Spermatogenesis of the Meadow Vole

(Microtus pennsylvanicus)

and

a

Comparative Study

of

Rodent Spermatogenesis

by

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Author's Abstract.

This study is composed of two parts.

Part 1 deals with the spermatogenesis of the meadow vole. There are 42 chromosomes in spermatogonia and 21 in primary spermatocytes. Amniotic cells were found to contain 42. The sex chromosomes are of the usual x-y type. The fate of the chromatin-nucleolus has been followed to the first maturation division except for a brief period in the late prophase of the primary spermatocyte.

Part 11 deals with a comparative study of the hhromosome complex of 5 rodents and the rabbit.

Acknowledgments.

I wish to express appreciation to Dr. W.J. Baumgartner, under whose supervision this problem was done, for his encouragement, friendly advice and criticism. I also wish to acknowledge the kindness of Dr. H.H. Lane for generous aid and the placing of many of the facilities of his department at my disposal.

Part I.

Spermatogenesis of the Meadow Vole (Microtus pennsylvanicus) and a Comparative Study of Rodent Spermatogenesis.

8 plates
90 figures

Introduction

To my knowledge, no cytological study has ever been made of the meadow vole, Microtus pennsylvanicus. The wide distribution of this species, together with the fact that these animals are easily bred in captivity, make it desirable that a study of the morphology of the chromosomes, the correct chromosome count and the type of sex chromosomes be determined.

There is a two-fold purpose in making a series of studies on the chromosomes of rodents, the first of which is to determine exactly the the number and morphology of the chromosomes

in the rodents commonly used by the geneticists in breeding experiments. The second is to lay the ground-work upon which future comparative studies of rodent chromosomes may be based in order eventually to plot the chromosome map of this order. It is hoped that this study of the meadow vole may prove to be a small contribution toward this larger problem.

Probably no element has caused more confusion in cytological investigations than the two large deeply-staining bodies usually called the chromatin nucleoli, which appear during the growth period of primary spermatocytes. Von Lenhossek ('98) was the first to draw the attention of cytologists to the 'internuclear body' of the white rat, and later investigations have shown that many, if not all mammals, exhibit a similar structure. These bodies have come to be regarded by many cytologists as being made up of the sex-chromosomes, but probably the evidence is not complete enough to form any definite conclusions in the matter. In this paper, the nucleolus has been traced up to the time of the spindle formation of the primary spermatocyte.

Materials and Methods

The testes of eight adult field mice, one half-grown specimen, and fragments of the amnion from three half-mature embryos form the material for the present study.

Allen's (1924) modification of Bouin's fluid, with certain minor changes, was used for fixation. The most satisfactory method of stirring the tissue while using the drop method for exhhange of alcohols was effected by using an electric stirring apparatus.

Sections were cut six to nine micra thick,--those cut at eight being the most satisfactory for study.

Iron-haemotoxylin was the principal stain employed. Several slides were stained with Fleming's triple stain, and these slides proved valuable in the study of the chromatin nucleoli. Eosin counter-stain was employed in some cases after iron-haemotoxylin, but the results obtained were not satisfactory for study. Slides should be

well destained for a study of the division stages, for it was found that chromatin stains much more darkly during the spermatogonial and spermatocyte divisions.

Perhaps one point in regard to breeding might be of interest to any one wishing to carry on breeding experiments. It was found that when individuals were mated and placed in a small cage, that only very seldom were litters obtained. The animals are quite gregarious in nature, and the best results were secured by placing several males and females in a large cage, 34"x36" in which about six inches of fine hay had been placed. They choose their own mates, and each pair makes a nest of its own before the birth of the young.

Description

That each species has a definite chromosomal complex has been shown by extensive work with insects, plants and animals. The number of chromosomes is practically constant, although certain exceptions occur due to fragmentation, polypoidy or fusion. A gradation in size is usually present in

the spermatogonial and spermatocyte metaphase. This series in the spermatogonia is double as there are almost invariably two of a given size and shape. Of these two paired chromosomes, one has been derived from the male, and the other from the female parent.

The germ cells in preparation for fertilization undergo a series of changes called spermatogenesis. A brief summary of the series of changes that take place in the nucleus of the field mouse, and the nomenclature employed may prove beneficial at this point.

A resting stage follows the last spermatogonial division and this is soon followed by the appearance of leptotene threads. These threads pair or synapse and form pachytene loops with ends lying, for the most part, near the nuclear wall. These loops thicken, shorten, and take the stain very intensely. The growth follows in which the size of the nucleus increases rapidly. The post-synaptic spireme consists of shorter and thicker threads usually called pachytene, of half the original number. In many cases the thick threads

show no external sign of duality as if the synaptic mates were completely fused to form a single pachynema. This condition exists in most mammals. In most insect material the duality is discernible. Probably the duality is always present in their internal structure, even when it does not appear on the surface. Later, however, the threads are plainly longitudinally double, (diplonema). These diplotene threads expand, diffuse and take the stain less intensely. This period is called the diffuse stage and is followed by the contraction of the diplotene threads and an increase in staining capacity. A further contraction of the chromatin into crosses, U's and circles ensues. This is called the diakinesis period. From this period, the chromatin constricts or shortens in some manner, and is soon ready to enter the first maturation spindle.

The maturation consists of two cell divisions of a somewhat different type. These are known as the first and second maturation divisions. Before the first maturation division, homologous chromosomes conjugate. Each member has already split as for an

ordinary division, so that the result is a chromosome called a tetrad composed of four parts or or chromatids which are usually not distinguishable parts (Fig. 32-34). At the first maturation division, every primary spermatocyte gives rise to two cells, each of which contains the same number of chromosomes as its parent cell, but instead of four parts, each chromosome now has two and is called a dyad.

At the second maturation division in the male, both of the cells derived from the divided primary spermatocyte divides, giving four spermatids in all, each containing one chromatid from every tetrad. That is, if a primary spermatocytic spindle had twenty-one tetrads, each of the four resulting spermatids would have twenty-one chromosomes but every one would be a single monad.

Spermatogonial Divisions

Two generations of spermatogonia can be distinguished in the meadow vole. The primary spermatogonia, seen more abundantly in the testes of the half grown individual, are larger, usually oblong or rectangular in shape and occur in the basal layer of

of the tubule. The nucleus appears round or oval, about 13.32 microns in diameter, and the chromatin is evenly distributed with traces of fine reticular structure. The nucleus of these cells contains two darkly staining bodies, the larger being approximately 2.2 microns in diameter, while the smaller one is about half that size. (Fig. 1). Jordan ('12) found only one nucleolus showing in the primary spermatogonia of the opossum, however, the presence of two is quite generally the case among the mammals.

The secondary spermatogonia are smaller cells that lie, for the most part, near the membrane of the tubule and appear more abundantly in the adult male. The average nucleus is about 11.1 microns in diameter and the chromatin is scattered in an indefinite granule-reticular network. These cells contain two nucleoli that are nearly always surrounded by what looks to be a faintly staining vesicle. (Fig. 2). The nucleoli appear of somewhat different shapes in the various cells, sometimes appearing closer together. This difference is probably due to different views one gets in various sections.

This condition possibly may favor the idea that the nucleolus functions in part as a storehouse of chromatin, an idea first stated by Haecker ('95) in his transportation hypothesis. On the other hand, many cytologists from the time of Flemming ('82) have considered the nucleoli generally as centers for storage of substances such as linin, plastin or chromatin, destined to play some part in later operations.[1]

Apparently spireme formation is initiated according to the second type mentioned by Wilson ('25), page 122. The nuclear framework is drawn together into localized areas and these areas condense into chromatin blocks in which the netlike structure is hardly visible. (Fig. 3). As the prophase proceeds, fine threads weave out from these blocks into the spireme stage. The events leading to the metaphase stage are practically the same as described for all the other mammals.

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[1] This is discussed fully in Wilson The Cell in Dev. and Heredity. p. 95.

A considerable amount of time has been devoted to a study of spermatogonial chromosomes so that they could be recognized in the equatorial plate views. The chromosomes at this stage have rather definite sizes and shapes and after a great many sketches have been made of cells and parts of cells in which each element can be clearly distinguished, the different chromosomes become somewhat familiar.

Drawings were made of fifteen metaphase equatorial plate views before a count was made in any of them. On checking up it was found that there were forty-two chromosomes present in nine of the cells drawn. Six of these cells are shown in Figures 6 to 9, and in each cell there are forty-two chromosomes. For the most part, the chromosomes in this stage appear as short thick rods, elongated rods or comma shape. With the exception of one relatively large pair and several quite small ones, the chromosomes are made up of medium sized elements that do not vary much in size. Figures 6 to 9 show rather late metaphase views in which the chromosomes have begun to move apart. It will be noticed that the chromosomes appear larger and

be noticed that the chromosomes appear larger and thicker due to the fact that a slight division has started. Figure 9 shows one chromosome in which a split can already be seen. As can be seen in the figures, the chromosomes have rather definite mates with the exception of two for which no mates of similar size and shape could be found. These are labeled the x and y. It will be noticed that the y is the smallest element in the cell, and is usually toward the center, surrounded by the other chromosomes. The x is a long, thin, medium sized element found near the edge of the cell. Due to the fact that there are an even number of chromosomes and that two elements fail to pair, it is concluded that the sex chromosomes are of the x-y type. An approximate alignment of the chromosomes in cells 4 and 7 are shown in figures 47 and 48. Because of so many medium sized elements so nearly the same size, any attempt at accuracy in alignment is futile.

Counts were made in several metaphase plate views in amniotic cells from about half mature embryos. One of these cells is shown in Fig. 10

and the number is undoubtedly 42. The cells of the amnion are larger than the germ cells, the one being 26.6 microns in diameter and the chromosomes are much longer. No x-y elements could be identified but it is possible that this cell is from a female embryo having an x-x complex.

In studies of the amnia and germ cells of the rabbit, Painter ('26) found that amnia over three weeks old showed deviations from the regular number of 44 found in the germ cells and in twelve younger embryos. On the basis of extensive studies made with the rabbit, and the examination of three Microtus pennsylvanicus embryos, it may be concluded that young growing amnion cells contain the same number of chromosomes as do the germ cells in these two species. More extensive studies should be made in order to form any general conclusions.

Primary Spermatocytes

Following the telophase of the last spermatogonial division, the chromosomes seem to

swell, stain less densely and begin to diffuse. (Fig. 11). One element stains more deeply than the rest and persists in a later stage, (Fig. 12), taken from a longitudinal section of the tubule with two cells intervening. Here the diffusion process has continued until the forms of only two of the chromosomes can still be distinguished. The diffusion process continues until a reticular resting stage is reached. (Fig. 13), in which two chromatin-nucleoli are seen. These stain quite black with iron haemotoxalyn, the smaller of the two staining more intensely.

After a resting stage, the primary spermatocyte enters into a growth phase that occurs along with synapsis. The reticulum of the resting nucleus changes to a delicate spireme, (Fig. 14). These leptotene threads thicken and stain more densely (Fig. 15-16). Two dark areas are still noticeable in Fig. 15.

The distinct bouquet stage described by so many investigators was not observed in the meadow vole material. Rather, the ends seemed to

terminate at different places along the nuclear wall, (Fig. 17). The spireme threads thicken and shorten into leptotene threads andthere is definite suggestion in many cells (Fig. 17), that these threads pair, beginning at the nuclear wall.

The first suggestion of a side by side conjugation came from Winiwarter in 1900 as the result of a study of mammalian cogenesis (rabbit, man), though he did not commit himself to this conclusion until several yeæs later (Winiwarter and Saintmont '09). In the meantime the theory of parasynapsis was placed upon a firm basis, in both plants and animals, by the work of many observers, prominent among them Janssens and A. and K. Schreiner. Since then the theory of parasynapsis has been adopted by many other observers, among them some who, like Montgomery ('11) or Robertson ('16), had long been strong advocates of telosynapsis.[1] The mammalian cytological investigators practically all favor

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[1] Wilson's Cell in Dev. and Heredity p. 550

the idea of parasynapsis occurring in mammals, with the exception of Jordan ('12), who thinks that reduction in the opossum takes place by telosynapsis. However, Jordan based his conclusions on stages that just precede the first maturation division,--and this is the phase which first led McClung and other competent cytologists to favor telosynapsis. No doubt a study of earlier stages would give the same results as have been obtained in other animals.

During the stages in which the pachytene loops stain so deeply (Fig. 17-18,) the nucleoli can not be distinguished.

The diplotene nuclei now undergo a remarkable transformation that has been found characteristic of many insects (Wilson '12) and other animals, in the course of which the double threads as such completely disappear from view, giving rise to a diffuse, slightly staining net-like stage in which the boundaries of the individual bivalents are hard to distinguish. The pachytene threads seem to loosen up and branch and the nucleus increases in size

to about 19 or 20 microns in diameter. (Figs. 19-23).

In the early growth period two oval bodies can be seen, the smaller one staining more intensely than the other (Fig. 19). The one marked p is thought to be the plasmosome for two reasons: first, it loses its affinity for stain as the growth period proceeds, and second, a definite clear space typically surrounds this element in all sections viewed from a certain angle. (Note especially figures 19 and 24.)

The chromatin nucleolus retains its staining capacity and seems to constrict in the center, sometimes taking on a curved shape. In no cell was an actual division seen to take place.

At the end of the diffuse period the nuclei undergo a rapid change in which there is a progressive condensation and contraction of the diffuse diplotene threads and an increase in staining capacity, the darker stain first appearing in definite knots along the threads, (Fig. 24). The chromatin further contracts into rings, U's and other shapes characteristic of the late

diakinetic period. In late diakinesis, Figures 26 and 27, the chromatin all stains with so nearly the same degree of intensity and attains such varied shapes, that it was impossible to locate the nucleolus with any degfee of certainty. However, in the first maturation division spindle the nucleolus can be distinguished again with its intense stain and clear cut outline. It can be followed to the first maturation division, (Fig. 29-30), where it divides early into two unequal parts. These have been designated as the x and y. (Fig. 31-34.)

Little positive evidence has been given in mammalian cytology, that the chromatin nucleolus goes to make up the sex chromosomes. This is probably due to the fact that until recently, methods of technique have been inadequate for mammalian material. Stevens ('11), who worked on the guinea-pig, favors such a conclusion, and Painter ('24), in his work with the opossum, gave a detailed account of the chromatin nucleolus from the time of its appearance in the growth period until the telophase of the first maturation division, and

is convinced that the nucleolus forms the sex chromosomes in the opossum. More evidence for such a hypothesis is found among insect material. E.B. Wilson ('12), in his studies on Hemiptera and other forms states:

> The history of the sex chromosome is very easily followed throughout, particularly in smear preparations, and affords a complete demonstration of their identity with chromatic nucleoli of the growth period.

Figure 31 shows a late prophase of the first maturation division in which the x and y have started an early separation. A side view of the spindle is shown in Fig. 32, whowing the x and y, and an early division of one tetrad, each dyad having passed to its respective pole. An early division of this same element appears in Fig. 30. In Fig. 33 and 34 the tetrads have been moved slightly aside in order to show their individual shapes. Each tetrad has a more or less characteristic size and shape, and by drawing these forms in a great many parts of cells in which their shape can be clearly distinguished, a thorough

acquaintance with each can finally be made. In no cell could more than twenty tetrads be distinguished, (Fig. 34). An alignment of the haploid elements (Fig. 46), shows the shapes for the most part to be long club-like, "derby hat," dumbbell and short thick rods. The short, thick, bent tetrad is also present. The sex chromosomes in Fig. 33 and 34 show an early division into unequal parts.

A small, round, intensely staining chromatoid body appears in many of the spindle views, (Fig. 34). In views where this element can be seen, it is always at one side of the spindle about 4.2 microns distant from the tetrads. Painter ('24) found such a body in the testes of the horse to appear as if it were hollow. In my material it stains deeply with no hollow appearance. Wodsedalek ('14) describes this body in detail.

Second Spermatocyte Divisions.

In the anaphase stage (Fig. 35), the dyads move toward their respective poles. The chromatin

appears condensed so that shapes and sizes can not be distinguished. A lagging element was observed in practically all divisions, but it is not thought to be the accessory chromosomes due to its shape, and to the fact that the x-y starts to divide relatively early. In the early telophase stage (Fig. 36), the dyads condense at the poles and in the late telophase (Fig. 37), the clumping of chromatin obscures almost entirely the outlines of the individual chromosomes. Figure 38 shows a late telophase view. The nucleus becomes reconstructed by a branching of the chromatin into rather heavy threads that arrange themselves around the periphery of the cell, leaving the center clear. Figure 38 shows a cross section of such a cell.

Metaphase plateviews of the second spermatocyte division were observed far mre rarely than the same views in the first spermatocyte division, but in four cases counts were made. Two early metaphase views were found (Fig. 41, 42), in which the chromosomes were well scattered and easily counted. Figure 41 shows 21 chromosomes. Figure 43 shows a late anaphase with 21 chromo-

somes passing to one pole but only 19 to the opposite pole. This is accounted for by the fact that the section is cut somewhat obliquely and it is quite probable that two of the chromosomes were sliced off at one end. (Fig. 43). In Figure 42, the large chromosome appearing at the extreme left in Figures 40-43, has already divided, making a total of twenty-two chromosomes in this cell.

A side view of a secondary spermatocytic spindle (Fig. 40) shows that the chromosomes are somewhat different in shape from those of the first spindle. Instead of having the quadripartite form, they have assumed the shape of short, thick rods with their long axis lying parallel to the long axis of the spindle. With the exception of two, all the chromosomes seem to divide at about the same time. As they migrate, they become massed together and by the time they have reached the poles few of the chromosomes can be distinguished in the mass of chromatin, (Fig. 44). Anaphase views are rare.

Guyer ('10), Wodsedalek ('13), Jordan ('11) and others working on various vertebrates have claimed a second pairing of the chromosomes previous to the secondary division. I have failed to find this fusion in the field mouse. Wodsedalek found that in this second pairing the accessory chromosomes do not pair. This process of pairing can not take place in the field mouse unless one of the autosomes should pair with an allosome since there is an uneven number of autosomes.

After the second spermatocytes divide, a reticular resting stage follows in which the number of nucleoli seems to vary from one to four, (Fig. 49-51).

The metamorphosis of the spermatide into mature sperms has not been followed at this time but will be given in a later study.

Summary

1. Fifteen spermatogonial metaphase views have been studied and 42 chromosomes found in 9 of them. Several dividing cells in the amnion of three embryos gave a count of 42.

2. The conclusion that the meadow vole has the XY type of sex-chromosomes is drawn from the fact that there is an even number of chromosomes, two of which fail to pair and that one tetrad divides early with an unequal division.

3. Young somatic cells show a constant diploid number.

4. Two chromatin nucleoli are found in spermatogonia, but their relation to the chromatin-nucleoli of the growth period is unknown.

5. In the metaphase of the first maturation division, a characteristic black, clear-cut element that appears to be a chromatin-nucleolus, divides into the X and Y elements.

6. A plasmosome and a chromatoid body were observed in certain stages.

Part II

A Comparative Study of the Spermatogenesis of Five Rodents and the Rabbit

5 text plates
32 figures

Historical Background

The first suggestion that sex determination might be hereditary was made by Mendel. This suggestion was adopted by Strasburger, ('00), Bateson ('02), and Castle ('03), but definite evidence was given by Correns ('07) in studies on hybrids between species of plants in which he found that all the eggs are sexually of one type, while the pollen grains are of two kinds, equal in number, one of them male producing and the other female producing. Genetic research on the heredity of sex-linked characters has borne out the above conclusion and has further shown that either sex may be the homogametic one.

For example, in mammals and diptera all the eggs are alike and have the constitution X, and are female producing, while half the spermatozoa are Y, and are male producing. In the birds and butterflies, however, the sperms are all of one kind, while the eggs are of two equal classes.

Stevens and Wilson announced in 1905 that the genetic phenomena are paralleled by certain particular chromosomes, so that the symbols X and Y may be applied to them in sex production in the same manner as to genetic factors that determine sex. These chromosomes are therefore called the sex-chromosomes, usually designated as X and Y. The conclusion from the facts just outlined would mean that a definite relation exists between germ cells and sex. Evidence is now conclusive that this determination of sexual genetic constitution is established during the maturation division in animals.

Cytology in so far as it deals with chromosomes and heredity in animals really began in 1883, with Van Beneden's discovery that eggand sperm of Ascaris megalocephala each contribute one-half of the chromosomes of each parent. He

also saw that this reduction in number occurred at maturation.

In the same year Roux advanced his hypothesis of linear arrangement of chromatin which is qualitatively different in various regions of the chromosomes. In 1887, Flemming, Carnoy and others reported that VanBeneden's observations applied to other animals and a year later Strasburger showed similar conditions to prevail in a number of flowering plants.

Flemming('87), recognized a difference in the appearance of chromosomes in the two maturation divisions. In the second, he saw that the chromosomes resembled those of an ordinary mitosis and called it homeotypic division. He also observed that the chromosomes of the first were different and called this division heterotypic. Most of the later writers have considered the heterotypic and reduction division as synonymous.

Weismann in 1887, in an essay based on Roux's work, of 1883, put forward what is known as the Roux-Weismann hypothesis which holds: (1) that

chromatin is the physical basis of heredity; (2) that it is differentially organized; (3) that the differentiated units are linearly arranged. He suggested two ways in which reduction might occur, either by a sorting out of chromosomes into two similar groups, or by a transverse instead of a longitudinal division of each individual chromosome. For this process he proposed the term "reduction division."

We have then these facts established; (1) that the body cells and early germ cells of most organisms contain twice as many chromosomes as the mature germ cells; (2) that reduction in the number occurs during maturation; (3) that the diploid number is restored by the unton of cells to form a zygote.

Boveri studied the larvae of sea urchins in which the normal combination of chromosomes had been altered by means of double fertilization. In such larvae, one part would be normal as regards skeletal development, for example, while others would lack any trace of a skeleton. From extensive work that he did along these

lines, Boveri concluded that not a given number, but a given combination of chromosomes is necessary for normal development and that this can mean only that the individual chromosome must possess different qualities.

McClung had previously reported the occurence of one chromosome which he called the accessory, in the Orthoptera, which differed from the others during spermatogenesis and was distributed in such a manner that half the mature sperm possessed and half lacked this chromosome. In 1901, he put forward his hypothesis that this chromosome was a sex-determinant and for the first time suggested an association between a particular chromosome and a particular group of characters.

Shortly afterwards Sutton ('02) published his critical analysis of the morphological character of the chromosome group of the short horned grasshopper, Brachystola magna. As his final suggestion Sutton wrote:[1] "I may finally call attention to the probability that the association of paternal and maternal chromosomes in pairs and their subsequent separation during the

reduction division, may constitute the physical basis of the Mendelian law of heredity."[1] This view he elaborated a year later and it has since received support from many lines of work by combined genetcial and cytological studies. Two of the best known instances are those of the fruit fly, Drosophila, by Morgan and the jimson weed, Datura, by Blakeslee and Belling at Cold Springs Harbor.

Thus the success of the method of interpreting genetic results in terms of a chromosome complex must be apparent to any one who has followed the literature of recent years. According to Morgan, 1926, this is not only true of the mechanism as applied to Mendel's law of heredity, but also to many of the novel situations that are continually presenting themselves where a parallel has been found between new genetic occurrences and an alteration in the chromosomal mechanism.

The study of chromosomes is of the highest importance for taxonomy in determining the re-

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[1] On the morphology of the chromosome group in Brachystola magna. Biol. Bull. 4:24-39.

lationships of species and genera. The time will probably come when the description of a species is not considered complete until the morphology of its chromosome group is known. Various types of change are indicated to have taken place in the phylogeny of chromosomes by the comparison of related species or genera. The most important of these changes follow:

1. Polypoidy, of the development of higher multiples of chromosome numbers.

2. Transverse segmentation or fragmentation of all the chromosomes or of particular pairs as in the Liliaceae.

3. End-to-end fusion of certain chromosomes. This has been found to be the case in certain species of Drosophila.

4. Irregular division or non-disjunction may lead to forms with additional pairs of chromosomes.

5. The recent genetic evidence from Drosophila and the behavior of the chromatin in such forms as Oenothera lata indicate that not only may chromatin fragments enter a nucleus and afterwards divide, but also that portions from

one chromosome may become attached to another and thus alter the genetic relationship of the factors concerned.

6. In the genus Carex, Heilbrunn ('24) has shown the haploid number in 44 species runs from 9 almost continuously to 44 and 56, but without multiple numbers. Heilbrunn suggests that one of the most important methods of chromosome increase in Carex has been by mutations of the Oenothera type followed by a division of the extra chromosome.

7. Certain species and even genera with new chromosome content have probably arisen through crossing.

The work of Tackholm, Harrison and Blackburn has shown that a study of cytological conditions is capable of yielding very useful data both from a taxonomic and general evolutionary aspect. It was found in Rosa mollis that the somatic chromosome number is 28, but at the metaphase the chromosomes are very slow to arrange themselves on the equatorial plate and when counts were made there

were 21 instead of 14. The central seven larger ones were bivalents and the peripheral 14 were univalents. The bivalents separated and passed to the poles, and the univalents divide and follow them more slowly. When daughter nuclei are formed, many of the chromosomes are left out and later form micronuclei. The next division is even more irregular. Only those which receive the seven chromosomes descended from the bivalents appear to survive and probably those with exactly seven are most fertile. We have in the roses a group of plants with a base number of 7 chromosomes in which there are several polypoid series; the first series comprises normally reducing forms with their somatic chromosomes in multiples of 14, giving diploid, tetraploid, hexaploid and in one type octoploid forms. If hybfids become fertile by doubling its chromosomes, it suggests a possible origin for all the known polypoid forms in the genus.

There has been a great deal of controversy in recent years over the subject of the determination of sex. Dr. Carl R. Moore states: "Our present

knowledge leads us to believe that sex in birds and mammals is determined at fertilization. Some would associate sex determiners with chromosomes, others with differences in metabolic rate, but it seems possible that the two conceptions are really different indicators of the same physiological condition."

Investigations of mammalian chromosomes have revealed in general a specific male and female chromosome constitution--an XX female condition, and an XY male type. The most modern chromosomal analysis of Painter ('22, '23, '24) on the opossum, monkey, man and horse would indicate such a condition to hold for mammals in general. Thus by segregation of the male XY elements and recombination with female XX elements, we have a condition that indicates zygotic sex determination. If we turn to differentiation of sex, we have the zygotic sex factors to balance with the hormonal ones which are a more complete development of zygotic determination. There is a great deal of evidence that the sex determining factors contain the potentialities of both sexes.

This is illustrated in hermophroditic forms. Bi-sexual potentialities are clearly demonstrated by grafting the opposite sex gland into an individual of the bird and mammal. Experimentally, then, sex differentiation can be modified.

Introduction to the Comparative Study

At the present time, mammalian chromosome studies, aside from the question of sex determination, have both a practical and a theoretical interest to biologists. From a practical side, the determination of the form, number and behavior of chromosomes of the most commonly used animals in the field of genetics, gives the cytological foundation upon which geneticists may work. From a theoretical viewpoint, perhaps the question that holds dominant interest at present is whether changes in the chromosome complex cause corresponding changes in the species. This general question is re-

ceiving a great deal of attention from the plant students. Many botanists think that new species are formed by gross changes in the chromosomes, both number and behavior.

In the insect group, the chromosome map has been quite well worked out, but there is little apparent order in the great amount of cytological data which has been accumulated during the last twenty-five years. Painter ('25), attributes this to the fact that the insects are more highly specialized than the mammals, together with the relative youth of mammals, would lead one to think that mammals would prove more favorable for determining the general trend in the behavior of chromosomes.

Painter ('25), in his study of mammalian chromosomes in which he discussed the chromosome constitution of at least one species in all the orders except Sirenia and Cetacea said:

> The average of these numbers (Ch. count) is a fraction over 50, but the number which I have most frequently found is 48. This is present in the most primitive eutherian order of insectivora and in such divergent orders as the primates and cheiroptera. The appearance of 48 chromosomes in three different orders has led me to believe and to

tentatively assume that this represents approximately the primitive eutherian number.

The next logical step is the extensive study of the chromosomes of the different species within an order. For this purpose the rodents were chosen at the University of Texas. Several studies have been made at that university as well as at other laboratories.

According to Flower and Lydekker, the Rodents have a wider range than any other of the land mammals, and occur in all parts of the globe, though they are poorly represented in Australia and Madagascar. The Rodents reach their greatest development as regards the number of families in South America, in which region occur also the majority of the largest members of the order. There are about 900 existing species. The Rodents were represented in the Tertiary period by nearly all the principal groups existing today, together with several extinct families. Some of the Tertiary Rodents attained a much larger size than living members of the order.

In this paper a comparison of five different rodents and the rabbit has been made. These studies were made by different authors that will be cited throughout the paper, and include three families and six species, as follows:

Order Rodentia

Family Muridae

M. musculus--house mouse
Rattus rattus L.--black rat
Rattus norvegicus--albino rat
Microtus pennsylvanicus--
meadow vole

Family Caviidae

Guinea-pig

Order Lagomorpha

Rabbit

The three families are quite widely separated and a comparison of the skeletons of the different rats and mice, leads one to believe that the relation between the species is not very close. The guinea-pig and the European rabbit (Lepus curriculus) have been crossed and such matings produce viable offspring: whether the latter are themselves fertile, I do not know.

Purpose

A comparative study of the spermatogenesis of rodents has been undertaken with two main aims in view: (1), to become familiar with the spermatogenesis of forms that have already been worked out in order better to interpret my own findings in studies of the meadow vole (Microtus pennsylvanicus); (2) to find whether there is any stability to be found in the chromosome complex of the rodents so far studied, and whether there is any evidence to show that differences in chromosome counts are due to any great addition or loss of chromatin, or to either fragmentation or end to end fusion of the original chromosomes.

No intensive comparative study of rodent spermatogenesis has been made up to this time. Painter, ('25) made a comparative study of mammals in which he included the rabbit. The same author also made a brief statement concerning what had been found in four rodents, namely the house

mouse, albino rat, guinea-pig and rabbit in his own laboratories.

A brief summary of the findings that have been made of six species of rodents together with copies of figures of certain important stages will be given. These copies were made with tracing paper and are as accurate as could be made in that manner. They are accurate enough for comparative purposes, I am sure. The following rodents will be discussed: guinea-pig, albino rat, meadow mouse, black rat, house mouse, and the rabbit. Some taxonomists do not classify the rabbit as a rodent but put it in a separate order.

Guinea-pig

The guinea-pig was studied by N.M. Stevens in 1911, who estimated the count at 56, and the sex chromosomes of the x-y type. League ('28) placed the number at 62 plus 2 and agreed as to the x-y type of sex chromosomes. This is the highest number so far encountered in the rodents or mammals. Two chromosomes conspicuous because of their large size were mentioned by both League

and Allen. The remaining elements are much smaller and range in shape from bent rods to somewhat oblong blocks. In the late prophase, the chromosome number was found to be lower than in the equatorial plate stage, approximately 40. The individual chromosomes at that stage reveal one or more cross constrictions in many of the elements. Later these constrictions in many of the elements break through, but the fragments are held together for awhile by chromatic strands and later entirely separate. An equatorial metaphase view is shown in Plate 1.

According to League, an acidophilic element and a chromatin nucleolus are associated together but these components later separate, the acidophilic body disappearing and the chromatin nucleolus persisting and entering the first maturation spindle. Plate VI, Fig. 2.

Frequently in telophase stages of first maturation divisions, a large element lags behind in the spindle and as division occurs it apparently breaks up into segments, suggesting

a compound chromosome. (Plate Vi, Fig. 1) This was given as a probable explanation of the discrepancy between the equatorial plate and late prophase counts mentioned above.

Stevens found two heterochromosomes to be clearly separated and a pale plasmosome also present. (Plate VII, Fig. 23).

A bouquet stage and parasynapsis were recorded by Stevens. A spindle side view is shown in Plate VI, Fig. 3.

Rabbit

The spermatogenesis of the rabbit has been recorded by several observers. The results given by Bachhuber ('16), and Painter ('26) will be discussed in this paper, both of whom found a count of 44 diploid, and the X-Y chromosomes.

Painter studied the amniotic cells from 15 embryos two weeks old, in which he found a constant chromosome number of 44. The embryos exhibit certain constant differences

to their sex chromosomes and on this basis can be divided into two groups, namely, embryos in which the chromosomes have mates of the same approximate size and shape; these are females carrying the xx chromosome elemtn--and embryos in which all the chromosomes match except one medium sized and one small element; these are males carrying the x-y chromosome. A retrace of a metaphase plate view is given. (Fig. 28 Plate 7.)

The spermatogonial cells of the testes are much smaller than the somatic cells. Counts show 44 chromosomes, each having a mate of about equal size and shape, except one medium and one small sized element. A copy of a metaphase plate view (Plate VII, Fig. 16), its serial alignment, (Plate VI, Fig. 13), are given.

No distinct plasmosome was observed in the general growth period which is so conspicuous in most of the other mammals. Neither was there any evidence of an acidophilic element being associated with the chromosome nucleolus as was found

in the guinea-pig and house mouse. Painter found the nucleolus prominent in the early pachytene and later stages and states that it is made up of the sex chromatin material. It typically appears as an oblong ring with a knob at one end. (Plate VII, Fig. 24).

A partial spindle as viewed from the side is made up of distinctive shapes. (Plate VI, Fig. 4). About 15 of the first maturation tetrads were identified. (Plate VIII, Fig. 36). The x-y components separated early.

Albino Rat

In the spermatogenesis of the albino rat, Allen ('18), shows 37 chromosomes in the metaphase, but was unable to differentiate an accessory. The chromosomes are all rod shaped, slightly curved and of about equal diameters. Both Painter and Pincus ('27), report 42 chromosomes for this species. The chromosomes, according to Pincus tend to condense into thick, short

rods (Plat VII, Fig. 17). A serial alignment of this cell is given in Plate VI, Fig. 11). The haploid number is 21, with the x-y chromosomes. The X and Y separate early in the first maturation spindle. For a spindle view see Plate VIII, Fig. 37.

Meadow Vole (Microtus)

No observations have been made on this rodent except those contained in this paper. A check up by another author would, of course, help to prove or disprove its validity. The count, 42, is based on nine spermatogonia, several amniotic cells and four second spermatocyte counts. For the most part, the spermatogonia show chromosomes as short thick rods, elongated rods or comma shape (Plate VII, Fig. 19). The accessores are the x-y type.

During the early primary spermatocyte stage, two chromatin nucleoli stain intensely, but during the late diakinesis the pachytene loops stain so heavily that the nucleoli could

not be identified. During the diffuse stage the plasmosome loses its affinity for stain and the chromatin nucleolus constricts and becomes curved. The tetrad shapes are shown in Plate VI, Fig. 6. A chromatoid body can often be seen in spindle views. The x and y divide early. A line up of the tetrads is given in Plate VIII, Fig. 39.

Black Rat

Only two investigators have reported on the chromosome number of the black rat. VanHoff ('11) reported 24 plus. Pincus ('27), placed the number at 40. (Fig. XX, Plate X). This rodent was also reported to have the x-y complex. Early metaphase spindles showed 20 tetrads mostly ring and rod shaped. (Plate VIII, Fig. 38).

The morphology of the chromatin nucleoli has not been worked out for this mammal.

House Mouse

A number of workers have reported the haploid or reduced number of chromosomes of both male and female mice as 20. Numerous counts made on dividing spermatogonia, (Cox and Yocum) show that the diploid number for the male is 40. Plate VII, Fig. 21 is a typical spermatogonial plate. The haploid number is 20. The sex chromosomes are of the x-y type. In the first maturation division the x and y appear first as joined, (Plate VI, Fig. 8) but later segregate to opposite poles. As a result, the sperms are dimorphic as regards the sex chromosomes.

A large heterochromosome was observed by Cox and Stevens in the early stages of the growth period. In the diplotene stage it was found separating into an acidophilic element which was irregular in shape. In the diakinetic stage these two elements were found entirely separate.

Each tetrad has an individual shape and size showing a certain amount of gradation from large to small (Plate VIII, Fig. 35). The tetrad showing an unequal division was designated as the x-y. The reduced number in the house mouse is 20.

Table I

Author	Date	Rodent	Diploid No.	Haploid No.
Allen	'19	Albino rat	37 spg.	18 and x
Painter	'26	Albino rat	42 spg.	21
Pincus	'27	Albino rat	42 spg.	21
Vanhoof	'11	Black rat	24 plus	16
Pincus	'27	Black rat	40	20
Flemming	'98	Rabbit	24	?
Winiwarter	'00	Rabbit	42	?
Barrett	'07	Rabbit	28-36	?
Bachhuber	'16	Rabbit	22	11
Painter	'26	Rabbit	44	22
Tafani	'89	House mouse	20 oocytes	?
Sabotta	'08	House mouse	16 oocytes	
Gerlach	'06	House mouse	12 oocytes	
Melissinos	'07	House mouse		88 mat. div.
Long	'08	House mouse		20 oocytes
Federly	'19	House mouse		20 oocytes
Yocum	'17	House mouse		20 spc.
Cox	'26	House mouse	40	20 spc.
Stevens	'11	Guinea-pig	56	?
League	'28	Guinea-pig	62 plus 2	15 spc.
Beach	'30	Meadow mouse	42	21 spc.

Discussion

The observations recorded above are of interest because of the light which they throw on the general question of the behavior of chromosomes in five species of one order and one of another. It is clear from the foregoing that there are marked similarities and some differences in the chromosome complexes of the six species compared.

Counts taken from Table I follow. These are the most relaiable numbers considering methods and technique. All the studies were made with Allen's modification of Bouins.

Guinea-pig	60 plus 2
Rabbit	44
Albino rat	42
Meadow vole	42
Black rat	40
House mouse	40

Table 2.

The lowest number found is 40 and the highest 60 plus 2, but in all cases there has been a similarity in the sizes and shapes of many of the individual chromosomes. The majority of the counts

are 40 and 42. In Plate VI, I have arranged in rows chromosome alignments from five species of rodents. It is apparent that the general seriation of the chromosomes is similar. It will be noticed that the chromosomes of the house mouse, albino rat and meadow mouse are more nearly alike with regard to shapes, there being no extremely large or extremely small chromosomes. The large U-shaped chromosomes, found in the rabbit and black rat, are not found in the other forms. An explanation for this difference may lie in the fact that spindle attachments for these large chromosomes are atelomitic while the short thick rods may be telomitic. It is possible that individual chromosomes have undergone changes due to translocation of parts or even by the loss of entire chromosomes or by the fragmentation of the other elements but there is no evidence to show that either of these processes has taken place. Even if the chromosomes appear alike in size and shape, it is possible that differences in species may occur by

the reshifting of genes, but the work done with plants seems to contradict this view.

An examination of the figures in Plate VII, Fig. 15-21, will show that there is a similarity in the chromosome constitution and behavior of the rodents. Their arrangement on the metaphase plate is much the same in all cases with the Y usually in the center. One finds by comparing the sex chromosomes in Plate I, that the Y is always one of the smallest elements in the cell, while the X is a medium sized chromosome which often assumes a bilobed condition. In only two animals are the somatic cells given (Plate VII, Fig. 28-29). The chromosomes are rod shape for the most part and are longer than are those of the germ cells.

Even though the chromosomes of the different species show a similarity in shape, the fact must be borne in mind that each cell was, in all probability, in a different or at least slightly different stage of development. If stages could be secured that are all in the same stage, the likenesses might be more marked.

A very interesting study has recently been made by Swezy, ('28) in which she compares the chromosome numbers in a mixed strain of rats. An attempt was made to estimate the amount of chromatin in nuclei with 62 chromosomes as compared with 42. Briefly, the result gave nuclei with 62 chromosomes a greater chromosome length by about one-third than those containing 42. Hance found for the pig where frggmentation had occured that the total amount of chromatin remained about the same.

It will be noted in Plate VI, Fig. 9-14, that observations tend to indicate a constancy in the size relations of thevchromosomal pairs in any particular species. It is of course, entirely possible that these dimensions may be merely hereditary carry-overs from generation to generation. It is difficult, through several comparisons of animals, plants and insects, not to be impressed with the likeness of pairs in sizes and shapes. Speculation as to the value of this to the cell soon proves futile. An in-

teresting study was made by Hance on Ascaris. Briefly, he found in the early cleavage stages of Ascaris, that the homologous chromosomes are of unequal length. Measurements showed that these homologues fall into two sharply defined groups suggesting their biparental origin. The shorter ones are considered to have come from the sperm. As the age of the embryo increases, these differences between the chromosomal mates tend to become less and it is suggested that at some later period in the history of the animal this difference will entirely disappear in response to the effect of continued existence in a constant environment. Hance attributes the large size of the egg chromosomes to a greater amount of nutrition in the egg.

A quotation has already been made of Painter's statement in which he places the primitive number of chromosomes of mammals at 48. This seems to me to be rather fruitless speculation; however, if such should be the case, then the only explanation of the rodent counts of 44, 42

and 40, would have to be end to end fusion or the loss of whole chromosomes.

How can the high number found in the guinea-pig be explained? League thought the fact that prophases of spermatogonia were found that showed a lower chromosome number than the equatorial plate stage and that cross constrictions were found in the individual chromosomes would tend to prove fragmentation. Painter suggests that the higher number is a late acquisition and has resulted from the breaking up of a number of elements. He further suggests the possibility that different strains of guinea-pigs may differ in the degree to which this fragmentation occurs and as a result the chromosome number may vary in well fixed material. It is noteworthy that Stevens ('09), working without our best modern method of fixation, found that there were approximately 56 chromosomes in the guinea-pig.

Recent work on the rat (Swezy '28) indicates that agreement between chromosomal behavior in that animal and the guinea-pig may be found. Swezy made seven matings in which

she used four females with 42-21 counts and two males, one having 42 diploid and 21 and 31 haploid chromosomes, the 31 appearing in the prophase of the second spermatocyte, and the other male having a 62-21-31 complex. The females were operated on, on the sixteenth day and the embryos showed 42 chromosomes. This indicates that only egg and sperm with same chromosome numbers can mate.

Matings were again made between two males with a chromosome count of 42-21-31- and two females, one with 62 chromosomes and the other count unknown. The first of these produced five embryos with 42 chromosomes and three with 62. In the second mating there were eight embryos with 42 and four with 62. These results[1] showing this remarkable behavior in two widely separated colonies of rats and a similar condition in the guinea-pig raise the question as to how wide spread such a condition may be. It is possible that we have here one of the factors in the evolutionary development of new species and that this new species will ultimately produce 62-31

1. For more complete results of this study see Jour. Exp. Zool. Vol. 51, p. 135.

chromosomes. Swezy concludes that the tendency of rats with 62 chromosomes to produce more secondary spermatocytes with 31 than with 21 would seem to favor this hypothesis.

Both processes of end-to-end fusion and of fragmentation of chromosomes have been reported for closely related species of animals and plants. Fragmentation has figured most prominently in plants, being espedially common in Liliacea. Hance ('18), found that in Oenothera scintillans, fragmentation may occur in different individuals of the same species. End-to-end fusion is well known in the Diptera as the work of Metz ('14) has shown.

Wilson ('25, p. 889) concludes that the number of chromosomes is of relatively minor importance, the essential thing being their constitution and that numbers may change from species to species without producing any other visible disturbance of heredity or development. Even so, it is surprising how the numbers and relative sizes of chromosomes within the species remains constant.

A comparison of the number and behavior of the chromatin nucleoli in the six forms has shown some differences. In the mamalia we usually find in the growth period two types of nucleoli, one a plasmosome, the other a chromatin nucleolus, which was found by Painter ('24) to be made up of the sex chromosomes. Of course with only one such record among the mammals, it would not be wise to form a hypothesis on this point, but from a study of the meadow vole, I am inclined to think that such is the case in this animal. Unfortunatley the work on the rat does not include a study of the nucleoli.

In the early growth period following the last spermatogonial division, the nucleus contains two deeply staining bodies, with the exception of the rabbit (Plate VII, Fig. 24), where the nucleolus appears as a ring with a knob at one end. No plasmosome was found but the author did not use differential stains to bring out another body, if one existed. In the guinea-pig and house mouse the nucleolus in each species was found to bfeak up into a basophilic and an

acidophilic body. In my own work on the meadow vole, I have come to the conclusion that the nucleolus does not break up but that it forms the x-y sex chromosomes in the first maturation spindle. The only point, then, that appears to be the same generally in this group, is the fact that there are a nucleolus and a plasmosome in the early growth stage. The fate of the nucleoli is at present rather uncertain in mammalian material probably due to the lack of efficient technique until recently.

Another point of interest in this group is the fact that the sex chromosomes are of the x-y type and in all cases divide early on the spindle unequally, to pass to the opposite poles. The fact that the X goes to one pole and the Y to the other, shows the rodents to be heterogametic, half the sperms carrying the X element and capable of producing females, and half the Y, capable of producing males.

In order better to compare the accessory chromosomes they were placed in a line in Plate VIII, Fig. 30-34. Here it will be noted that

their general shape, size and behavior is much the same. Perhaps we should expect these elements which determine sex to be the most stable of the chromosomes due to the fact that any great loss or gain of material in the sex chromosomes might lead to a sexually abnormal individual.

Plate VIII shows an alignment of the tetrads of five rodents. There are certain general shapes that prevail throughout, as for instance the "derby hat", the long rod, the dumbbell and short thick rod shapes. Whether these tetrads would appear the same if they could be shown at exactly the same stage will probably never be known. In all the species compared, the haploid count is one-half the diploid count.

Certainly the number of species used in this study is too small to warrant any broad generalizations, yet, we may perhaps note certain observations.

1. A comparison of the spermatogenesis of five rodents and the rabbit shows that in all major details the process is the same.

2. The XY sex chromosomes exist in all six animals, and their behavior is the same.

3. Differences in chromosome number probably arose by fragmentation or end-to-end fusion.

4. The somatic cells (amnion), in early stages of development show the same count as do the germ cells.

5. In all the forms where synapsis was discussed, rat (Allen), house mouse (Yokum), guinea-pig (Stevens), and rabbit (Bachhuber), parasynapsis was discussed and recorded.

6. A chromatin nucleolus and a plasmosome are present.

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Plate I.

Explanation of Figures.

Fig. 1. Primary spermatogonia, resting stage.

Fig. 2. Secondary spermatogonium.

Fig. 3. Prochromosome stage of secondary spermatogonium.

Fig. 4. Metaphase plate views of secondary spermatogonia.

Fig. 5. Metaphase plate views of secondary spermatogonia.

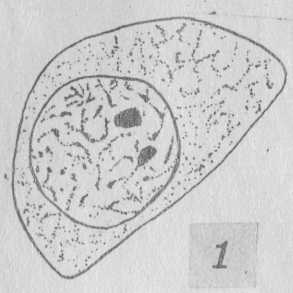
Fig. 6-9 Late metaphase plate views.

Fig. 10. Equatorial plate view from the amnion of an embryo.

Fig. 11. Late telophase of spermatogonial division.

Fig. 12. Early resting stage of primary spermatocyte.

Plate 1



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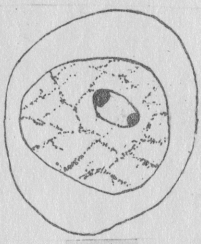

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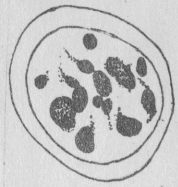

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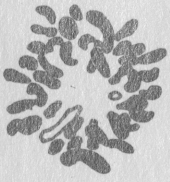

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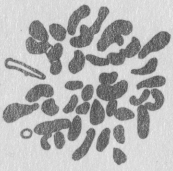

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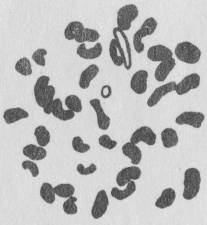

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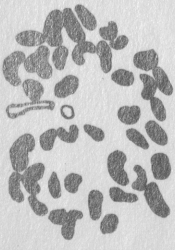

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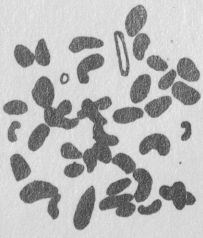

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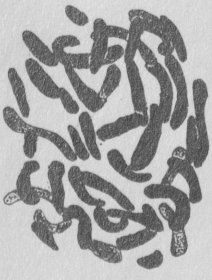

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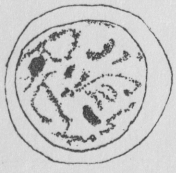

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Plate II

Explanation of Figures.

Fig. 13. Resting stage, primary spermatocyte.

Fig. 14. Early spireme stages.

Fig. 15. Early spireme stages.

Fig. 16. Leptotene threads.

Fig. 17. Leptotene threads.

Fig. 18. Pachytene stage.

Fig. 19. Diffuse stage.

Fig. 20. Diffuse stage.

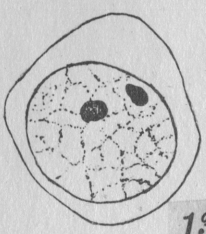
Fig. 21. Diffuse stage.

Fig. 22. Diffuse stage.

Fig. 23. Diffuse stage.

Fig. 24. Diplotene stage.

Plate 2



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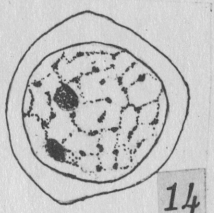

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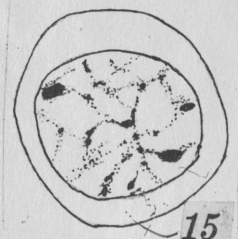

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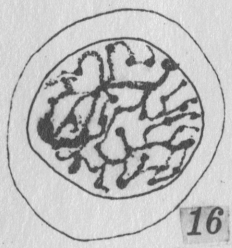

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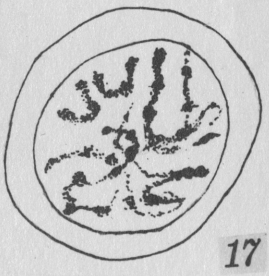

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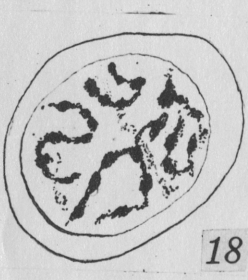

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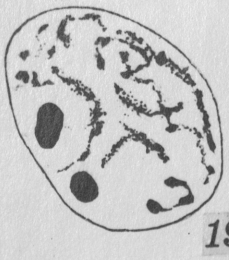

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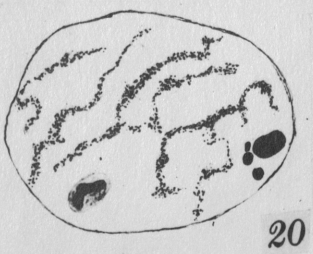

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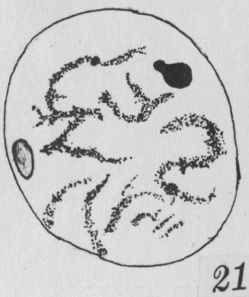

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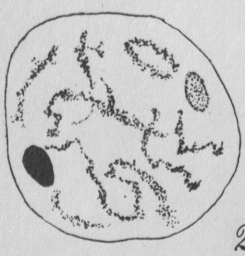

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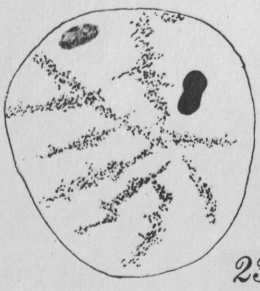

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Plate III

Explanation of Figures.

Fig. 25. Early diakinesis.

Fig. 26. Late diakinesis.

Fig. 27. Late diakinesis.

Fig. 28. Early prophase of first maturation division.

Fig. 29. Early prophase of first maturation division.

Fig. 30. Late prophase of first maturation division.

Fig. 31. Late prophase of first maturation division.

Fig. 32. Side view of first maturation spindle.

Fig. 33. Spindle dissection of primary spermatocyte.

Fig. 34. Spindle dissection of primary spermatocyte.

Fig. 35. Early anaphase.

Fig. 36. First spermatocyte telophase.

Plate 3


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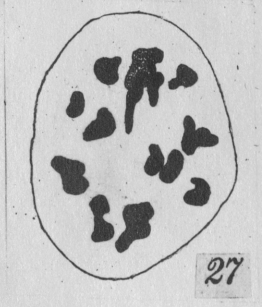
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Plate IV.

Explanation of Figures.

Fig. 37. Telophase of first spermatocyte.

Fig. 38. Late telophase of primary spermatocyte.

Fig. 39. Early prophase of second spermatocyte.

Fig. 40. Side view of spindle of second maturation division.

Fig. 41. Metaphase plate views of second spermatocyte.

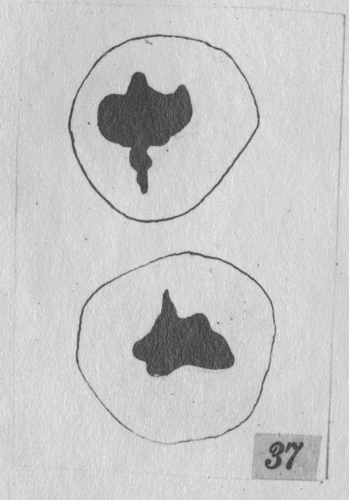
Fig. 42. Metaphase plate views of second spermatocyte.

Fig. 43. Early anaphase of second division.

Fig. 44. Telophase of second spermatocyte division.

Fig. 45. Early spermatids.

Plate 4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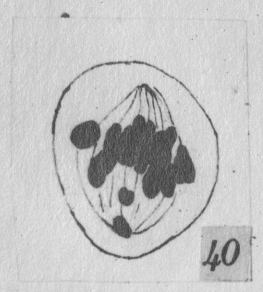
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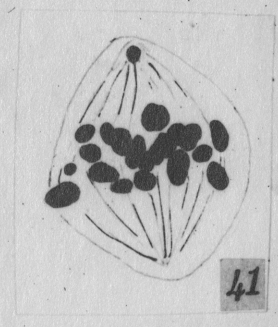
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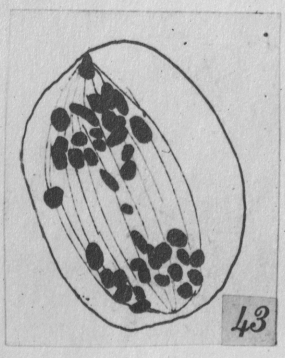
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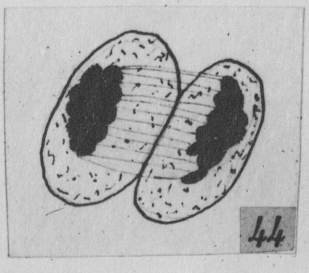
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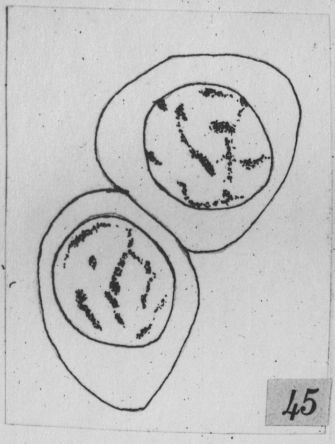
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Plate V.

Explanation of Figures.

Fig. 46. Tetrads from first maturation spindle.

Fig. 47. Serial alignment of chromosomes from cell Fig. 4.

Fig. 48. Serial alignment of chromosomes from cell Fig. 7.

Fig. 49. Early spermatids.

Fig. 50. Early spermatids.

Fig. 51. Early spermatids.

Fig. 52. Early spermatids.

Plate 5

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Plate VI.

Explanation of Figures.

Fig. 1. Suggestion of a compound chromosome. League. 1928.

Fig. 2-8 show:
sex chromosomes as they appear in the first maturation spindle as follows:

Fig. 2. Guinea-pig from League.
Fig. 3. Guinea-pig from Stevens.
Fig. 4. Rabbit from Painter.
Fig. 5. Albino rat from Pincus.
Fig. 6. Microtus meadow vole-Beach.
Fig. 7. Black rat from Pincus.
Fig. 8. House mouse from Cox.

An alignment of the spermatogonial chromosomes are shown in Fig. 9-14:

Fig. 9. House mouse from Cox.
Fig. 10. Meadow mouse from Plate I, Fig. 7.
Fig. 11. Norway rat from Pincus.
Fig. 12. Black rat from Pincus.
Fig. 13. Rabbit from Painter.
Fig. 14. Rabbit somatic cells from Painter.

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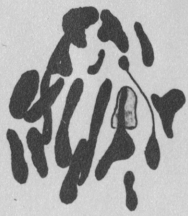

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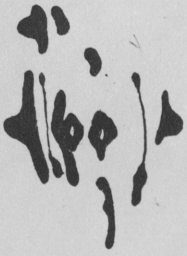

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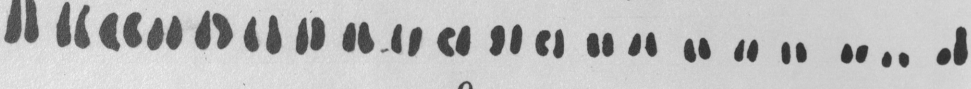

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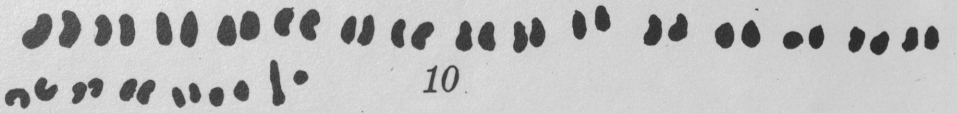

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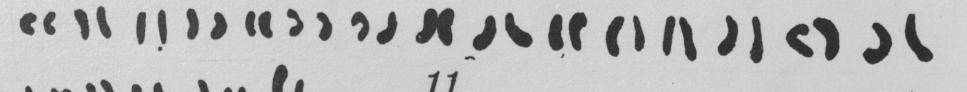

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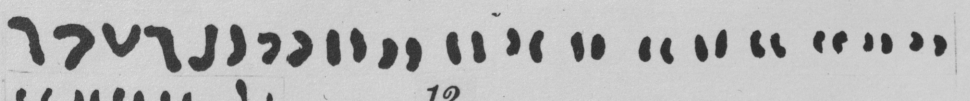

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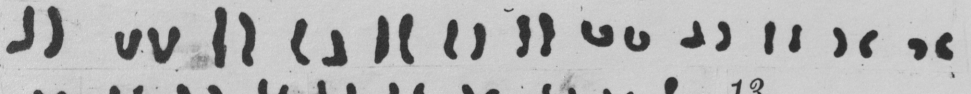

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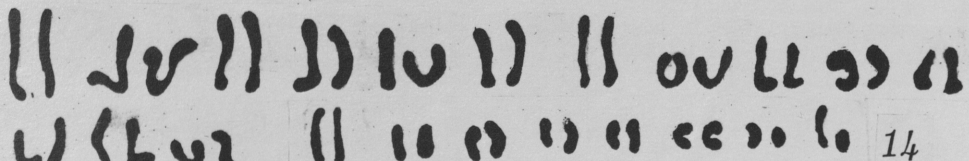

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Plate VII

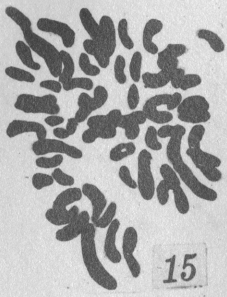
The spermatogonial metaphase views of six different rodents are represented as follows:

Fig. 16. Rabbit from Painter.
Fig. 17. Albino rat from Pincus.
Fig. 18. Albino rat from Painter.
Fig. 19. Meadow vole from Beach.
Fig. 20. Black rat from Pincus.
Fig. 21. House mouse from Cox.

The chromatin-nucleoli appear in the growth period as follows:

Fig. 22. Guinea-pig from League.
Fig. 23. Guinea-pig from Stevens.
Fig. 24. Rabbit from Painter.
Fig. 25. House mouse from Cox.
Fig. 26. Albino rat from Pincus.
Fig. 27. Meadow vole from Beach.

Fig. 28. Somatic cells from the rabbit from Painter.
Fig. 29. Somatic cells from the meadow mouse.



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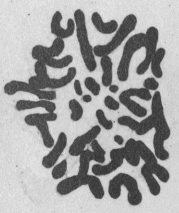

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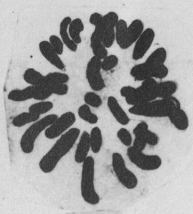

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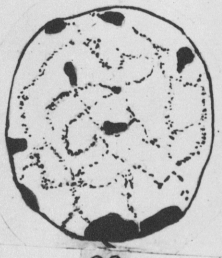

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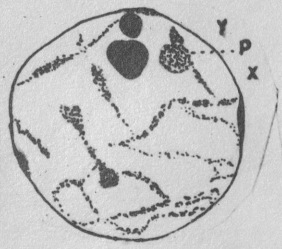

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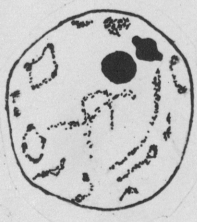

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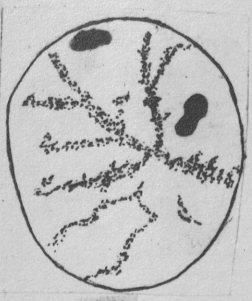

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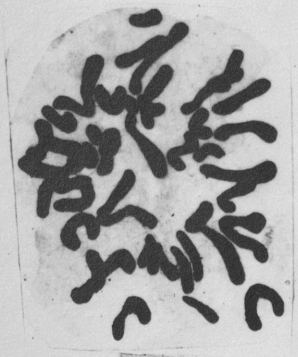

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Plate VIII

Division stages of the accessory chromosomes are shown in figures 29-33:

Fig. 30. House mouse from Cox.
Fig. 31. Guinea-pig from League.
Fig. 32. Guinea-pig from Stevens.
Fig. 33. Rabbit from Painter.
Fig. 34. Meadow vole.

An alignment of the tetrad shapes is shown as follows:

Fig. 35. House mouse from Cox.
Fig. 36. Rabbit from Painter.
Fig. 37. Norway rat from Pincus.
Fig. 38. Black rat from Pincus.
Fig. 39. Meadow vole.

Plate 8

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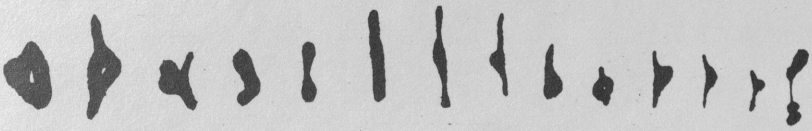

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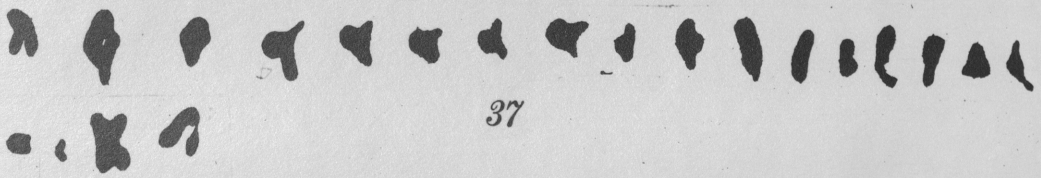

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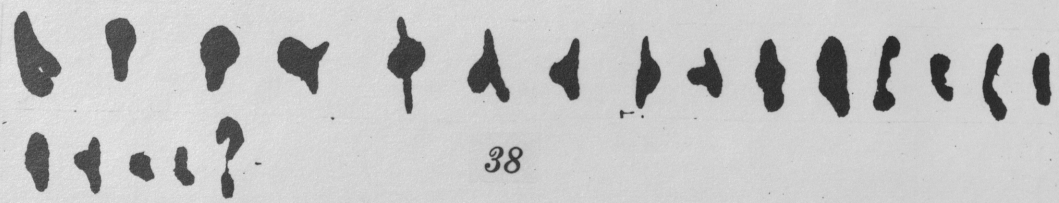

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