoid element is left in one of the daughter cells. It is usually seen in the cytoplasm of the daughter cell near the massing chromatin of the nucleus. At the division of the secondary spermatocyte into the spermatids, the body again goes over entire to one of the daughter cells. It can be easily seen, therefore, that only one-fourth of the spermatids receive the element. It can not be considered as an acroblast, since all of the spermatozoa possess acrosomes, or perforatoria. It may be chromatin in a degenerate condition which has been ejected from the nucleus to be absorbed by the cytoplasm. Weight is lent to this consideration by the fact that the body is much smaller in the secondaries than in the primaries, thus tending to show that the structure is

a disappearing one.. A clear space around the chromatoid body in some of the cells is evidence which may betoken a gradual digestion of the structure.

Considering all of the evidence on this body at hand, it seems to me a settled question that the chromatoid body is of no consequence in the spermatogenesis. It is probably degenerating "chromatin-like" material which is absorbed before the end of the series.

It will be noted that no accessory chromosome was observed in this spermatogenesis. The chromosomes are all paired at synapsis, and there is no aberrant element during the reduction divisions. In short, there is no evidence of the peculiar and sex-determining chromosome of other animals.

In seeking an explanation for this condition, attention must be called to the fact that in other Sauropsida, notably the birds, the breeding results indicate that there is only one class of spermatozoa. The eggs of the species are of two kinds. Breeding results in the chicken, for example, prove this to be a fact. Guyer has found an accessory in the chicken, but Boring and Pearl just as firmly deny the findings of Guyer on this form. There is no reason, then, why the reptiles may not also conform to the condition as found in the chicken. Attempts were made to settle this point decisively for the lizard by a study of the female cells, but suitable material was not forth-

coming. I shall try to clear up this phase of the work on the lizard when I secure some embryos.

Strong support is given to the expectation of female dimorphism when one considers the measurements which were made of the adult spermatozoa. In this work, it was noted that there was only one large class of spermatozoa. In other words, actual measurements show that all of the spermatozoa are alike. On the other hand, no consideration was given to the widths of the individuals in this experiment. The latter consideration can probably be eliminated entirely since no accessory was observed.

The conditions as seen in the secondaries are very puzzling. I have no explanation to advance for finding three kinds of spermatids. More material will probably clear this point up. At present, however, I am inclined to the belief that the chromatoid body has complicated the stage, although the manner is not clear. The secondary and the spermatid stages have not been numerous in the present material. Confusion in the study of the stages mentioned is increased by much crowding and indistinctness.

Jordan has recently allowed me to use his manuscript and plates on two turtles, the only other reptiles which have been investigated. Comparisons for similarity are almost impossible, since his forms are in no way like

the lizard. In fact, Chrysemys does not even agree with the closely allied genus, Cistudo. This would lead to the conclusion that there is great variation in closely allied reptiles. However, proof is set against this idea by my own incomplete observations on Phrynosoma, the horned lizard. The horned lizard resembles very closely in chromosome content the common fence swift.

Jordan attacks the belief that the interstitial cells are correlated with secondary sexual characters. In the turtle, there are many interstitial cells and few secondary sexual characters. The lizard, on the other hand, shows practically no interstitial cells and few secondary sexual characters. Jordan's facts may point to a seasonal variation in interstitial cells, but the lizard shows a dearth of these structures at all seasons. The lumina of the tubules are always large in the lizard and the tubules are compactly placed, a condition which permits of no room for interstitial cells.

Amitosis seems to be the case for some of Jordan's spermatogonia and spermatids. On this phase of his work, he is not clear yet, so conclusions are not drawn from these observations. There is no evidence of any kind for amitosis in the lizard. The method of indirect cell division is strictly adhered to.

In keeping with my results on Sceloporus, the accessory is not found in Chrysemys. Cistudo, however,

shows a definite structure which looks like an accessory chromosome. Uncertainty veils the whole question of the accessory, since no ~~argument~~ agreement can be reached in three different genera of reptiles.

No synezesis figure was observed in Chrysemys although Cistudo is a clear case with the typical figure. The lizard exhibits a definite synezesis stage similar to that described for other vertebrates. Synapsis is undoubtedly the case for the lizard. It seems even to go the extent of being a partial conjugation in fours. Jordan's turtles offered no suggestion of a quadrivalent grouping, but this work is incomplete and inconclusive at the present time.

In the lizard there is some evidence for a theoretical discrepancy which seems to make this form different from any other studied heretofore. The nearest thing to a reduction is the second synapsis of the chromosomes which was observed in the primary telophases. Synapsis gives bivalent chromosomes on the metaphase plate of the primary spermatocyte. Equational division of these bivalents, followed by the second synapsis of the chromosomes, shows seven or less chromosomes on the equatorial plate of the secondary spermatocyte. These elements have the appearance of bivalents. Now, although the secondary division stages are not clear, it is easy to see that no matter what happens, the sum total of the chromosomes in

terms of univalents will be seven of less for each spermatid. Under normal conditions of spermatogenesis, eleven chromosomes of the univalent kind should be present in each spermatid. Under Guyer's principles of secondary synapsis, the chromosomes resulting from secondary synapsis are quadrivalent in nature in conditions where the first division is reductional. Equational division of the secondaries then sends bivalents to the spermatids. But, as was seen, the number of univalents is not great enough in the lizard to adhere to this hypothesis. Eleven is the theoretical number and seven or less are to be seen in the trimorphic spermatids.

This aberrant condition as seen in the lizard may be a case where consideration should be taken of chromatin quality and not of quantity. Under this idea, so far as heredity is concerned, the reduced number of univalents in the spermatids is the qualitative equivalent of eleven univalent chromosomes. These univalents would then be the potential equals of a much larger number.

The evidence as brought forward in this study seems to point toward a modification of the chromosome theory of sex-determination. There is no definite accessory chromosome at any stage of the spermatogenesis. The only other hypothesis remaining open is that of female dimorphism. The solution of this problem will be pushed as rapidly as possible by the author.

## Summary.

1. The spermatogonia show twenty-two differently sized chromosomes in metaphase plates, all of which pair as synaptic mates. There is no unpaired accessory chromosome.

2. During synapsis, the eight small chromosomes condense into two. Prophase then shows a precocious division of these two again.

3. Lagging is evident during the prophases of the primary spermatocyte, notably in the case of the largest tetrad.

4. The metaphases of the primary spermatocyte show eleven differently sized chromosomes.

5. Primary division is equational, eleven going to each daughter cell.

6. Secondary synapsis of the chromosomes takes place during telophase of the primaries and resting stage of the second spermatocyte.

7. There is a seeming trimorphism in the secondary spermatocytes. One kind shows seven; a second, six; and a third, five. The secondary distribution of chromosomes to spermatids is not clear.

8. Transformation into spermatozoa is direct by elongation of the cytoplasm and the nucleus.

9. The adult spermatozoon conforms to the reptilian type in having a long perforatorium. A middle piece was not seen.

10. Measurement of adult spermatozoa reveals one kind with respect to size.

11. A chromatoid body is present which has a peculiar behavior.

12. There is active cell ingestion in the ovary by the developing ova.

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## Explanation of Plates

### Plate I.

All drawings on this plate were made with a Zeiss 1-30 objective and a No. 12 apochromatic ocular. The camera lucida was used to get the outline in each case. The drawings seem to show discrepancies in size, but all are based on actual specimens. Some cells are evidently larger than others at the same stage.

Figure 1-----Primary spermatogonium, abundant in resting testes. Two chromatin nucleoli show here.

Figure 2-----Secondary spermatogonium showing the beginning of the chromatoid body. Increased nuclear size.

Figure 3-----Spireme stage of the spermatogonium.

Figure 4-----Prophase of spermatogonium. Spireme has segmented and condensed into chromosomes.

Figures 5,6,7,--Metaphase plates of spermatogonia showing 22 chromosomes. Figures 5 and 7 lettered for synaptic mates.

Figure 8-----Telophase of spermatogonium showing two or three elements aberrant in behavior. Condition is not rule in these stages.

Figures 9, 10--Early growth stages of the first spermatocyte. Both are leptitene stages, Figure 10 is

an exceptionally large cell. The chromatoid body may be noted.

Figure 11----Pre-synapsis stage. Chromatin tangled in the middle of the nucleus. Nucleoli still dimly seen in the mass.

Figure 12----Synezesis. Chromatin masses at the side of the nucleus. Nucleoli disappearing.

Figure 13----Amphitene stage. Double thread can be seen at places.

Figure 14----Pachytene stage. Thread shorter and thicker.

Figure 15----Beginning of chromosome formation. Condensation of the pachytene segments. Only a part of the nucleus is shown.

Figures 16, 17, 18---Chromosome formation. Tetrads form by condensation.

Figures 19, 20, 21, 23----Prophase side views. Figures 19 and 20 show 11 chromosomes in side view. Note lagging of tetrads. Figure 22 is a diagram of tetrad in side and top view.

Figures 24, 25, 26----Metaphases of primary spermatocytes. Eleven chromosomes. Five tetrads, two intermediates, and four microsomes.

Plate II.

Drawings made under same conditions as in Plate I.

Figures 27, 28, 29, 30--Side views of anaphases of first spermatocyte. Equational division of all chromosomes.

Figures 31, 34--Polar views of anaphases just completed. Eleven small chromosomes may be seen.

Figures 32, 33--Two anaphase groups in same cell. Eleven in each group.

Figure 35----Telophase polar view. Chromosomes massing up for second synapsis.

Figure 36----Late anaphase of first spermatocyte. Synapsis has evidently proceeded precociously in this specimen.

Figure 37----Resting stage of the second spermatocyte, Much chromatin and two nucleoli present.

Figures 38, 39, 40, 41--Metaphase second spermatocyte. Seven chromosomes present.

Figures 42, 43----Metaphases second spermatocyte. Five chromosomes present.

Figures 44, 45----Metaphases second spermatocyte. Six chromosomes can be noted.

Figures 46, 47a---Other cells with six chromosomes much smaller than in Figs. 44-45.

Figure 47b---Telophase second spermatocyte. Lagging body present.

Figures 47c, 48, 49, 50, 51, 52, 53, 54--Stages in spermiogenesis. The disappearance of the chromatoid body is pictured in 47c to 53 inclusive.

Plate III.

Figures 55--67 inclusive were made under the conditions of Plate I. Figures 68--74 inclusive were drawn with camera lucida, Zeiss 1/30 objective and Zeiss No. 6 ocular.

Figures 55--57. Completion of spermiogenesis. Figure 57 is adult spermatozoon. In the measurements made in Plate Iv, only the dark chromatic part was considered.

Figures 58-59. Sertoli nuclei. Large, dimly staining nucleolus and several strongly staining nucleoli. The cytoplasm of these cells extends to spermatozoa between the germ cells. Location, near periphery of tubule.

Figures 60-67 inclusive. Conduct of the chromatoid body.

Figures 68-74 inclusive. Ingestion of cells by developing ova. Figure 69 shows entire small cell in cytoplasm. Yolk nucleus in relation to it. Figures 70 and 71 demonstrate nucleoli of digesting cells. Figure 73 is an egg where yolk granules seem to be shot off from the yolk nucleus.

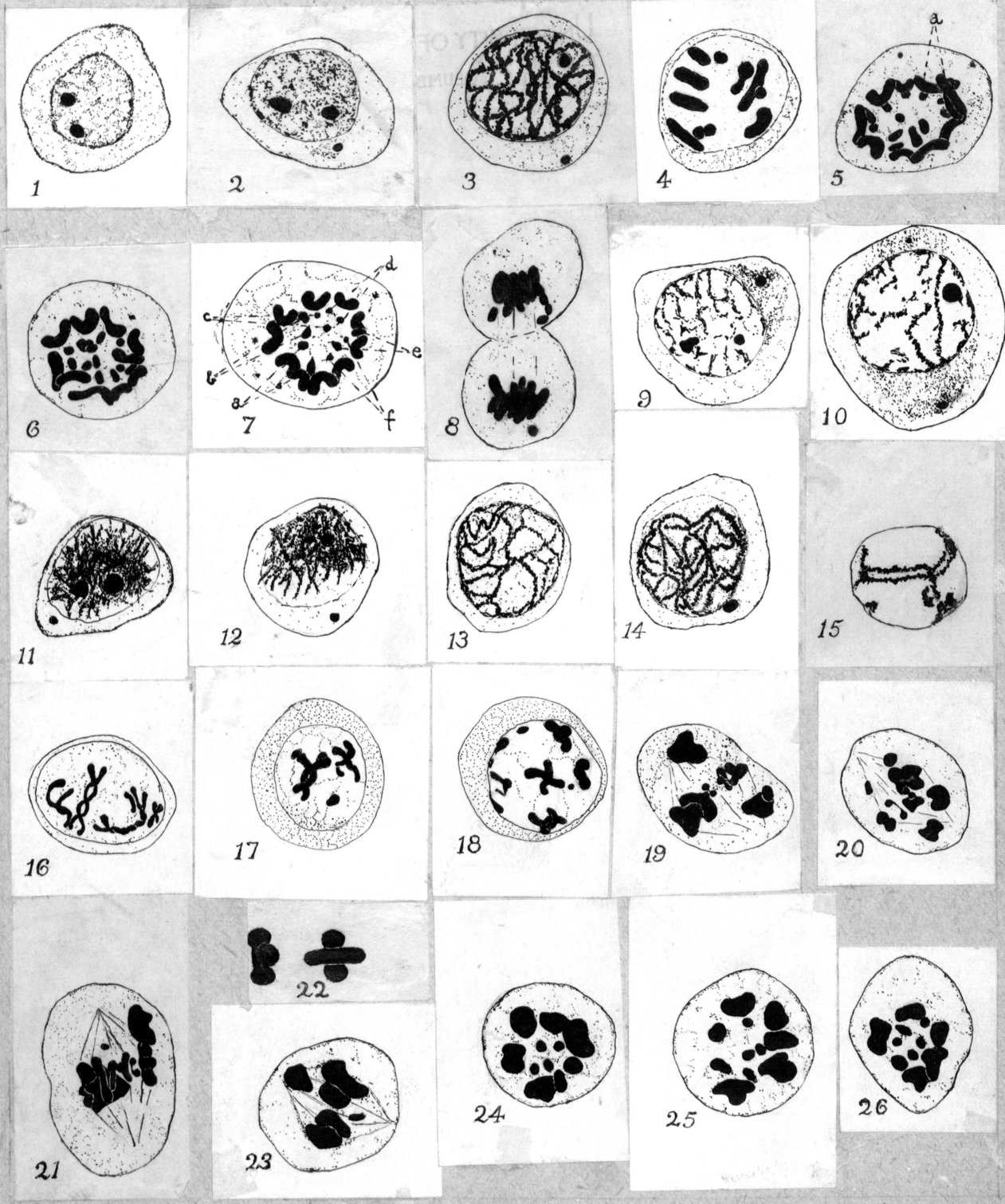
Figure 75. Diagram illustrating the method of tetrad formation.

Plate IV.

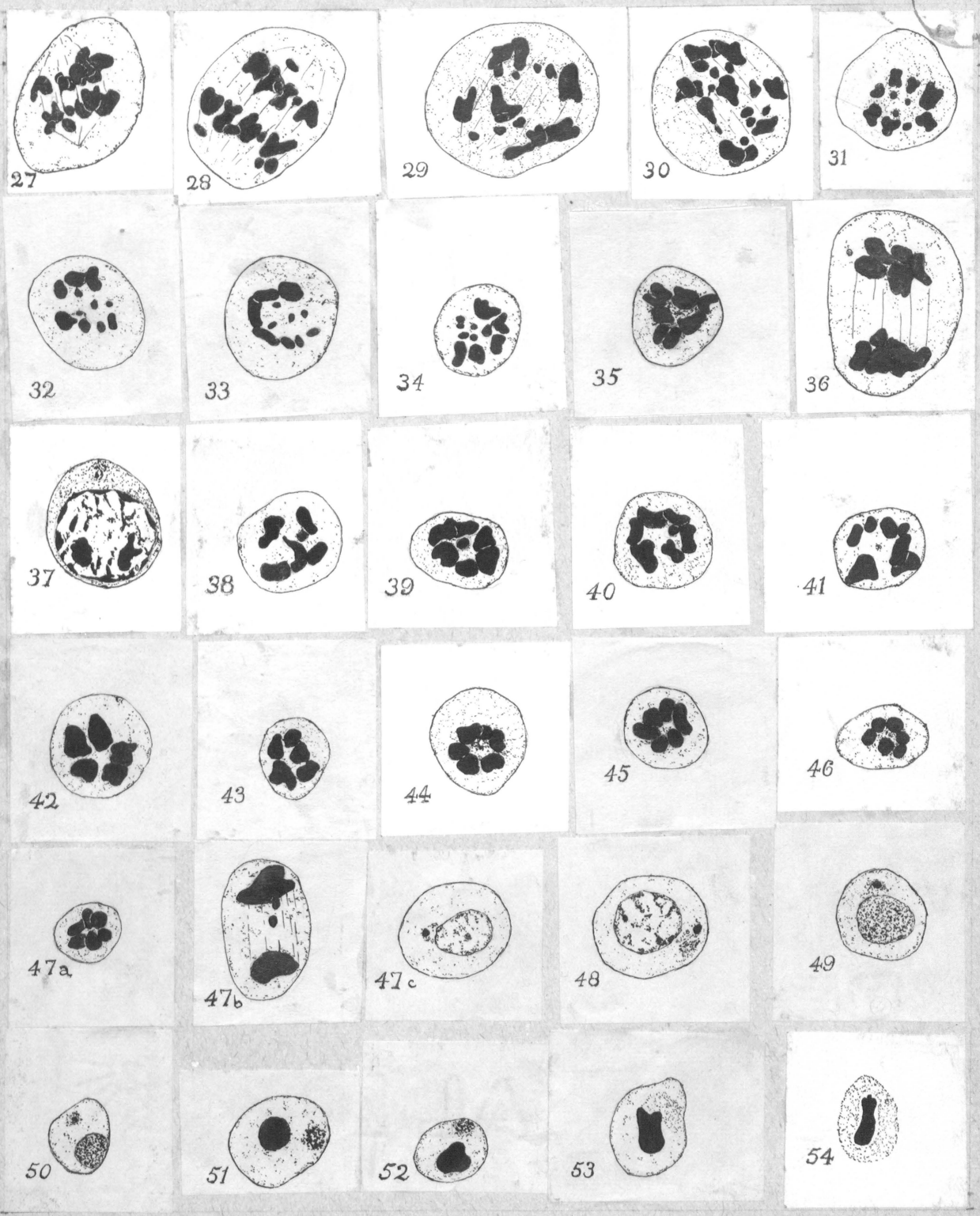
Curve showing actual sizes of 185 adult spermatozoa. Ordinates are numbers of individuals. Abscissae give the length in micra.

# Plate I





# Plate II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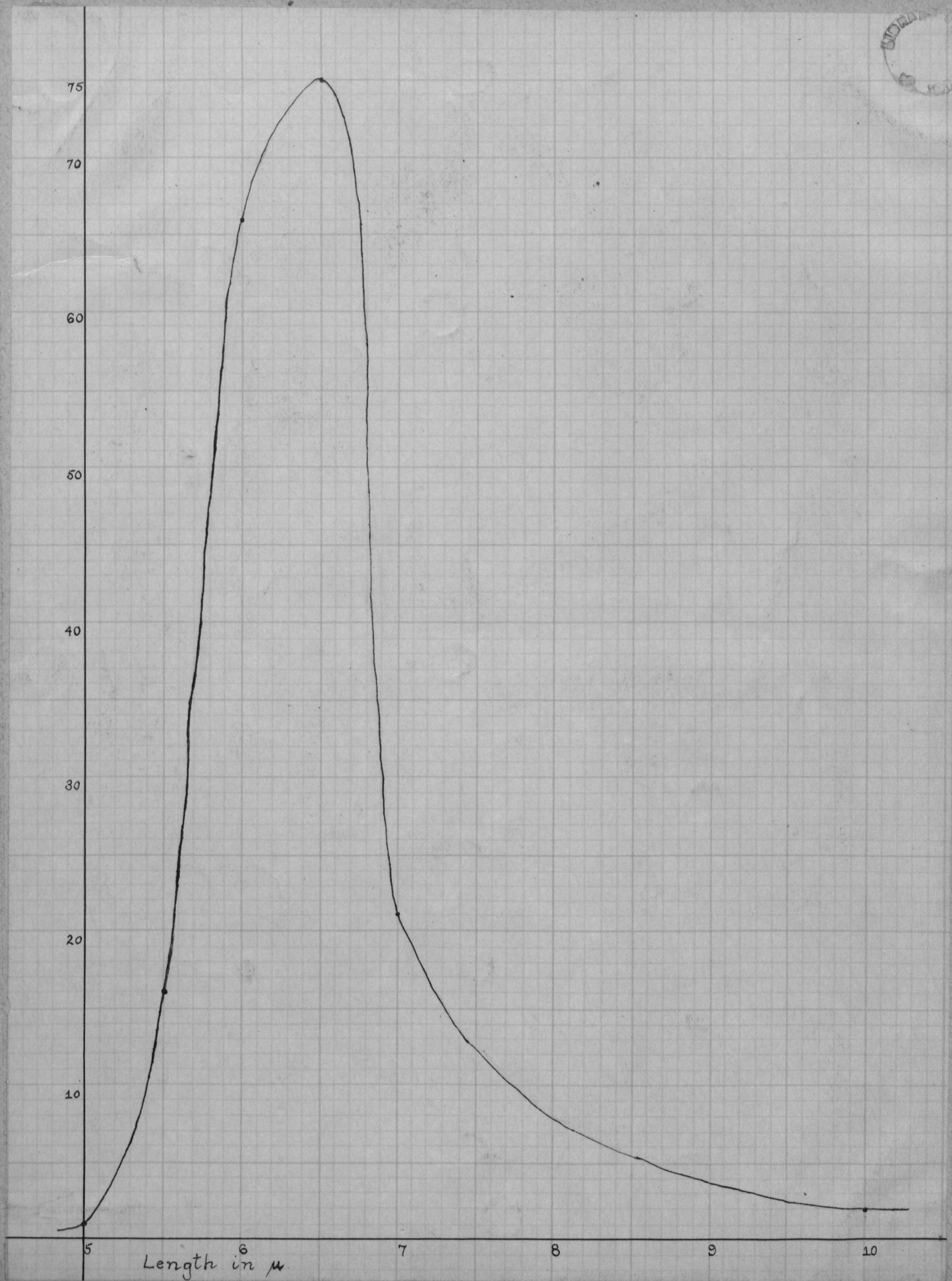
# Plate III

# Plate IV



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Chairman of Graduate Committee.

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The thesis of Mr. E. E. MacMorland is a strong paper, in my judgment, well meets the general standards followed in the Biological Sciences group.

Very truly,

Chas. W. Greene

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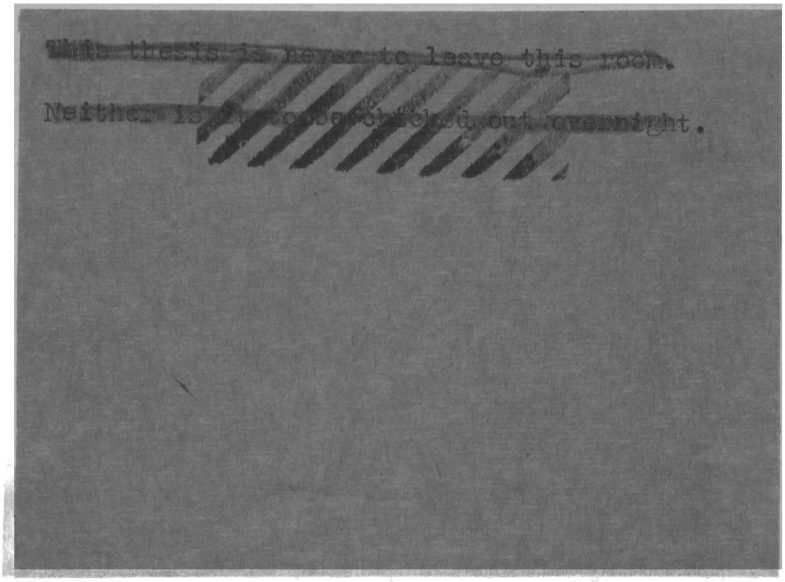

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