A STUDY OF CHROMOSOME NUMBERS AND SPERMATOGENESIS OF PEROMYSCUS LEUCOPUS NOVEBORACENSIS.

By

Marie Scott

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Approved by: H. H. Lane

Head of the Department.

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H. H. Lane

Instructor in charge.

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INTRODUCTION

Very little cytological work has been published on mammals in comparison with that done on other forms. This is not because cytologists have lacked an interest in this field, but it is due to the fact that only in recent years has technique developed to the point where the conditions in the higher vertebrates could be investigated. Besides studies published on rodents, most of the work has been done on the pig, opossum, armadillo, cat, cow, man, and perhaps a few others. Among the rodents, the guinea pig, white rat, Norwegian rat, and house mouse have been favorites. No such work has been done on any wild species of rodents common in this vicinity. For this study of spermatogenesis, the small white footed deer mouse, Peromyscus leucopus noveboracensis, has been chosen. A few having been kept in cages in the laboratory through the winter, it has been possible to observe many of their habits, an account of which has been included in this paper. Therefore, the paper shows observations on (1) habits of the animals and (2) spermatogenesis.

ACKNOWLEDGEMENTS

I wish to express appreciation to Dr. H.H. Lane under whose supervision this problem was done. The placing of many of the facilities of his department at my disposal; and his generous aid, friendly advise, and criticism have made this work possible. It is also with pleasure that I find this opportunity to acknowledge the kindness of Mr. Phil B. Powers. For his continued encouragement, constant interest, and valuable assistance have helped me greatly in this work. I thank Mr. W.R. Lynn for the drawing of plate I. and to all others who have assisted in any way, I express thanks.

LITERATURE

Among the first to count mammalian chromosomes was Flemming, who used human material for this purpose in 1882.[1] Tafani and Hermann counted chromosomes of mice in 1889.[2] A survey of the literature on chromosome-counts for mammals since these dates shows more work done on rodents than on all the other mammals. Table II is a summary of papers published on chromosomes of rodents since 1878.[3]

Much of this work has been done on the guinea pig and none of the workers agree as to the number. The counts range from sixteen to sixty two diploid. Von Bardeleben ('92) records sixteen, and Flemming ('98) finds twenty-four somatic chromosomes. Moore and Walker ('06) describe thirty-two. Stevens ('11) counts fifty six; Athias ('12) reports twenty-four to twenty-eight. Lamas ('13) finds only sixteen; Harmon and Root ('26) report thirty eight, and the most recent count gives sixty two plus or minus two. This was done by League ('28).

Most of the disagreement is explained by "poor methods of technique." Harmon and Root describe eight U shaped chromosomes, twenty-eight slightly bent rods, and two unequal in size in the spermatogonia of the guinea pig. The latter are judged to be the X and Y factors. The first division is reductional.

1. Arch. Mikr. Anat. Vol. 20. no. 1. 1882.
2. Arch. Mikr. Anat. Vol. 34. no. 1. 1889.
3. Taken from Harvey '28. I have supplemented all articles listed for dates since 1918, for her tabulation extends only to that date.

League describes sixty-two plus or minus two in the spermatogonia, and says "the primary spermatocyte number is approximately thirty-one." She does not mention the work of Harmon and Root's in counting the various sizes of chromosomes, but simply states that their low number is doubtless due to poor fixation and to the counting of compount elements as single chromosomes.

Stevens ('11) discusses the heterochomosomes of the guinea pig, saying that the guinea pig shows no synapsis or numerical division. He also describes a rest period between the two maturation divisions.

Work done on the rabbit does not show such wide variations in the numbers of chromosomes. Flemming ('98) gives twenty-four as the somatic number; Von Winiwarter ('00 & '01) finds forty-one to forty-three. Barrat ('07) reports twenty-eight to thirty-six and Bachhuber ('16) can find only twenty-two. Masui ('23) reports the largest number, forty-eight, but Painter ('25) finds only forty-four. The latter says that the Eutheria may have come from a common stem which at one time had the same chromosome constitution and genetic complex. He suggests that forty-eight is probably the primitive Eutherian number and animals having this number have likely retained more of the primitive characteristics. The reason he assigns for the rabbit possessing a smaller number is that either it has lost whole chromosome pairs, or some chromosomes have undergone end to end fusion, or non-disjunction. A larger number than forty-eight may be due to fragmentation

according to this theory.

Rats and mice have long been favorite animals for studies of spermatogenesis as Table II shows. This is doubtless due to the fact that they are so numerous and are so easily raised in captivity. The counts of the chromosomes range from sixteen to forty-two for rats; and from twelve to forty for mice. Of these papers on the mouse, only three discuss the accessory chromosome. Yocum ('17) describes the XO type in second division reduction. Masui ('23) states that the mouse has XY type of accessory chromosomes with the first division reductional. Painter ('26) finds forty chromosomes, but otherwise agrees with Masui. The only available report of chromosome count on a wild mouse, is that of Athias ('12) on Microtus.

Table III gives all the results on mammalian spermatogenesis in which the sex chromosomes have been figured or described up to 1923. Since that date, the number of workers in this field has greatly increased.

Observations on Habits of Peromyscus in Captivity.

Nelson ('18) says of Peromyscus, "their exceedingly quick and graceful movements and their beauty of form and color would make them generally attractive were it not for the prejudice against all their kind resulting from the offensive ways of the house mouse." They show many color variations in different parts of the country. Although one of the largest genera of mammals, Peromyscus is found only in the Americas. And the white footed deer mouse is found only on this continent. Summer ('26) captured Peromyscus from various parts of the United States and compared color variations. Those of the desert and beach country are much lighter than the ones taken here. These are grayish brown color above, with a broad band of darker brown along the spine. Under parts are pure white and this extends up on the sides a short way. The feet are white and the tail is white below and brown above.

As a rule, they are not very shy or nervous, but neither are they bold. They soon become adjusted to their surroundings. In one night they will construct a comfortable nest of cotton inside their little tin house and by morning be apparently happy.

They have many interesting habits. One is that of drumming with their fore feet. I can give this no other interpretation than that it is a danger signal. Seton ('20) says that the males do all the drumming, but I have observed many females run to the top of their small house and drum

for several seconds with one fore-foot. As a rule, a mouse always drums with the same foot; sometimes this is the left, and sometimes the right. Once I took six females from their cages and put them in traps on the table, preparatory to killing them. All six exhibited this habit. One would start and then another and another until every one had drummed on the sides of her trap. This procedure was usually followed by a pause after which it was repeated in the same fashion. I do not know the age at which they begin this, but I observed one young male, three weeks old, drumming on the sides of his trap.

M.A. Walton first recorded this drumming, but he said that these animals were dumb. Seton ('09), Nelson ('18), Hiskey ('17) and others have mentioned their singing. One evening I was working late in the laboratory alone, with no light except the sub-stage to my microscope, when I had the pleasure of hearing one sing. It was rather prolonged and very musical, somewhat like the song of a canary, only softer. I did not discover whether it was a male or female because I was afraid the song would cease if I moved nearer the cages.

Seton ('09) says Peromyscus is nocturnal. Dunkelberger ('20) reports that her specimens were not altogether so. Johnston ('26) provided experiments to prove them nocturnal. So far as I could tell, mine were nocturnal and, unless disturbed, would remain inside their little houses with the doorway well closed with cotton throughout the day.

Yet in one cage I had a pair that apparently did not appreciate their house and were outside all day. I kept them from November until May and not more than twice did I observe them inside the house. A nail projected from one corner of their cage near the floor, and they stayed around this nail, often sitting on it for long periods of time. They were much more nervous than any others, and no matter what type of house I tempted them with, or where I placed it in the cage, they could not be induced to make a nest. Their cage was beside a table on which a light burned most of the day and part of the night, and this may be one explanation for their nervousness. Yet, another cage was nearer the light, and at different times I had four pairs in this cage; each made a nest and seemed no more nervous than mice in the cages that were more secluded and darker.

The fighting instinct is very evident in Peromyscus. They fight standing on their hind feet, snapping at each other; or rolling over and over, and uttering shrill little squeaks. As a rule, Peromyscus that have been in captivity for some time may be moved from cage to cage without any disturbance. But often if animals that have just been trapped are placed in the same cage or put in with mice that have become accustomed to captivity, a fight ensues. Dunkelberger ('25) says that at no time during her observation did a male attack a female. But this occured in one instance with my mice. A male and female, trapped in the same vicinity, on the same day were placed in the same cage. Almost im-

mediately he attacked her and she fought in self-defense. This continued until she was completely exhausted and offered no resistance when I picked her up, but lay limp and panting in my hand. I do not know whether he would have killed her, but sympathy forbade further experiment. Sometimes a female will live in a cage with several males, or a male with several females. But in one instance I had occasion to regret such an arrangement. A female was placed in the cage with three males about eleven o'clock in the evening. Since the hour was so late, and there seemed no tendency on the part of any to fight, I observed them for only a few minutes. But the next morning I found evidence of a gruesome scene. The little female was in the center of the cage with her head half severed from her body, and the three males were placidly occupying the house.

Peromyscus females take excellent care of their young. If the nest is disturbed, the mother crouches over them and endeavors to bite anything that comes near. She is not easily frightened; but if she moves, she carries her entire family with her; because the young remain attached to the mother for the greater part of the first two weeks after birth. If one is pulled away and placed across the cage from her, she may carry it back to the nest between her teeth, or roll it over and over with her fore feet until it is in the place where she wishes it to be. If she carries it between her teeth, she may take hold of any part of it and drag it along; but usually she catches it on the abdomen or back of the neck

and carries it much as a cat does her kittens. On one occasion, I continued disturbing a mother whose offspring were very young and she buried them one at a time in a corner opposite the nest, beneath shavings that were scattered over the floor of her cage.

The animals were paired and each pair placed in a separate cage when brought in from the field. As it became necessary to kill them, changes were often made and pairs separated. One pair had been undisturbed for about five weeks but the female had not become pregnant, so I moved another female in with them. The second female had been in a cage with two males for three months and showed no signs of pregnancy. Immediately upon her introduction to the cage, the male became very inquisitive, following her over the cage smelling, sometimes the anterior and sometimes the posterior parts of her body. She seemed to resent this and occasionally fought back, uttering shrill squeaks. I was endeavoring to keep very still only two feet from the cage; and if I made the slightest movement, both mice would assume a listening, observing attitude for several minutes. Then the male always ran to the top of the house and beat a tatoo on the tin roof with his right fore foot. This happened six times during the hour that I observed them. The female would run from him and crouch down between the house and the wire of the cage, where there was just room for her slender body. At three different times, he stood on the house and tried to lift her with his teeth; catching the

skin on her neck between her shoulders or in the middle of her back. She was almost his size, but once he dragged her whole weight, placing her beside himself on the house. She made herself limp, offering neither resistance nor assistance to his efforts. During all of this time, he had also succeeded in keeping the other female inside the house. If she so much as stuck her head out, he would rush to the entrance and snap at her or strike at her with his fore feet and then return immediately to the female that had just come into the cage. After almost an hour, he apparently tired and went into the house, nor would he come out again no matter how much the visitor ran about outside. Not once did she endeavor to enter the house, although it was just like the one that she had occupied for some time. They usually use their houses as a refuge when I put my hand in the cage to catch them, but she did not try to enter even then. After removing her, I endeavored to capture him, but he always ran straight to the house as soon as my hand was inside the door of the cage.

METHODS

1. Trapping.

The traps used in capturing the animals were like those first made by Burt ('27), and illustrated in plate I. The top was cut from a medium sized tin can and this was fastened to an ordinary mouse trap. A square of heavy hail screen large enough to cover the end of the can was then fastened to the spring. When the trap is sprung, this wire flies up and covers the open end of the can so that the mouse is caught inside but not injured. Bits of cotton were placed in each trap, partly to attract the mice, and partly to provide a means for keeping them warm. If they are permitted to remain in an empty trap when the weather is cold, they soon freeze to death. The bait was placed well back in the can, in front of the cotton. I tried several kinds of bait; Miss Dunkelberger's mixture of peanut butter, raisins, and bacon; various kinds of grain; and finally a mixture of oats, sunflower seeds and corn. I caught more mice on the nights that I used the mixture of grains, but this may have been due to other influences than the bait.

Most of the animals were taken on dark nights, although some were caught on moonlight nights. None were captured on stormy nights, but snow did not keep the mice from searching in the traps for food. Since I was interested in collecting a series of embryos, much more trapping was done than would have been necessary just for a study of spermatogenesis. I trapped through the months of October, November,

February, and March. The following table gives a record of mice caught each month.

	Table I			
Month	No. of traps.	Males	Females	Total
Oct.	57	6	3	9
Nov.	154	9	6	15
Feb.	75	2	2	4
Mar.	543	19	15	34
Total	849	36	26	62

Traps were set in numbers ranging from 6 to 40 per evening, the usual number being 20 to 25. The above table represents 40 nights trapping. The greatest number captured at one time was seven. Twenty traps were set and five males and two females were caught. Two of the mice were in one trap.

2. Technique.

The following statement from Bachhuber, although made in 1916, is applicable to studies of this type today. "Mammalian spermatogenesis seems to offer greater difficulties for study than any other form. This is due to the impossibility of securing, by means of existing regents and methods, proper fixation. In nearly all preparations it has been found that chromatic structures have a tendency to mass so that individual details are lost." He found this true in his work with the rabbit, rat, and guinea pig. I also encountered many cells in various stages of spermatogenesis in which chromosomes were so massed that a count was not possible. Testes from some twenty two Peromyscus of different ages were taken for this paper. But through various faults of technique, especially at first attempts, only ten were prepared well enough to be of use.

Painter ('22) recommended the modification of Bouins as suggested by Allen ('16 & '19), and Flemming's cold as recommended by Hance ('17), as fixatives for mammalian chromosomes. Harmon and Root ('26) did not find Allen's modification successful, and Masui ('23) obtained unfavorable results from Flemming's cold. My experience was that one was about as good as the other. About half the drawings in this work were made from tissues fixed in Flemming's and the other half made from those fixed in Allen's modification of Bouin's.

This modification is made as follows: to one hundred cubic centimeters of Bouin's, made up of seventy five parts

of aqueous picric acid with fifteen parts of formalin and ten parts of glacial acetic acid, add one and one-half grams of chromic acid and three grams of urea. This solution was heated to thirty-eight degrees centigrade and tissues added. This temperature was maintained from two to three hours to insure penetration.

Flemming's strong solution was made each time immediately before using. To fifteen parts of chromic acid, one per cent aqueous solution, were added: four parts of osmic acid, 2 per cent aqueous solution; one part glacial acetic acid; and urea. This was cooled to fifteen degrees centigrade and tissues kept in it for twenty-four hours. After fixing, tissues were washed in running water twelve hours and dehydrated gradually.

All animals were killed by decapitation and one testis removed immediately to the fixing agent. Not more than thirty seconds were required for the operation, and most of the time it took only ten seconds. The testis was removed and cut in half as it was immersed in a small vial three-fourths full of the fixative. By rapidly replacing the glass cover and giving the bottle a vigorous shake, the tubules are forced out into the liquid in much less time than if they are teased apart; and penetration is better.

All tissues used in this study were dehydrated and cleared by the drop method. Powers had previously tried this method as suggested by McClung ('16), and in attempting to simplify the apparatus devised the following which

proved most successful. A tank of compressed air as shown in Plate II (a) had a hollow tube (b) and hose (c) attached to it in manner illustrated. The glass pipette (d) at the end of the hose was immersed in the liquid containing the tissue. In this way, a stream of air could be regulated through the liquid, keeping it thoroughly mixed. By using the burette (e) having a straight stop cock (f), the drops could be timed and an accurate account kept of the change of fluids. A siphon (h) on the side of beaker (g) removed excess of fluid into a waste jar (j) so that the concentration of the liquid which was being added rose constantly.

When tissues had been carried to seventy five per cent alcohol, part of each was stored in eighty per cent alcohol and a small amount tied in a bag made of lens paper, with each tissue numbered. In this way, parts of several testes may be handled at the same time. The seventy-five per cent alcohol was replaced by a solution of half analine and half seventy-five per cent alcohol. This was followed in the same manner by a solution half absolute alcohol and half analine. Then pure analine was dropped in. This was replaced by oil of wintergreen until all analine was removed. After the tissue was in pure wintergreen oil it was run through a series of eight bottles containing graded amounts of wintergreen oil and paraffin until it was in pure paraffin. These bottles were warmed just enough to keep the paraffin melted, and the tissues remained in each from five to eight minutes. After this, it was embedded in pure paraffin.

Sections were cut from five to ten micra in thickness; those about 7.5 were the best. Several stains were tried, but Heidenheim's iron haematoxlyn gave the best results. The triple stain of safranin, gentian violet, and orange G, as used by Kingery ('17) did not bring out the structures of the chromosomes as clearly as the haematoxlyn. The same was true of Mallory's phosphotungstic haematoxlyn.

Smear preparations were also made. These were fixed in Flemmings strong at room temperature, and stained with Heidenheim's haematoxlyn.

Discussion.

The present problem was undertaken with a view of determining, if possible, the number of chromosomes in Peromyscus leucopus noveboracensis. But such a problem could not be completed without involving the whole subject of spermatogenesis. Therefore, the following discussion includes more than chromosome numbers.

A. General Arrangement of Germinal Cells.

The structure of Peromyscus testis is much like that described for other mammalian testes. There are very few interstitial cells, especially in young testes; it differs in this respect from the pig as described by Wodsedalek ('13). The usual four types of cells are present in adult tissue, but in the testes of a mouse three weeks old no mature spermatozoa and few spermatids were present. In older tissues, some tubules seem to have an abundance of spermatogonia, primary and secondary spermatocytes, but few spermatids or spermatozoa. Other tubules are almost filled with the latter and very few cells of earlier stages are present. Usually all four types may be seen in one tubule. The spermatogonia are near the periphery of the tubule. They are different sizes and show numerous mitotic figures. They divide and form primary spermatocytes. These later divide giving rise to secondary spermatocytes. In Peromyscus, this heterotypic division is the true reduction division; the resulting cells containing the haploid number of chromosomes. Both primary and secon-

dary spermatocytes were abundant in almost all the tissues. The reduction division is followed by homotypic division and the secondary spermatocytes form spermatids. The latter are found in clusters of five to eight, perhaps more, attached to Sertoli cells. As they go through spermatid transformation stages, these clusters come nearer the lumen of the tubule. Finally they separate from the Sertoli cell completely and become mature spermatozoa with long tails extending into the open part of the tubule. In some cases, this lumen was almost filled with mature sperm and cast off cytoplasm.

B. Spermatogonia.

Spermatogonia lie along the wall of the tubule as a rule, but sometimes they are crowded out of place and are found farther inside the tubule. One and sometimes two nucleoli are visible in the spermatogonial resting stages. (Fig. 1) Wodsedalek ('13) describes two such nucleoli in the spermatogonia of the pig, and traces them through developmental stages until they become the X and Y chromosomes of the spermatocytes. Bachhuber ('16) also describes these nucleoli in the germ cells of the rabbit and says, "they may be the X and Y chromosomes," but he has not sufficient evidence to name them as such. Jordan ('11) can find no trace of them in spermatogonia of the opossum. Masui ('23) describes only one nucleolus in this stage, so he concludes that in the mouse, this is not the sex chromosome because it "gradually disappears in the prophase." Allen ('18) finds no accessory chromosome in any of the sperm-

atogonial divisions of the rat. Besides the nucleoli, there are numerous chromatin granules scattered through the nucleus of the spermatogonia of Peromyscus. At the close of the resting stage, these collect along fine threads and form a spireme of woolly appearance. This shortens and thickens finally breaking up into chromosomes in the later prophase. (Fig. 2 & 3). In tissue that has been well fixed and stained these may be counted. Figures 2 to 11 and figure 24 show an average count of thirty. Of these ten cells, 5 have 30, 1 has 28, 1 has 29, 2 have 31, and 1 has 34 chromosomes. Figure 13 has 28 chromosomes arranged in almost perfect pairs but none have characteristic size and shape of the X and Y chromosomes seen in figures 11 and 12. These are evidently autosomes and the accessory chromosomes are missing from this one cell. This does not agree with Painter's ('26) count for the house mouse. He finds 40 chromosomes in the spermatogonia and suggests that this may be a number common to all rodents. According to his counts, all rodents should have 40 2. He discusses these differences in counts of germ cells of the same animal and explains them mostly on the basis of poor technique. Wodsedalek ('13) finds 18 chromosomes as the diploid number for the pig, but Hance describes 40. The total amount of chromatin in each case was about the same. Since I found more cells showing 30, none having as many as 40, and somatic cells from ovary having 30 (Fig. 57), I feel justified in saying that the diploid number in Peromyscus is 30.

During spermatogonial metaphase, the chromosomes

arrange themselves on the spindle. A polar view may be counted, but side views are more difficult. The differences in numbers occuring in figures 5,6,7 & 8 may be due to the fact that chromosomes were at different levels and it is possible to count two as one or, if they are bent, one may appear as two. The accessory chromosomes are usually distinguished by their size, shape and actions. Most of the time, they appear in the metaphase as may be seen on figures 10 & and 11. But if their position is such that a true polar view of each is revealed, they appear as in figure 12. The X-chromosome is larger than Y and not so round. Figures 14 and 15 are side views of a metaphase. A count was not possible because the chromosomes tend to form a mass, or lie so closely together that they cannot be distinguished. Neither can the accessory chromosomes be distinguished in this stage because they undergo mitotic division the same as the autosomes. Each new cell receives both X and Y chromosomes. Figures 17 and 18 are side views of the anaphase. A correct count cannot be made of either, but spindle fibers are evident in each, and the X and Y chromosomes may easily be distinguished at one pole in Figure 18. Figure 24 is one end of a cell in late telophase showing 30 chromosomes. Immediately following this stage, the cell completes its division and each daughter cell goes into a resting stage. Thus are created the primary spermatocytes.

C. Primary Spermatocytes.

Following the brief resting stage, the cell grows in

size and the chromatin forms a loose spireme as shown in figure 19. The resting stage is held to be brief because so few cells are seen in this condition. We know that the spireme stage persists much longer because so many of them are present. Gradually this irregular spireme begins to condense and collects at one pole of the cell, forming synezesis. This is often called the boquet stage. Figure 20 is an example of this feature. Duesberg ('08) says that there is no synezesis in the rat. Allen ('18) in writing of the same animal, mentions a slight gathering of chromatin threads at one pole of the cell. But he says "no synezesis has been observed with any method of fixation." Jordan ('14) did not often find synezesis in the mouse but Masui ('23) reports synezesis as occuring frequently in his mouse material.

As these threads form chromosomes, they pass almost immediately into the metaphase. The characteristic tetrads are formed as in figures 25 to 35. Figures 25 and 31 show cells having large tetrads. As these divide, they are preceded along the spindle by the X and Y chromosomes as in figure 35. The ones near the poles in figures 32 and 33 are likely tetrads instead of accessory chromosomes, because they do not have the same size or shape as either the X or the Y. Figure 36 shows an early anaphase with very evident spindle fibers. A late anaphase is seen in figure 37, but all the chromosomes are not present on this spindle. Soon after this the cell divides and again the daughter cells

present a brief resting stage.

D. Secondary Spermatocytes.

The resting stage of the secondary spermatocytes (fig. 38 and 39) must be very brief because frequently both primary and secondary spermatocytes may be seen dividing in the same field. The large nucleolus is again present. Smaller chromatin masses also exhibit their deep staining qualities. They are very dark and remain about the same size in the early prophase stage (fig. 40-41). Since no spireme was found in any of the tissue for this stage, probably none is formed. Figures 42 to 46 exhibit dimorphism in the secondary spermatocytes. Half of them received the large X chromosome at reduction division, and the other half received the smaller Y. Figure 45 has 16 chromosomes, one of which is the Y from the primary spermatocyte. The others received the larger X chromosome. A count was rather difficult in these stages because the chromosomes were seen at different levels. Figure 42 was the easiest of all to count and there is no doubt about its containing 15 chromosomes. I judge this to be the haploid number since 30 is the diploid.

Few side views of metaphase were found and in none was it possible to find all the chromosomes spread apart so that they could be counted. Figure 47 shows the spindle fibers but all the chromosomes are not present. Figure 48 is an anaphase and figure 49 is a telophase. No exact counts were made of these stages. The sex chromosome cannot be distinguished because it acts in the same manner as the autosomes.

In telophase, they are always massed into a big lump of chromatin.

E. Spermatids.

Following this division, these chromatin massed become broken up into irregular chromosomes, but no count was possible. (Fig. 50) But in most of the cells of this stage the nebenkern is prominent. "The nebenkern is a cytoplasmic structure formed by the aggregation of mitochondria, or chondrioconts, and ultimately drawn out to form the envelope of the axial filament in the flagellum". (Wilson '25) No detailed study of spermatid transformation of Peromyscus has been made to date, but a few general observations were made in this study. The chromosomes break up completely and for a short while, chromatin granules may be observed as in fig. 51. The centrosome in this figure has divided. Part of it remains in its present position, but the larger part migrates around the nucleus of the cell to the opposite side. Here it forms the acrosome as shown in figures 52, 53, & 54. These cells were still attached to the Sertoli cells. The nucleus during this time begins to elongate and the cytoplasm is drawn down into a tail filament. That which is not used in the filament is sloughed off. For a long time the spermatids may be observed among these masses of cytoplasm, with their tails streaming into the lumen of the tubule.

F. Mature Spermatozoa.

The mature sperm-head stains dark slate blue and decolorization is very difficult. Figures 55 and 56 show the

general features of mature sperm of Peromyscus. The head is flat but becomes decidedly hooked with the darker staining chromatin in the hooked region. Part of the centriole has gone into the formation of the neck region. The middle piece exhibits a granular spiral appearnce and with the flagellum is very evident.

RODENTIA

Species	Diploid And Partheno-Genetic	1st - Cyte	2nd - Cyte
Cavia			
'Meerschweinchen'	16 spg		
'Meerschweinchen'	Prob. 24 som		
'Guinea-pig'	32 spg	16 ♂ (gemini)	16 ♂
'Guinea-pig'	56? spg	28 ♂	
Cavia Porcellus		24-28 ♀	24? ♀
'Cobaye'	16 som	8 ♀	8 ♀
Eliomys quercinus		16 ♀	16 (10-16) ♀
Lepus			
'Kaninchen'	24? som		
'Lapin'	41-43 oog 36-46 som (Mostly 42)	10-12 ♀ (from Honore')	
'Rabbit'	28-36 spg	14-18 ♂	
'Rabit'	22 spg	12 ♂ (= 11)	11 ♂
Microtus incertus		28-34 ♀	28-34 ♀
Mus decumanus (see also Mus rattus)			
'Rat'	32 spg	16 ♂ (gemini)	

RODENTIA

-Tid	Remarks	Observer	Reference
8♂		Von Bardeleben, '92	Verh.Anat.Gesel, '92,p.202.
		Flemming, '98	Anat.Anz.,14,p.171
		Moore and Walker, '06	Liverpool Univ.Rep., '06, p. 1.
	XY to poles in 1st	Stevens, '11	Biol.Bull.,21,p.155
		Athias, '12	Arch.R.Inst.Bacter. Cam.Pest.Lisbonne, 3, p. 287.
8♀		Lams, '13	Arch.Biol.,28,p.229
		Athias, '09	Anat.Anz.,34,p.1
		Athias, '12	Arch.R.Inst.Bacter. Cam.Pest.Lisbonne, 3, p. 287.
		Flemming, '98	Anat.Anz.,14,p.171
		Von Winiwarter, '00	Arch.Biol.,16,p.685
		Von Winiwarter, '01	Arch.Biol.,17,p.33
		Barrat, '07	Proc.Roy.Soc.London,79B,p.372
	XY to poles in 1st XY sometimes double in 1st	Bachhuber, '16	Biol.Bull., 30,p.294
		Athias, '12	Arch.R.Inst.Bacter. Cam.Pest.Lisbonne, 3,p. 287.
	Earlier accounts of Moore corrected in '05	Moore, '93	Anat.Anz.,8,p.683
		Moore, '94	Intern.Monatschr.,11, p.129.

RODENTIA (Cont'd)

Species	Diploid And Partheno-Genetic	1st -Cyte	2nd -Cyte
'Ratte'		12 ♂ (8-12)	
'Wanderratte'	16?spg	8 ♂	8 ♂ (8-16)
'Rat'	20-30 spg	ca. 12 ♂	
'Mus decumans var. albinos'	24 som(prob)	12 ♂ (prob)	12 ♂ (prob)
'Weisse Ratte'		16 ♀ (10-20)	16?♀ (8-16)
'Mus decumans albinos'	More than 24 spg	16 ♂	Prob.16 ♂
Mus musculus 'Mus musculus, var. blanche et noire'	20? cl	20 ♀	20 ♀
'Maus'		16 ♂	

RODENTIA (Cont'd)

Tid	Remarks	Observer	Reference
		Moore and Arnold, '05 Moore and Walker, '06	Proc.Roy.Soc.,London,77B,p.563 Univ.Liverpool Reports, '06,p.1.
		Lenhossek,'98	Arch.mikr.Anat.,51, p.215
		Von Ebner,'99	Sitz.Ber.d.k.Akad. Wissen.Wien,108 (3), p.429.
		Von Ebner,'02	Kölliker's'Geweblehre des Menschen,'III
		Regaud,'01 Regaud,'01	C.R.Soc.Biol.,53,p.406. Arch.d'Anat.micros., 4,p.231.
		Regaud,'09	Arch.d'Anat.micros., 11,p.291.
		Duesberg,'08	Arch.Zellf.,1,p.399
		Sobotta,u.Burkhard, '10	Anat.Hefte,42,p.433
		Van Hoof, '11	La Cellule,27,p.289
		Tafani, '89	Atti R. Accad.Lincei, Rendiconte,Ser.4, vol.5,p.119(also in Pub.Inst.St.sup. Firenze, Med-chirurg.= Arch. Anat. Norm.e path.,5,p.1.
		Hermann,'89	Arch.mikr.Anat.,34. p.58.

RODENTIA (Cont'd)

Species	Diploid And partheno-Genetic	1st -Cyte	2nd -Cyte
'Grau Maus'	24? oog	6 groups of 4♀	
'Maus, weisse, grau und Tanz'	30 + cl	16♀ (10-19)	16 ?♀
'Souris blanche'	12 spg (10-12)	12 ♂ (double). Some cells 16 (double)	12♂ (single). Some cells 16
'Mouse'	24 spg		
'Mus musculus'		12♀	12♀
'Mus musculus=souris blanche'		12♀(12-13)	12♀
'Mus musculus var. alba'		8♀	8♀
'White mouse'		12♀ (12-24 due to precocious division)	12♀ (=24 Univ)

-Tid	Remarks	Observer	Reference
		Holl,'93	Verh.Anat.Gesel. Göttingen,'93,p.122
		Holl,'93	Sitz.Ber.d.k.Akad. Wissen.Wien,102(3), p.249
	Earlier accounts corrected in '07	Sobotta,'93	Verh.Anat.Gesell. Göttingen,'93,p.111
		Sobotta,'95	Arch.mikr.Anat.,45, p.15
		Sobotta,'07	Anat.Hefte,35,p.493
		Sobotta,'08	Verh.phys-med.Gesell.Wurzburg,39,p. 241
6 ♂ some cells 8		Lukianow,'98	Arch.Sc.Biol.St. Petersburg,6,p.285
		Moore and Arnold,'05	Proc.Roy.Soc.London, 77B,p.563
		Moore and Walker, '06	Univ.Liverpool Reports,'06,p.1.
		Gerlach, '06	'Uber die Bildung der Richtungskorper bei Mus musculus,'Wiesbaden.
12♀		Lams et Doorme, '07	Arch.Biol.,23,p.259
		Melissinos, '07	Arch.mikr.Anat.,70 p.577.
12♀		Coe and Kirkham,'07	Science,25,p.778
		Kirkham,'07	Biol.Bull.,12,p.259
		Kirkham,'08	Trans.Connecticut Acad.Arts and Sc., 13,p.65

RODENTIA (Cont'd)

Species	Diploid And Partheno Genetic	1st -Cyte	2nd -Cyte
'Mouse, white, black and hybrid white X gray'		20♀	20♀
'White mouse'		12-24♀	12-30♀
'House mouse'	39 spg	20♂	20♂
Mus norvegicus albinus (=white rat)	40 (pachytene threads of oocyte		
Mus norvegicus albinus	37 spg 37♂ som	19♂	18,19♂
Mus rattus, see also Mus decumans			
'Mus rattus albus'		8♀	8♀
'Mus rattus albinos'	More than 24 spg	16♂	Prob. 16♂
Sciurus 'Ecureuil'	24 + som	ca. 16♂	
'Rabbit	44		
'Guinea-pig'	38		19
'Mouse'	40		20
'Rabbit'	48		24
'Norway rat'	42		
'Black rat'	40		

RODENTIA (Cont'd)

Tid	Remarks	Observer	References
20♀		Long, '08 Long and Mark '11	Science, 27, p.445 Carnegie Institute Pub., 142, p.1
		Kingery '14	Biol.Bul., 27, p.240
19, 20♂	X to pole in 2nd	Yocum, '17	Univ.California Pub. 16, p.371
		Pratt and Long, '17	Jour.Morph., 29, p.441
	X X to pole in 1st	Allen, '18	Jour.Morph., 31, p.133
		Melissinos, '07	Arch.mikr.Anat., 70, p.577
		Van Hoof, '11	La Cellule, 27, p.289
		Van Molle, '07	La Cellule, 24, p.257
	X Y type	Painter, '25	Science N.S.Vol.LXI.
	X Y type	Harmon and Root '26	Jour.Morph. & Phys. Vol.47, No.1
	X Y type	Masui '23	Jour.Col.ofAgni.Imperial Univ. of Tokyo, Vol.8, No.2
	Norway rat has K shaped chro. not present in Black rat.	Pincus, '27	Jour.Morph.Vol.44

RODENTIA (Cont'd)

Specie	Diploid and Partheno-Genetic	1st -Cyte	2nd -Cyte
Mixed strain of rats	42		21
'Guinea Pig	62 2	31	
'House mouse'	40	20	
'House mouse'	40	20	

RODENTIA (Cont'd)

-Tid	Remarks	Observer	Reference
	X Y type	Swezy, '28	Jour.Exp.Zool. Vol.,51,No.2.
	A possible sex chromosome may be identified	League,'28	Jour.Maryah & Phys. Vol.46, No.1
	X Y type	Cox, '26	Jour.Morph.& Phys. Vol.43,No.1
	X Y type	Painter,'26	Science N.S.Vol.64.

TABLE III

Form	Diploid Chromosome No.	Type of sex Chro.	Behavior of sex chro.	Author
Homo sapiens (Negro)	22	X 0	1st.div.reduction	Guyer, '10
Homo sapiens (Negro)	23-24	X 0	1st.div.reduction	Montgomery '12
Homo sapiens (White)	47	X 0	1st.div.reduction	Winiwarter '12
Homo sapiens (Negro & White)	24	X Y	2nd.div.reduction	Wieman '17
Dog	21	X 0	1st.div.reduction	Winiwarter Sainmont
Armadillo	31?	X 0	1st.div.reduction	Newman & Patterson '10
Opposum	17	X 0	1st.div.reduction	Jordan '11
Guinea Pig	56?	X Y	1st.div.reduction	Stevens '11
White rat	37	X 0	1st.div.reduction	Allen '18
House Mouse	39	X 0	1st.div.reduction	Yocum, '17
Horse	37	X 0	1st.div.reduction	Wodsedalek '14
Pig	18	X 0	1st.div.reduction	Wodsedalek '13
Cattle	37	X 0	1st.div.reduction	Wodsedalek '20

SUMMARY

1. The usual four types of cells are present in the tubules of Peromyscus testes.

2. During spermatogonial divisions the accessory chromosomes divide just as the autosomes.

3. The diploid number of chromosomes is probably thirty.

4. The haploid number is fifteen.

5. Sex chromosomes are of the XY type, the X chromosomes being the larger.

6. The heterotypic division is reductional and half the secondary spermatocytes receive X chromosome and half receive Y.

7. Second spermatocyte division is non-reductional.

PLATES

All drawings were made with the aid of the camera lucida, using Spencer 1.5 mm. oil immersion objective on a B. & L. microscope. A 20X compensating eye piece was used for all the figures. All drawings have a magnification of approximately 2,260 diameters.

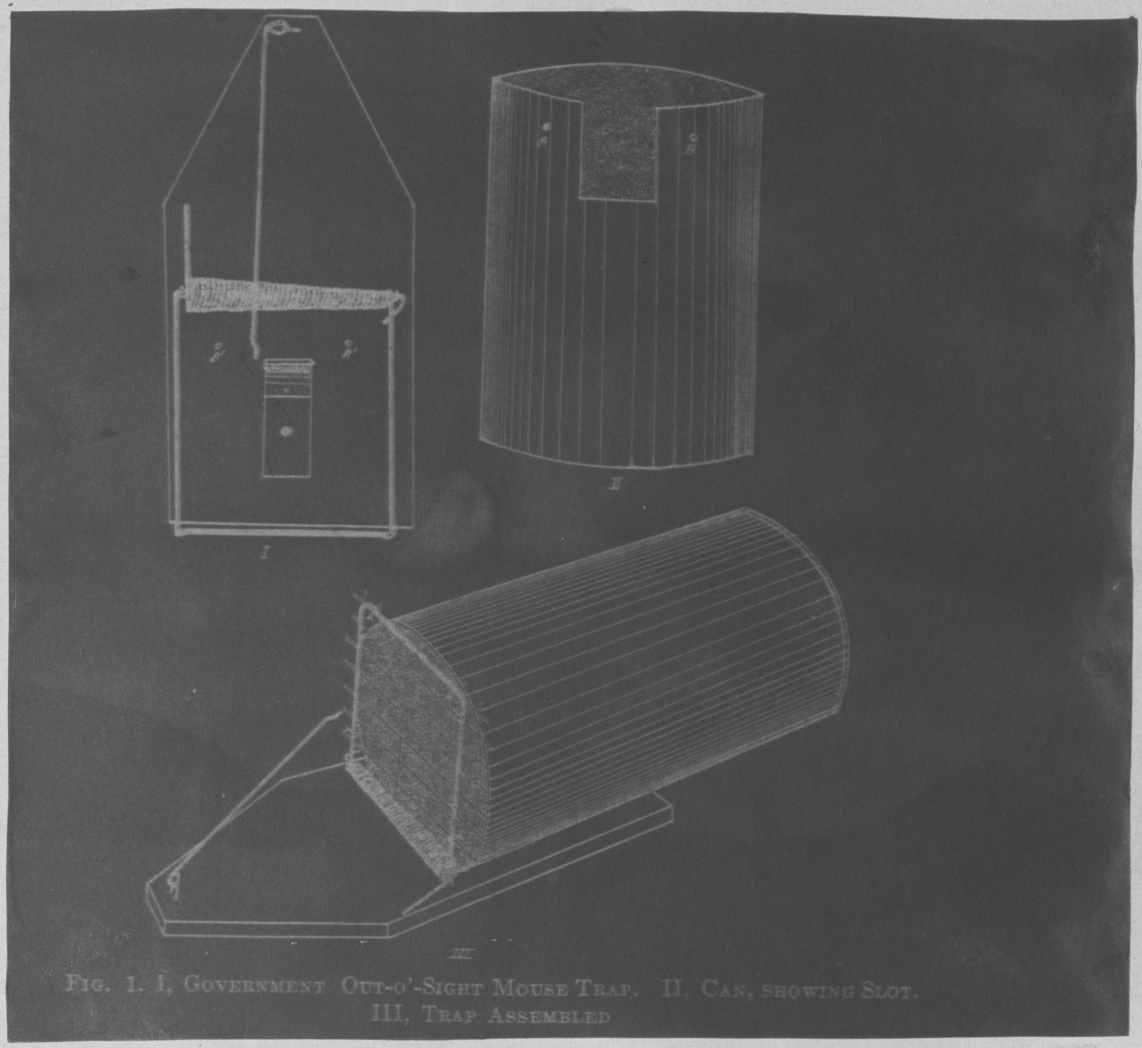
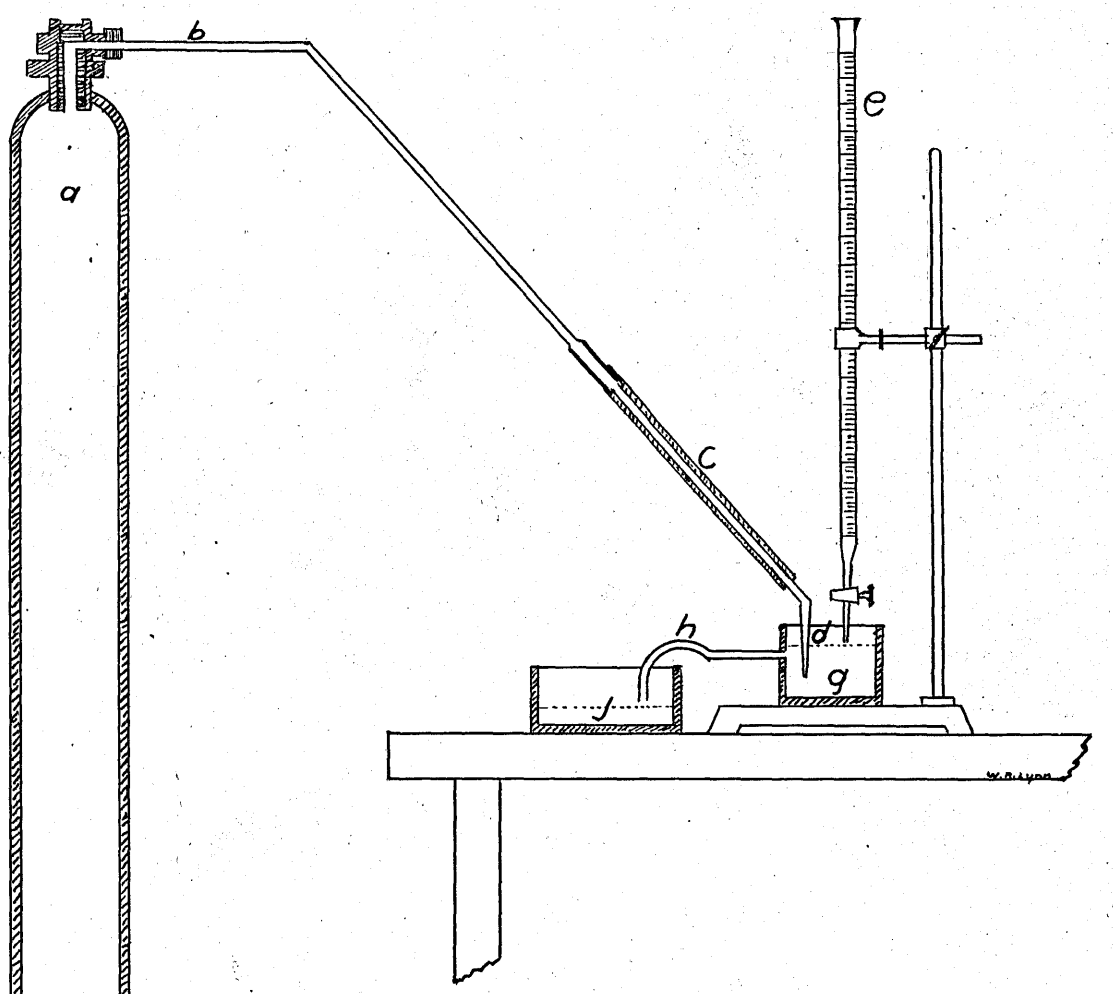


FIG. 1. I, GOVERNMENT OUT-O'-SIGHT MOUSE TRAP. II, CAN, SHOWING SLOT. III, TRAP ASSEMBLED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Explanation of Plates.

Plate III.

Fig. 1. Spermatogonium in resting stage showing nucleolus and chromatin masses. Made from smear.

Fig. 2 and 3. Prophase stages of spermatogonia, each showing 30 chromosomes.

Fig. 4. Late prophase of spermatogonia showing 30 chromosomes in almost perfectly corresponding pairs.

Fig. 5. Prophase of spermatogonia showing 28 chromosomes.

Fig. 6. Same stage as fig. 5 with 29 chromosomes.

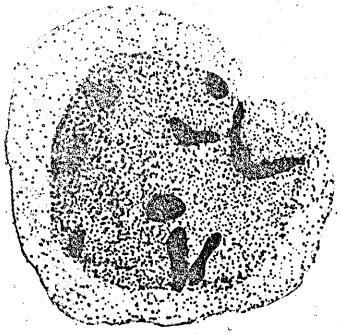
Fig. 7. Same except with 31 chromosomes.

Fig. 8. Prophase having 34 chromosomes.

Fig. 9. Same as fig. 8. showing 31 chromosomes.

Fig. 10. Metaphase of spermatogonia showing 30 chromosomes.

Fig. 11. and 12. Polar views of metaphase showing chromosomes in pairs. The X and Y are seen in the center because they precede the others to the poles. All chromosomes are not present on these spindles.



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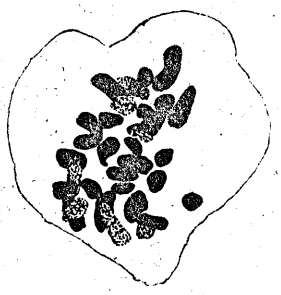

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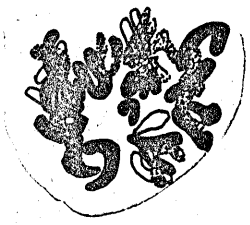

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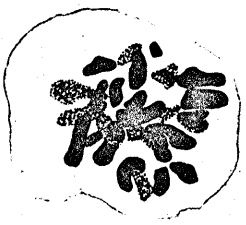

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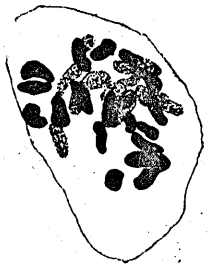

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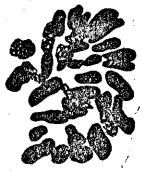

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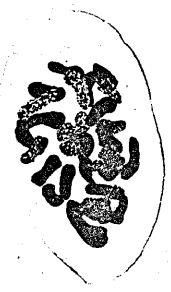

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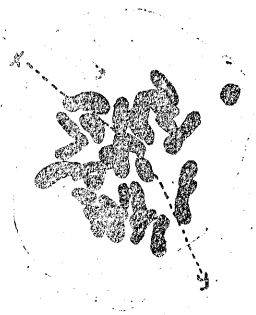

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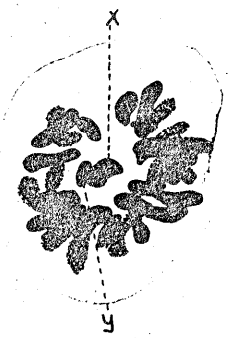

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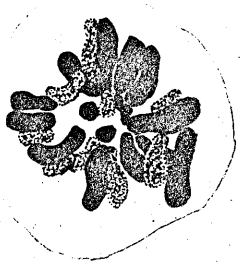

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Plate IV.

Fig. 13. Polar view of spermatogonial metaphase with 28 chromosomes. The X and Y are missing.

Fig. 14 and 15. Side views of metaphase. No count of chromosomes was possible, but spindle fibers are plain in 14. Fig. 15 is an example of chromosomes massed together.

Fig. 16. Side view of reduction division of primary spermatocyte. Chromosomes X and Y are seen preceding the others on the spindle fibers.

Fig. 17 & 18. Side view of late anaphase of spermatogonia. All chromosomes are not present, but X and Y are evident in fig. 18.

Fig. 19. Primary spermatocyte, pre synaptic or leptotene stage.

Fig. 20. Primary spermatocyte in synezesis, boquet stage.

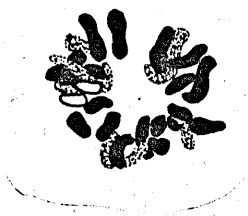
Fig. 21. Primary spermatocyte, post synaptic stage. Spireme threads are thicker than in synezesis and nucleolus is again visible.

Fig. 22. Post synezesis showing spireme breaking up into chromosomes.

Fig. 23. Prophase of primary spermatocyte showing odd shaped chromosomes.

Fig. 24. One end of a late telophase in spermatogonia showing 30 chromosomes. X and Y can not be definitely distinguished.

Fig. 25 & 26. Tetrads in primary spermatocytes. X and Y visible. (see fig. 16).



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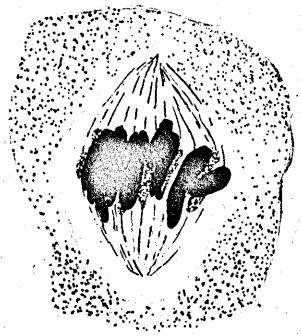

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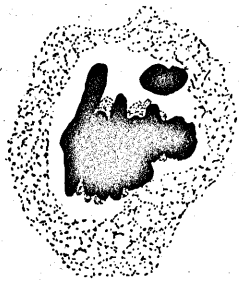

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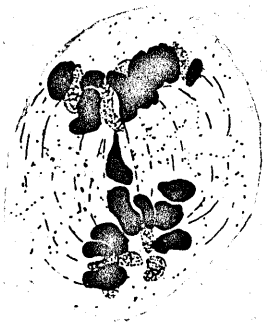

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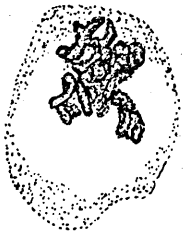

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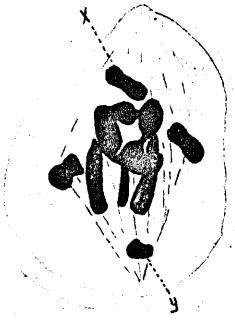

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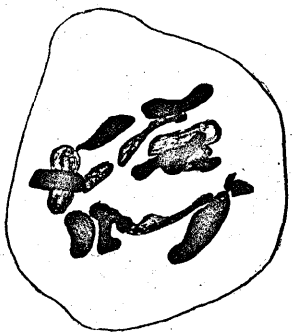

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Plate V.

Fig. 27-28. Primary spermatocytes showing tetrad formation. Fig. 28 drawn from a smear.

Fig. 29-30 Side view of primary spermatocyte metaphase.

Fig. 31 Side view of chromosomes dividing, one large tetrad is visible.

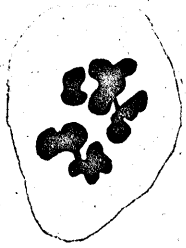
Fig. 32-33-34-35. Reduction division of primary spermatocytes. In Fig. 35 the X and Y chromosomes are preceding all others to the poles.

Fig. 36. Early anaphase of primary spermatocyte. Spindle fibers show clearly.

Fig. 37. Early telophase of primary spermatocyte.

Fig. 38-39. Resting stage of secondary spermatocytes.

Fig. 40-41. Early prophase of secondary spermatocytes. The large nucleolus is still visible.



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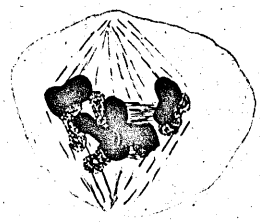

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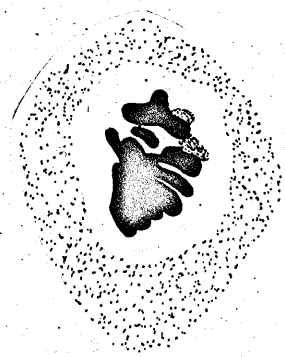

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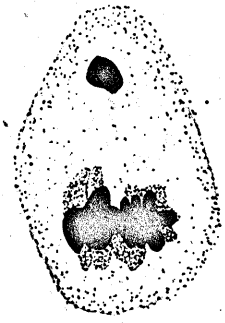

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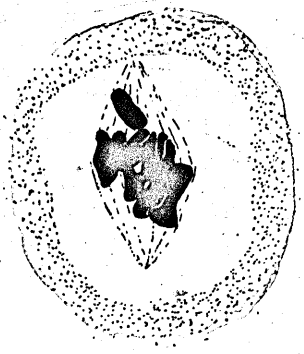

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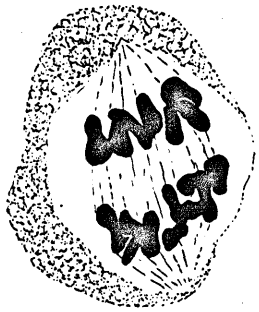

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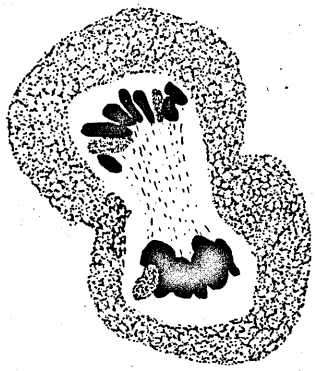

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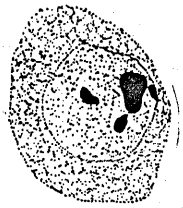

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Plate VI.

Fig. 42. Polar view of secondary spermatocyte showing 15 chromosomes. This one received the large X chromosome.

Fig. 43. Late prophase of secondary spermatocyte. 17 chromosomes appear, one of which has size and shape characteristic of X.

Fig. 44. Same stage as Fig 43--but 16 chromosomes are present, one of which is X.

Fig. 45. This secondary spermatocyte received the Y chromosome, 16 are present in this cell.

Fig. 46. Another cell showing 16 chromosomes one of which is X. (Drawn from a smear.)

Fig. 47. Metaphase of secondary spermatocyte.

Fig. 48. Anaphase of secondary spermatocyte.

Fig. 49. Telophase, no count is possible in this stage.

Fig. 50. Early spermatids with irregular and broken chromosomes. Nebenkern is visible. (From smear).

Fig. 51. Spermatids showing division of centrosome.

Fig. 52. Spermatid showing beginning of the axial filament and acrosome.

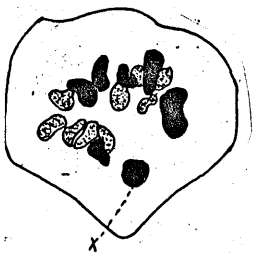
Fig. 53. Side view of about the same stage as Fig. 52.

Fig. 54. Developing spermatozoan showing formation of axial envelope.

Fig. 55. A spermatozoan almost fully developed. Middle piece has beaded appearance. Sloughed off protoplasm visible.

Fig. 56. Side view of mature spermatozoan. Showing head, middle piece and tail.

Fig. 57. Somatic cell from Peromyscus ovary showing 30 chromosomes.



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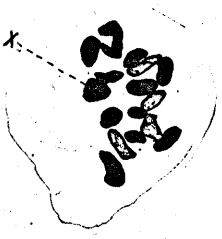

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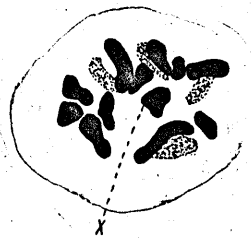

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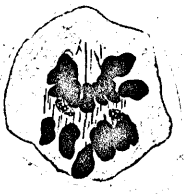

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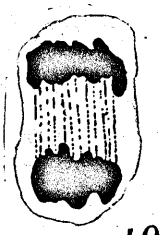

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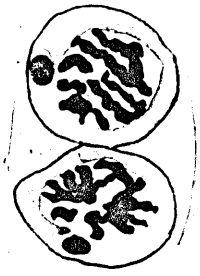

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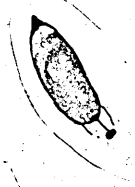

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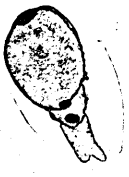

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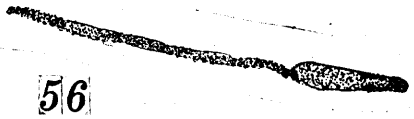

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