

SPERMATOGENESIS AND OOGENESIS OF HAEMONCHUS CONTORTUS,
A NEMATODE PARASITIC IN THE ABOMASUM OF SHEEP
AND OTHER RUMINANTS

By

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INTRODUCTION

Haemonchus contortus, otherwise known as the "Barber Pole Worm", "The Wire Worm" or simply as the large stomach worm of sheep, has been the subject of much scientific interest for the past quarter of a century. Considerable work has been done which has established this parasite as a factor of economic importance and its presence as a pathological agent in the abomasum of ruminants is generally well recognized. This worm is one of the largest of the Strongloid group, measuring sometimes an inch in the females and three-quarters inch in the males, and its general anatomy is fairly well known. The literature does not, however, reveal any work which has been done in connection with the development of the reproductive cells of this species.

In the present work a study has been made of spermatogenesis and oogenesis with some attention directed to methods of copulation, the passage of the sperm cells upon entrance into the vulva and the anatomical location in the female where the sperms are collected for the purpose of fertilization of the ova. No histologic section, depicting the entrance of the sperm into the ova, has been observed but developmental stages immediately following this penetration of the ova are shown in this paper.

Even though a considerable amount of material has been examined which has afforded opportunity for studying the entire

2.

reproductive organs of both male and female worms, several developmental stages which have been reported as occurring in Nematods, have not been observed in this study. Sufficient evidence, however, is herein presented which indicates very clearly and definitely the normal chromosome number.

3.

REVIEW OF LITERATURE

A compilation of publications dealing with the chromosome number of parasitic nematodes is available in Wilson's (10) The Cell In Development and Heredity. To expedite a comparison of results of other workers and those described in this paper this list is recorded below:

| <u>Author's Name</u> | <u>Date</u> | <u>Species</u> | <u>Chromosome Number</u> |
|----------------------|-------------|--|--|
| Van Benedin | 1883-84 | <u>Ascaris bivalens</u> | N = 2, 2N = 4 |
| Boveri | 1887 | <u>Ascaris megalcephala univalens</u> | 2N = 2 in the male |
| Montgomery | 1904 | <u>Paragordius varius</u> | N=7, 2N=14 |
| Edwards | 1910 | <u>Ascaris lumbricoides</u> | 2N=43, N=19 and 24 in the male |
| Gulick | 1911 | <u>Heterakis vesicularis</u> | 2N=9 in the male, 2N=10 in the female |
| Boveri and Schliep | 1911 | <u>Angiostomum (Rhabditis) nigrovenosum</u> | 2N=II in the male (Schliep in the male N=5 and 6) - 2N=12 in the female |
| Mulsow | 1911 | <u>Ancyracanthus cysti</u> | 2N=II in the male 2N=12 in the female |
| Vejdovsky | 1912 | <u>Gordius tolsunus</u> | N=2, 2N=4 |
| Goodrich | 1914 | <u>Ascaris incurva</u> | N=14, and=21 2N=35 and 42 |
| Walton | 1916 | <u>Ascaris canis</u> <u>Ascaris felis</u> | 2N=36 in the male 2N=9 in the male |
| Homedes (4) | 1933 | <u>Ascaris suilla</u> | 2N=43, N=19 and 24 in the male, N=24, 2N=48 in the female |
| | | <u>Ascaris vitulorum</u> | 2N=9 in the male |

*This does not appear in Wilson (10)

5.

INVESTIGATION

Procedure

Methods of Obtaining Specimens

During the course of this investigation several heavily infested sheep were slaughtered and the abomasa were removed. The contents of each abomasum were washed out into a jar of physiological salt solution at 40° c. The stomach was then split longitudinally and placed, with the mucosa surface down, in a Baerman's apparatus which had previously been filled with physiological salt solution at 40° c. After a short time the worms freed themselves from the mucosa and descended to the neck of the funnel where they were drawn off from time to time. These worms were then picked out according to species with the aid of a binocular microscope.

Some worms were picked directly off of the mucosa and were fixed immediately, and fifty of these were imbeded. About seventy-five or one hundred were taken from the Baerman apparatus or from the mucosa and placed in Ringer's solution and kept in a water bath at 40° C. from which three worms of each sex were removed beginning at 7:00 p. m. and fixed at intervals of one hour each for a period of twelve hours. This was done in view of the possibility that mitosis and meiosis in these worms might occur in cycles or at regular intervals.

Histological Technique

Previous unpublished work done by Holden (6) in this laboratory indicated that hot (about 70° C.) Carnoy's fixative was suited for this particular type of tissue, in that the worms fixed nearly straight and otherwise less contorted. The fixing action was allowed to continue over a period of twelve hours, then removed and stored in 70 percent ethyl alcohol.

Upon removal from 70 percent alcohol the worms were placed in diaphanol for from four to six hours, in order to soften the cuticle which envelopes each animal. Zirkle's (10) method for the preparation of woody plant tissue, by the use of n-butyl alcohol was used in order that the cuticular covering would remain soft during dehydration and infiltration. In this case the worms were started in the 50 percent solution and kept only 30 minutes in each concentration.

From the absolute butyl alcohol the specimens were removed to a mixture of half absolute butyl and half paraffin, (50-52°) and allowed to remain for from two to four hours. The next change was to soft (50-52°) paraffin, for from two to four hours, then into medium (53-55°) paraffin in which they were imbedded. Hard (56-58°) paraffin was used but was found to be too brittle for this type of tissue. Tissuemat also was employed as an imbedding medium but

difficulty was experienced in maintaining the desirable consistency for sectioning.

Smears were made from macerated gonads according to Belling's iron-aceto-carmin method. This method, according to the technique employed in this study, was not successful.

Male and female specimens were sectioned at six microns. While this thickness was suitable for the male germ cells, the female germ cells were rendered too thin for proper study. Female worms cut at ten and thirteen microns proved satisfactory for examination. Cross sections and longitudinal sections were made of both sexes. The longitudinal sections of the males were decidedly superior to the cross sections due to the morphology of the testes and their location with respect to the alimentary canal. These organs parallel the canal antero-posteriorly thus affording complete sections in which development from the earliest germ cells to the mature sperm could be studied. In the female the cross sections seemed best suited for study due to the fact that the ovaries of the female form a spiral around the alimentary canal, hence sections cut lengthwise afforded a less number of cells in one plane and the material was more easily torn in preparation.

The sections were mounted on slides in serial order and allowed to dry for twenty-four hours or longer before they were

stained. They were then run through two changes of xylene for five minutes each, then through the regressive ethyl alcohol series for one minute each to distilled water. From the water they were mordanted in 2.5 percent iron alum for thirty minutes, washed in tap water for fifteen minutes and removed to Hydenhain's hematoxylin, to which one drop of turgitol #7 had been added for every sixty cc. of staining fluid. The turgitol was used after Hance's (5) method, with exception that thirty minutes time was allowed for staining. The slides were then rinsed in tap water for fifteen minutes, destained in saturated picric acid solution until differentiation was attained. The sections were then washed in running water for fifteen minutes, run back up the alcohol series allowing one minute in each concentration with exception of absolute alcohol in which they were kept for fifteen minutes, two changes of xylene for five minutes each were allowed for clearing the sections from whence they were removed and mounted in balsam. The dioxan method of dehydration as described by Guyer (4) was used on several slides, and while the method saves time it apparently acted somewhat as a bleaching agent, since slides so treated either presented less differentiation or the stain appeared very weak. A total of thirty male worms and fifteen female worms were examined in the study herein described.

Results

Spermatogenesis

The development of the primary spermatocytes from the spermatogonia has not, in this study, been observed with any degree of accuracy. It is believed that the transitory stages involved in the development to the sperm in Haemonchus contortus are exceedingly brief and that the resting stages which will be described are the resting stages which will be described are the resting stages of the primary spermatocytes.

From twelve slides of longitudinal sections of male specimens in which each section showed the entire length of the testis and, therefore the complete development of the reproductive cells. It is apparent that the development proceeds from the anterior portion to the posterior region of the testis. The cells appear very small in the anterior end of the testis. The nucleus is made up of a single nucleolus and linin threads. These structures are located just within the nuclear membrane and stain lightly as compared with the later stages of their development. As progressive development increases the cells become larger, more elongated, possess a definite nuclear membrane but as yet no cytoplasmic membrane can be observed, Figs. 1, 20 and 21. From this point there follows a gradual change without any indication that a mitotic division takes place. A syncytium persists, the nuclei become more spherical and

larger and the linin threads heavier with rather large granules arranged irregularly on these threads. Other workers have described these granules as having arisen from the large nucleolus. This seems highly probable since with the appearance of these granules the nucleolus disappears, Figs. 2, 3 and 22. Condensation of chromatin material, Fig. 3, proceeds, forming heavy spireme-like structures which are similar in appearance to structures which have been described in the literature as the diplotene stage. From this stage there develops immediately four large diads and one accessory chromosome, Figs. 4, 5, 6, 7, and 23. The nature of these diads become more apparent as they resolve into eight small monads and one more body which obviously is the sex chromosome. Fig. 8, is a camera lucida drawing depicting these monads. No photomicrograph could be obtained for this developmental stage since the chromosomes did not appear in one plane. From Fig. 7, on to the complete development of the sperm, no sex chromosome can be differentiated. Fig. 9, shows a division or anaphase of meiosis. These stages, were observed frequently after the groups of nine monads have made their appearance, but this observation was not entirely consistent as there appeared in some sections, groups of four and five chromosomes without any evidence of the presence of these division stages, Figs. 10 and 24. These groups, because of their continuous occurrence, in the material examined are regarded as representing the normal

haploid chromosome number. Figs. 12, 13, 25 and 26, represent the characteristic condensation of these groups of four and five chromosomes into spermatids which develop into spermatozoa, Fig. 14 and 27. In two slides from two different worms there were found chromosome aberrations, involving a deviation from the regular haploid number of four and five to a rather unbalanced condition of three and six. This is diagrammatically illustrated in Fig. 11, while Fig. 28, is a photomicrograph of this abnormal condition. This stage was observed quite often in the reduction stage but these same groups or similar ones cannot be found in that stage where the normal groups are condensing to form the spermatids. In view of this it is believed that these abnormal groups are either absorbed or do not mature to become functional sperm.

Oogenesis

In this study the investigation relating to development of the female germ cell has not been completed, but the stages showing several interesting phases of this development are described.

The very earliest cells are characterized by appearing elongated and perpendicular to the long axis of the worm, with a definite cell wall, and a comparatively small heavily stained nucleus. The morphology of these cells was observed to change but little with the exception that they appear much larger as the

ovary approaches the seminal vesicle. Differentiation of the chromatin material was observed in the primary oocyte. Figs. 15 and 29, show the arrangement of the dyads within the nuclear membrane.

With the penetration of the egg by the sperm reduction division or the formation of polar bodies is usually stimulated. In Figs. 16 and 17 respectively there are seen two bodies which are definitely composed of five chromosomes, and despite the fact that no sperms appear in the cells nor indeed any chromatic material which might resemble the pronucleus of a sperm, there is no other alternative to follow but to conclude that these bodies are polar bodies. The five chromosomes in the lower boundary, in Fig. 17, indicate that reduction division has been accomplished.

The cells in Figs. 18 and 19 were located in the uterus slightly distant from the seminal receptacle. The position of these cells clearly indicated that fertilization has been accomplished. Fig. 19 is here described as depicting the early anaphase of the first somatic division of the fertilized egg. Twenty chromosomes appear, each daughter cell receiving ten. In Fig. 18, is seen a polar view of a stage similar to that in Fig. 19. Figs. 30 and 31 are microphotographs of the same cells described in Figs. 18 and 19.



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Plate V

COPULATION

The photomicrograph, Fig. 32, shows a male and female Haemonchus worms. These specimens were obtained from an abomasum of a sheep in 1935. They were fixed in hot 70 percent ethyl alcohol and mounted in glycerine and later photographed by Elizabeth Conner.

The phenomenon is fairly common where the abomasum is heavily parasitized. Fixation often causes the worms to separate. During the present investigation similar specimens to those described above were successfully fixed but manipulation of the worms

in preparation for imbedding proved disastrous.

The Fig. 32, illustrates definitely the use made of the bursal rays and the spicules of the male during copulation. The latter are inserted well into the vagina while the bursa is pushed backward and closely applied to the body of the female. Sections through the posterior region of the male show that the spicules are hollow and also that, when they are placed together, as is shown in the picture that another hollow tube is thus formed. It could not be determined from a study of these specimens as to the method by which sperms are transferred from the male to the female.

Great numbers of sperm appear in sections cut through the ovifectors and may be found in the entire length of the uterus. They are particularly plentiful in the seminal vesicle and where this organ is constricted it is most probable that here the one called ova are fertilized. Female cells which have passed the seminal vesicle and have entered the uterus have already begun to segment, hence it is quite evident that this particular part of the female reproductive system is the site of fertilization. Since this structure is very short it is exceedingly difficult to observe this process.

SUMMARY AND CONCLUSIONSSpermatogenesis

1. Examination of long sections, saggital sections, and cross sections of the male Nematode, Haemonchus contortus indicate that the development of the sex cells proceeds from the anterior end of the testis towards the posterior end.
2. The first type of germ cells which could be definitely recognized in the material studied are spermatocytes of the first order.
3. No sex chromosomes could be recognized in the resting stage of the primary spermatocyte.
4. The leptotene stage is represented by numerous fine, weakly staining linin threads as shown in Fig. 2.
5. The diplotene if present is so transitory that its nature could not be determined with any degree of accuracy.
6. The pachytene stage is represented by Fig. 3. These heavy threads do not split but resolve themselves quickly into heavy and thicker chromosome bodies. From these bodies four dyads are formed and one accessory or sex-chromosome designated as an X type chromosome, Fig. 7.
7. From the dyad forms nine monads result which separate into two groups of fives and fours. These groups of chromosomes

condense to form the spermatids.

8. Chromosome aberrations were observed to occur in which groups of six and three chromosomes were segregated. These groups were not observed to form spermatids.

9. While copulation has for many years been known to occur in this species, observation of this phenomenon has not been reported. The above observation has been made in these laboratories in 1936 and again during this investigation in 1938. The vagina of the female is definitely entered by the spicules of the male.

10. There is no evidence that there is a division stage in the secondary spermatocytes to form the spermatids. Observations, however, do indicate that spermatids result by condensation of the chromosomes in the secondary spermatocytes.

Oogenesis

11. Three stages in the oogenesis of the female Nematode, Haemonchus contortus were observed, namely the primary oocyte depicting five dyad chromosomes; a secondary oocyte containing one polar body of five chromosomes; an early anaphase stage of somatic division just after fertilization. Twenty chromosomes appear in this stage, each daughter cell receiving ten.

DESCRIPTION OF PLATES

PLATE I

Camera Lucida Drawings

The magnification of the camera lucida drawings are not stated because accurate figures cannot be given since the drawings were transferred by free hand to the plate and the plate photographed.

Figure 1. Cells of early resting stage or primary spermatocyte found in the anterior end of the testis.

Figure 2. Cells of a later stage, found more posteriorly in the testis, showing the morphological developmental process over those in Figure 1.

Figure 3. Cells of a still later or pachytene stage.

Figures 4, 5, 6, and 7. Characteristic cells showing the possible chromosome formations which were observed in the secondary spermatocytes with four dyads and one sex-chromosome.

Figure 8. Forms of nine monads which result from the four dyads and one sex-chromosome.

Figure 9. Reduction division stages observed, resulting from the groups of nine monads.

Figure 10. Secondary spermatocytes formed through reduction division showing normal haploid numbers.

Figure 11. Chromosome aberrations resulting from abnormal reduction division.

Figure 12. A condensation of the normal haploid numbers of chromosomes showing four and five in each group.

Figure 13. Further condensation of the chromosomes to form immature sperm, showing also a definite cell wall.

Figure 14. Mature sperm.

Figure 15. Primary oocyte showing five dyads.

Figure 16. Secondary oocyte showing polar body.

Figure 17. Secondary oocyte showing a polar body and five functional chromosomes.

Figure 18. Polar view of early anaphase stage of somatic division just after fertilization.

Figure 19. Long sectional view of early anaphase stage of somatic division showing twenty chromosomes.

PLATE II

Photomicrographs (x 3500)

Figures 20 and 21. Early resting stages of male germ cells as is shown in Figures 1 and 2.

Figure 22. Later stages of germ cells as shown in Figure 3.

Figure 23. Dyad groups which are represented in Figures 4, 5, 6, and 7.

PLATE III

Figure 24. Haploid groups of four and five chromosomes as is shown in Figure 10.

Figure 25. Condensation of the groups of four and five chromosomes, shown in Figure 12.

Figure 26. Condensed immature sperms with definite cell walls, shown in Figure 13.

Figure 27. Mature sperm.

PLATE IV

Figure 28. Results of abnormal reduction division, chromosome aberrations, as shown in Figure 11.

Figure 29. Primary oocyte showing five dyads as in Figure 15.

Figure 30. A cell from which Figure 18 was taken.

Figure 31. A cell from which Figure 19 was taken.

PLATE V

Figure 32. Described on page 13.

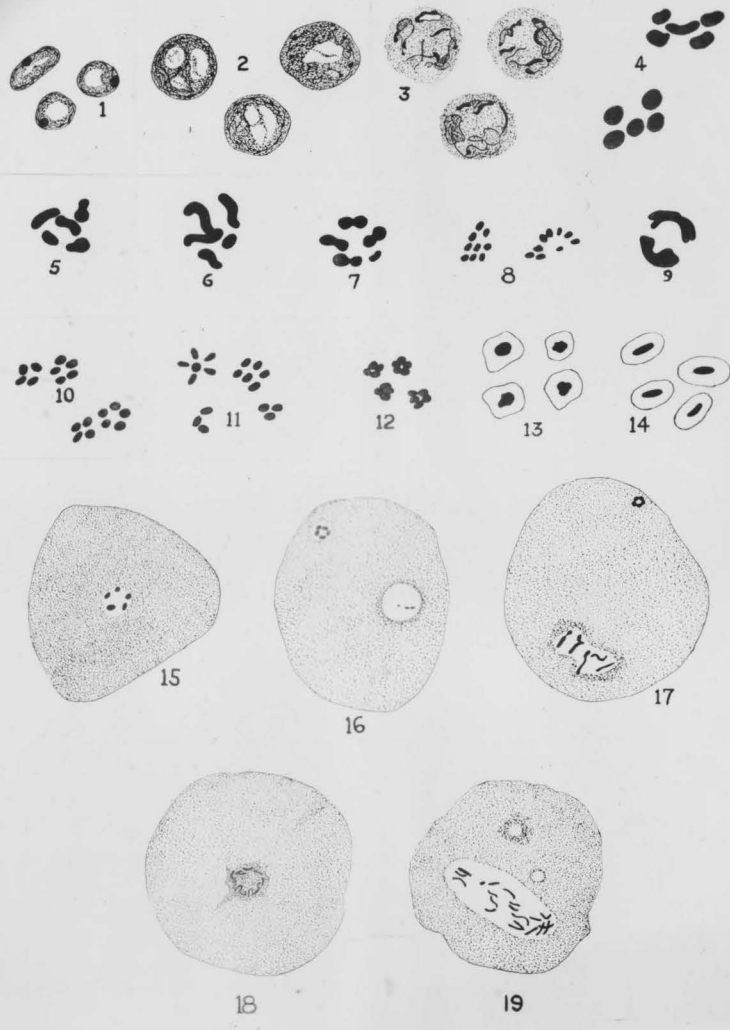
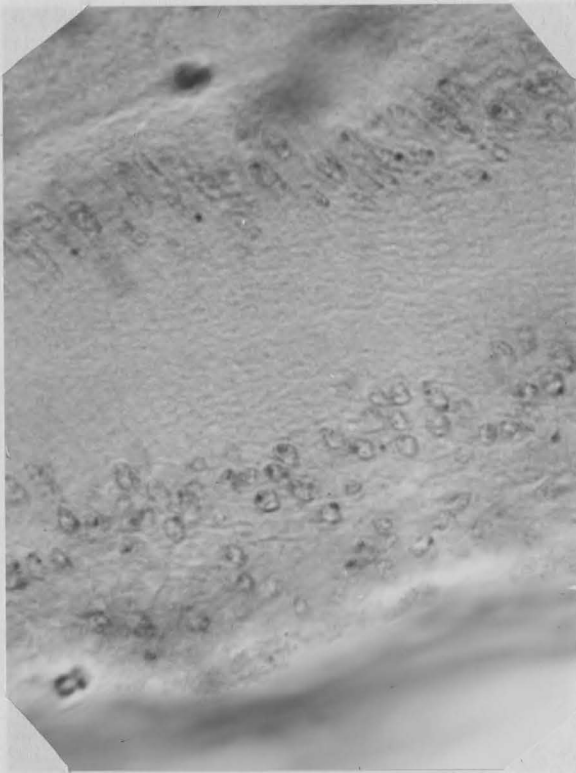
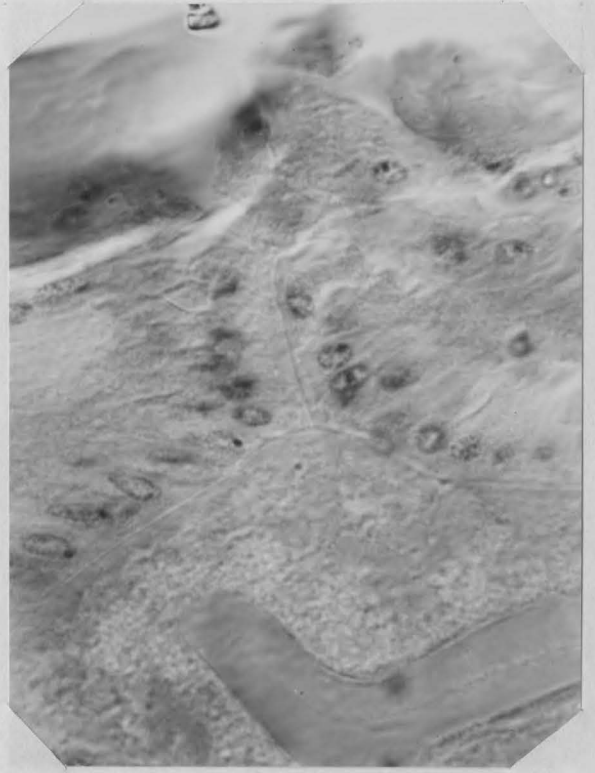


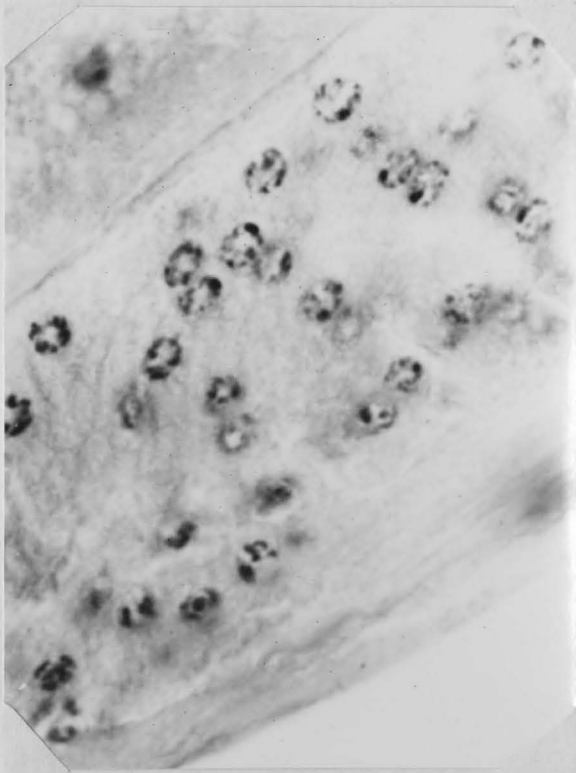
PLATE I



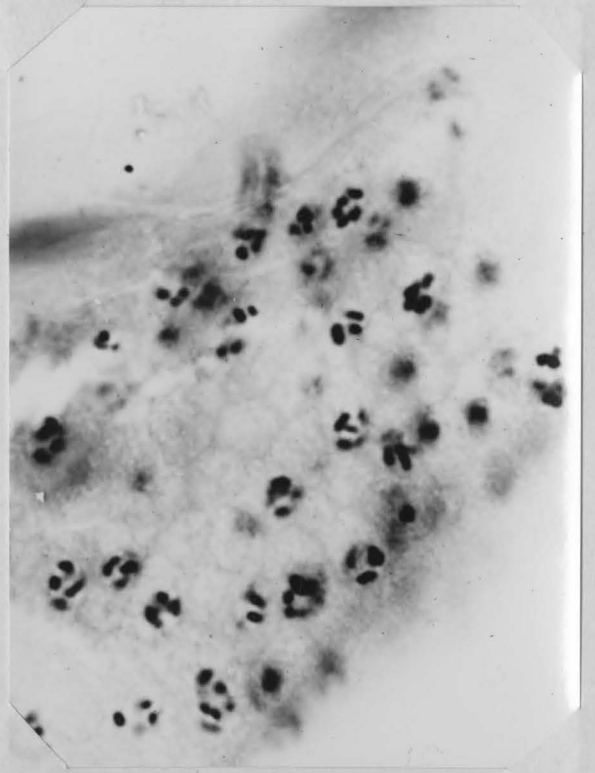
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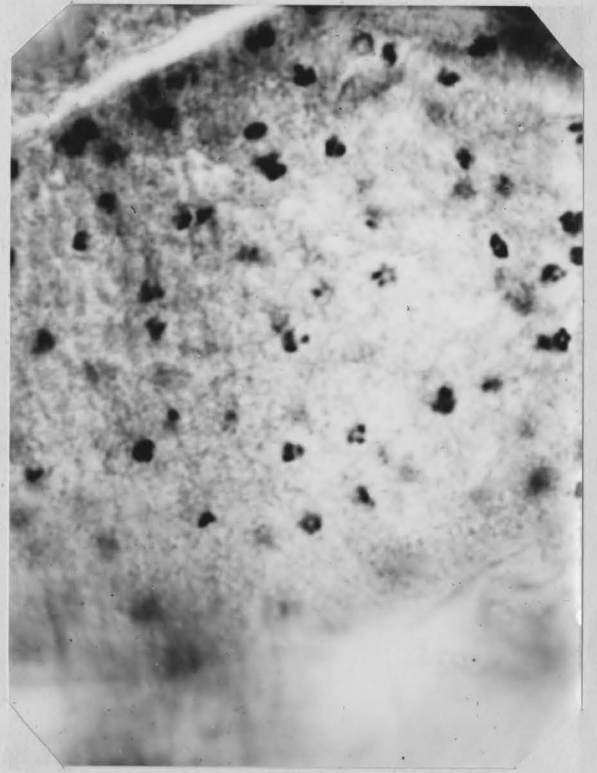
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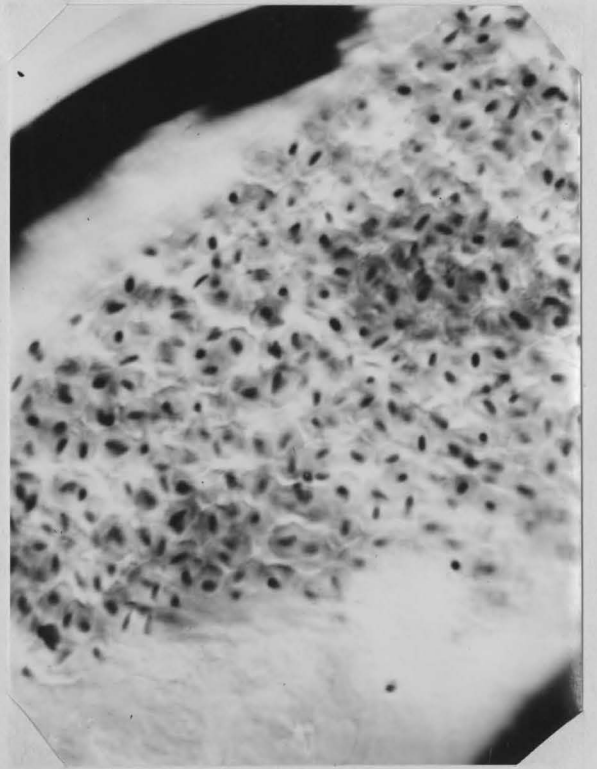
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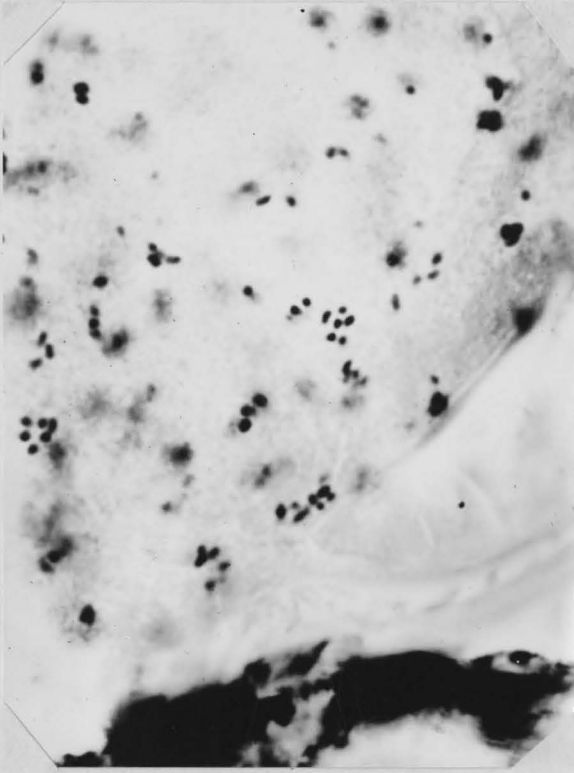
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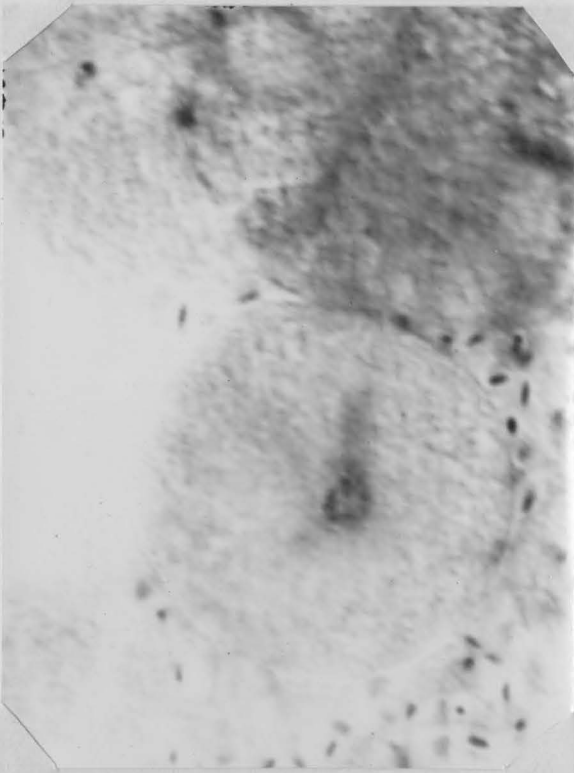
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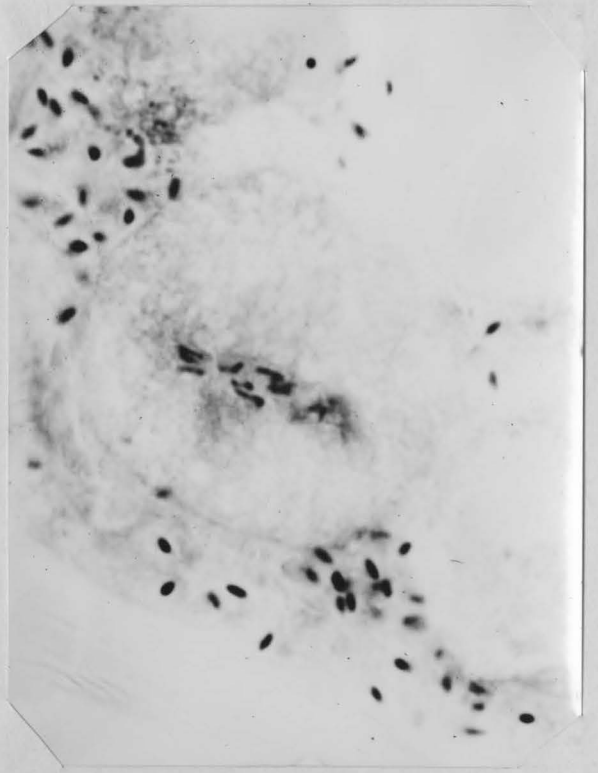
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