OOGENESIS OF *OSTERTAGIA CIRCUMCINCTA*, A PARASITIC NEMATODE OF THE ABOMASUM OF SHEEP

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INTRODUCTION

Ostertagia circumcincta, a parasitic nematode of sheep, known collectively with the O. trifurcata, the smaller trichostrongyles and several other worms of the genus Ostertagia, as the "brown hair worms of sheep", has held a rather obscure position in the evaluation of the importance of the sheep and goat parasites. This fact and their small size account for the elusive past of this nematode. The status of economic importance, however, to which O. circumcincta has now risen, not only in this state, but in the western section of the United States, Europe, New Zealand, and Australia, suffices as an explanation for further researches bearing on the classification, anatomical organization and cytological study of this parasite.

This paper deals quite briefly with the anatomy of the female Ostertagia circumcincta, and somewhat more in detail with the changes observed in the development of the germ cells from the primordial stage through the first cleavage division.

REVIEW OF LITERATURE

York and Maplestone (1) give a very brief description of the genus Ostertagia and only mention the various species and their hosts. Ransom (2) gives a more detailed description of the anatomy of both male and female Ostertagia circumcincta.

A summary of the chromosome number of parasitic nematodes from Wilson's (3) The Cell in Development and Heredity is included in the unpublished works of Holden and Henderson.

The original papers of Edwards (4), Goldsmith (5), Homedes (6), Gulick (7), and Schliep (8) were read in their entirety. Those of Gulick and Schliep were the only ones dealing with oögenesis; however, both papers brought out details strikingly similar to those observed in Ostertagia circumcincta.

PROCEDURE

Methods of obtaining specimens

Several sheep known to be heavily infested with *Ostertagia* were slaughtered from time to time and the abomasum or fourth stomach immediately opened and washed with physiological salt solution at 40°C. The residue was examined for living worms which were transferred by means of a steel needle to fresh salt solution. The abomasum was further examined as herewith described. It was placed in a Baerman's apparatus with the mucosa resting on a screen. The worms were washed down into the funnel, drawn off and placed in fresh salt solution. The best specimens were secured in this way. Finally, the mucosa was carefully examined under a binocular microscope and worms still clinging to the wall were picked off with a needle. Great care was taken to select only *Ostertagia circumcincta*. It might be here noted that it was observed to be impractical to put more than five or six worms in one watch glass; if too numerous they knot themselves together in such a way as to make them almost impossible to separate and therefore of little value as individual specimens.

Histological Technique

Several fixing agents and various methods of fixing were used during the course of this work. The best results were obtained by quickly but carefully drawing off the salt solution

from the worms in a watch glass and immediately pouring hot Carnoy's over them. Very good specimens were also obtained from the use of Flemming's (strong) fixing fluid. Other fixing agents employed (with varying success) were hot 70% alcohol, hot Bouin's fixative, hot mecuric chloride, and hot water. Several fairly straight specimens were obtained by immersing the worms individually in the fixing agent. A third method used, but with little success, is as follows: one worm at a time was removed from the salt solution, straightened out on a cover glass and then covered quickly with the hot fixing agent.

For the most part the worms were fixed immediately after being recovered from the sheep. Utilization of another method consisted in the incubation of adult worms at 37.5°C. in Ringer's solution. Four worms from this lot were fixed every hour from 7 P.M. until 5 A.M. to check the possibility of developmental stages occuring in cycles.

Seventy per cent ethyl alcohol was employed as a storing agent.

From 70% alcohol, the nematodes were transferred to diaphanol, and allowed to stand from two to twelve hours. Six hours were sufficient to prevent distortion of tissues while sectioning.

Zirkle's (9) method of dehydrating plant tissue with the use of butyl alcohol was utilized in dehydrating Ostertagia

with modifications: the lower first two dilutions in the series were omitted and iso-butyl alcohol was added at the top. Thus the worms were transferred from the diaphanol directly into 50% dilution, and they were allowed to stay in each vial 30 minutes instead of one hour. A small amount of Eosin was added to the first 95% alcohol in order to give the worms a traceable amount of color.

About 40 whole mounts were made. The stains used were Fast Green, Crystal Violet, Eosin, Aceto-Carmine, and Delafield's Haemotoxylin. Several worms were cleared and mounted in glycerine without being stained. The best whole mounts were obtained from the use of Delafield's haemotoxylin.

The process of embedding worms for sectioning employed the use of three grades of paraffin; soft (50°-52°C.), medium (53°-55°C.) and hard (56°-58°C.). Infiltration with soft paraffin was most successfully accomplished by filling a small vial half full of soft paraffin and then covering it with iso-butyl alcohol into which the worms were transferred by means of a pipette from the iso-butyl alcohol bath. The vial was then placed in a paraffin oven. After the paraffin melted, thus allowing the Ostertagia to come in contact with dilute paraffin, they were transferred again by use of the pipette into a vial of melted soft paraffin. The worms were allowed to stand two or three hours in each grade of paraffin and finally embedded in the hard paraffin.

Sections were cut 5 and 7 microns thick, fixed on slides following the usual method and allowed to dry 24 hours before staining. About 100 worms were cut; the longitudinal sections proved to be more informative for this study than the cross sections.

Fast Green, Delafield's haematoxylin and Heidenhain's iron-alum haematoxylin were employed as stains. A 5% solution of Heidenhain's afforded the clearest definition of chromatin material. The procedure used is as follows: from the drying oven, slides were run through two jars of xylene, five minutes in each, in order to dissolve and remove the paraffin. From the xylene they were run down a series of ethyl alcohols (100%, 100%, 95%, 80%, 70%, 50%, and 35%) into tap water, standing two minutes in each jar, then mordanted in a 2.5% solution of iron-alum for 20 minutes, washed in running tap water 15 minutes and stained for 1 hour.

Following the staining, the slides were run through three changes of water, destained in a saturated aqueous picric acid solution, allowed to wash for 15 minutes and then run back up the series; one minute in each of the lower alcohols, 5 minutes in 95%, 10 minutes in each of the absolutes and 5 minutes in each of the xylenes. Great care was taken to insure thorough dehydration before mounting; several drops of n-butyl alcohol were added to the absolute alcohol as a water remover, and the xylene

baths were changed whenever the slightest amount of milkyness appeared. The slides were mounted in balsam as usual.

Painter's aniline oil method of dehydration before embedding was used when Flemming's fixing fluid was employed but this method was not found to be as satisfactory as Zirkle's method according to the technique employed in this study.

Description of Female Reproductive System

Observations and measurements made in this laboratory add little to Ransom's description of female Ostertagia circumcincta. As is typical of parasites, the reproductive system is well developed and fills the greatest part of the body cavity. The germ tube was observed to make its appearance just below the distal end of the oesophagus, the distance from the oesophagus being nearly constant in all worms; however, not always does the first appearance of this tube indicate its origin, as shown in Figure 20, but rather the tip of a loop one side of which terminates a short distance laterally and caudally. Numerous other irregularities distort the position of the intestine so that the normal relative positions of the intestine and ovary cannot be determined. Furthermore, one becomes confused in attempting to trace the development of the eggs through one of the loops, both from sections and from whole mounts. The germ tubes or ovaries are connected with the amphidelph uteri by a short oviduct and an enlarged portion, the seminal receptacle (Figure 21) which merges into the uterus.

uterus. The uteri are united to the vagina by a muscular ovijector. (Figure 22). The vagina is located in the latter one-third of the worm and may or may not be protected by a flap. An attempt was made to determine whether females bearing vulva flaps displayed any difference in chromosome counts from females which were devoid of vulva flaps. Because of the difficulty encountered in obtaining sufficient material of both types it was impossible to complete this comparison. The chromosome number of those specimens having flaps is the number recorded in this paper.

RESULTS

Oögenesis

The primordial germ cells at the tip of the germ tube were observed to be almost spherical in shape and to have a nearly centralized nucleus, the chromatin material being so closely packed together as to give no insight into a probable chromosome number. It was impossible in this study to follow the division of the germ cells as reported by other observers to take place in these early stages of oögenesis.

Following the so-called "resting stage" the chromatin material of the oogonia takes on the appearance of the leptotene and diplotene stages (Figures 3 and 4) in preparation for the division which follows.(See Figure 5). In division, here again no count of the chromosomes could be made. Cellular structure as shown in Figure 6 was observed in several sections at this stage. Such a structure has been reported by other observers to be fragmentation of nuclear material which occurs before division and that the number of fragments bears no relation to the chromosome number.

Oöcytes of the first order are very similar to the oögonia in general appearance as will be noticed when comparing Figures 6 and 7. These oöcytes increase in size, their nuclear material fragments as before (Figure 9) and again the diplotene formation appears, preceding the division which results in the formation of

the secondary oöcytes.

The oöcytes of the second order resemble the fully developed eggs except that they are still retained in their consecutive arrangement in the lower portion of the ovary and have a rectangular rather than an elliptical shape (compare Figure 10 with Figure 16).

As the "resting" nuclei of the secondary oöcytes become more active, they fragment in a very characteristic fashion, the nucleoli often appearing to be vacuolated (see Figure 11). The diminution is followed by a reorganization and condensation of the nucleoli, and with that the appearance for the first time of definite chromatin strands. (Figure 12a). The nucleolus becomes paler and the chromatin strands condense to form six well defined bivalent chromosomes (Figure 12b). The bivalent chromosomes divide to form twelve as shown in Figure 12c. (The several dark staining bodies are bits of chromatin fragments not yet absorbed). The presence of the haploid number of chromosomes and the position of the cell in the ovary indicate that the egg is ready for fertilization. Gulick and Schliep both state that the chromosomes are present in their haploid number at the time of the first direction division (formation of polar body). In this study, it was observed that as the egg approaches the seminal receptacle the chromosomes are agin present as six tetrads (Figure 13); however, in this study as in Schliep's, the question of how the

fusion of the twelve individual chromosomes to form these tetrads occurred could not be decided.

Gulick reports that at the entrance of a sperm into the cytoplasm of the egg, the egg nucleus moves "as if by repulsion" to the distal apex of the egg and there gives rise to the first direction division. It was not possible in this study to determine the introduction of a sperm into the cytoplasm of the egg; however, it was observed that the nucleus of an egg in the seminal recepticle had assumed its position in one end of the egg (Figure 14 and 19), and that its six tetrads split into their monovalent halves thus giving rise to a polar body containing six diads (Figure 15).

With the completion of the first and second direction divisions the egg comes to lie in the uterus. The egg is characterized by the presence of two reticulated nuclei lying very close to each other. (Figure 16). Following the resting stage of the fertilized egg (Figure 17), the chromatin resolves itself into individual chromosomes and the achromatic figure of the first cleavage division is formed. (Figure 18).

SUMMARY AND CONCLUSIONS

1. The primordial germ cells arise at the tip of the germ tube which lies just posterior to the oesophagus.

2. The period of growth following each stage in the development of the egg is accompanied by a fragmentation of the chromatin material before division takes place.

3. Chromatin strands make their first appearance in the secondary oöcytes.

4. The chromatin strands condense to form six bivalent chromosomes or tetrads.

5. The six tetrads split to form twelve individual chromosomes then fuse to form six bivalents which are present when the egg enters the seminal receptacle. $N = 6$ $2N = 12$

6. The eggs remain in a one-celled stage until they reach the seminal receptacle where fertilization takes place; immediately thereafter, that is to say, in the uterus, they begin their clevage divisions.

DESCRIPTION OF PLATES

Plate I

Camera lucida drawings with a magnification of 1700x.

Figure 1. Three primordial germ cells as they are grouped in the tip of the germ tube.

Figure 2. Oögonia in the "resting stage".

Figures 3 and 4. Nuclei of oögonia showing chromatin material in leptotene and diplotene stages.

Figure 5. Oögonia with chromatin material pulled apart into two masses.

Figure 6. Oögonia with chromatin material completely fragmented.

Figure 7. Oöcytes of the first order in "resting stage".

Figure 8. Fragmentation in nuclei of primary oöcyte.

Figure 9. Diplotene stage of primary oöcyte.

Figure 10. Oöcyte of the second order in "resting stage" (only nucleus and a portion of surrounding cytoplasm shown).

Figure 11. Nucleus of secondary oöcyte showing the nucleolus fragmenting.

Figure 12a. Chromatin strands before condensation.

Figure 12b. Oöcyte showing six bivalent chromosomes.

Figure 12c. Nucleus of an oöcyte of the second order showing eleven monovalent chromosomes.

Figure 13. Ovarian egg in seminal receptacle. Chromo-

somes again present as six tetrads.

Figure 14. Portion of ovarian egg showing the nucleus lying in one end of cytoplasm.

Figure 15. Portion of egg showing the formation of a polar body.

Figure 16. Ovarian egg showing the two pro-nuclei just before fusion.

Figure 17. Fertilized egg in resting stage.

Figure 18. Achromatic figure showing metaphase stage of the first cleavage division.

Plate II

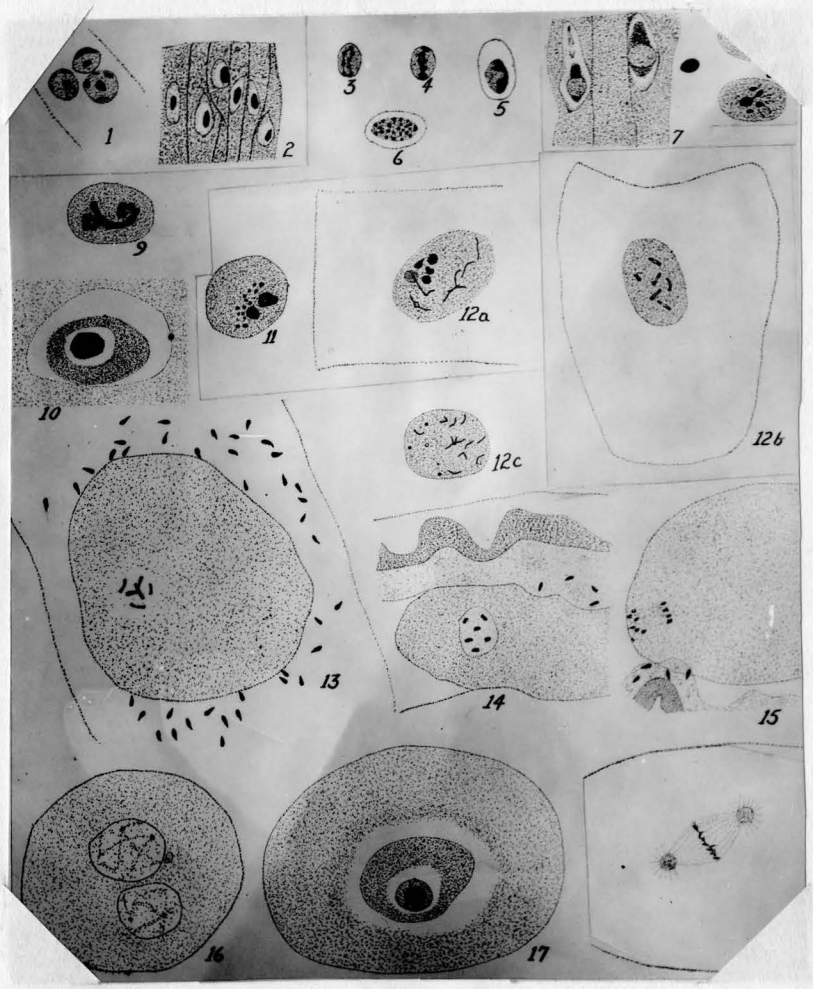
Photomicrographs

Figure 19. Portion of ovarian egg showing the nucleus lying in one end of the cytoplasm. x720.

Figure 20. A section from the anterior portion of a female Ostertagia circumcincta showing the origin of a germ tube. x80.

Figure 21. A section from the latter half of a female Ostertagia circumcincta showing the seminal receptacle. Note the centralized nuclei of the eggs in the oviduct (to left of seminal receptacle). x80.

Figure 22. Ovijector showing an egg just before emission. x80.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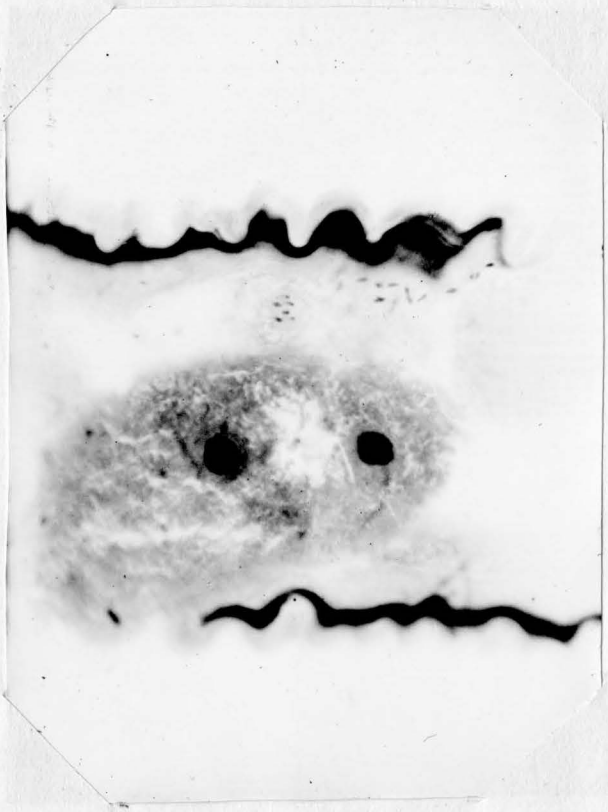

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