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GAMETOGENESIS IN THE FINAL GENERATION OF *SCHISTOSOMATIUM DOUTHITTI* (CORT, 1914) PRICE, 1931 (TREMATODA: SCHISTOSOMATIDAE)

by

Ariel Adrean Roth

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Abstract

GAMETOGENESIS IN THE FINAL GENERATION OF SCHISTOSOMATIUM DOUTHITTI (CORT, 1914) PRICE, 1931 (TREMATODA: SCHISTOSOMATIDAE)

Ariel Adrean Roth

A study of gametogenesis in S. douthitti was made by the use of sectioned and stained specimens. Spermatogenesis follows the general pattern found in the hermaphroditic trematodes in that the dividing germ cells remain attached to each other and finally form a cluster of 32 spermatids. This is unlike the schistosomes of man where the dividing germ cells do not remain attached to each other. The three spermatogonial divisions appear to be normal mitotic ones. During the prophase of the first meiotic division, preleptotene splitting is evident. Pseudoreduction by parasynapsis takes place during the zygotene stage while the chromatin forms a condensed mass which is located on one side of the cell. This mass opens up to form the pachytene bouquet. During the metaphase which follows there appear to be 7 or 8 bivalents. Precession of one of the diads seems to take place on only one side of the spindle. Spermiogenesis takes place in the spermatid by elongation of the nucleus into a long filamentous chromatin body. Most of the cytoplasm of the spermatid is left behind as the sperma-

tozoon emerges, leaving a residual body which shrinks and later disintegrates. The spermatozoon appears to have a thin cytoplasmic layer surrounding it, and a narrow filamentous tail.

Meiosis in the oocytes is similar to that found in the spermatocytes. The process begins in the ovary and proceeds through to the pachytene stage. It then usually enters a diffuse stage, where the chromatin forms a fine network distributed throughout the reconstructed nucleus. Further stages take place in the eggs located in the veinules of the hepatic portal system of the host. During the metaphase of the first meiotic division there are apparently 7 bivalents. The first polar body resulting from the first meiotic division is occasionally seen to become bilobed, but does not divide.

The pronuclei which follow the second meiotic division are at first unequal in size, the male being the smaller. After they become equal in size karyogamy takes place forming the metaphase of the first cleavage division. This division is unequal, one of the cells being slightly smaller than the other. No centrioles were found in any of the divisions of gametogenesis. However, one questionable case of their presence was found in the first cleavage division. The diploid number of chromosomes for the male lies within the range of 14 to 16, and is probably 15. That of the female is probably 14.

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS. ii

TABLE OF CONTENTS iii

LIST OF TABLES. v

LIST OF PLATES. vi

INTRODUCTION. 1

HISTORICAL REVIEW 5

MATERIALS AND METHODS 10

THE MALE REPRODUCTIVE SYSTEM. 14

SPERMATOGENESIS 19

Primordial cells and primary spermatogonia . . 20

Secondary spermatogonia. 24

Tertiary spermatogonia 25

First meiotic division 26

Second meiotic division. 35

Spermiogenesis 37

THE FEMALE REPRODUCTIVE SYSTEM. 41

OOGENESIS 44

Primordial germ cells and oogonia. 44

First meiotic division 46

Second meiotic division. 56

Pronuclei. 59

KARYOGAMY AND CLEAVAGE. 61

MITOTIC CHROMOSOMES 63

MISCELLANEOUS OBSERVATIONS. 66

Cells of unknown function. 66

Other miscellaneous observations. 68
DISCUSSION 69
Effect of various factors on the rate of spermatogenesis 69
Grouping of male germ cells 71
Spermiogenesis and the residual body. 72
The female reproductive system. 75
Growth of the oocyte as related to nutrition granules. 75
Locale of oogenesis 76
Differentiation of primordial cells from more advanced stages 78
Centrioles. 80
Prophase of the first meiotic division. 81
Reduction and equation divisions. 86
Chromosome numbers. 86
SUMMARY. 92
LITERATURE CITED 94
EXPLANATION OF PLATES. 103

LIST OF TABLES

Table 1 Comparison of the Degree of Fulness of the Testicular Follicles and the Relative Rate of Meiosis with Other Factors Listed in the Table. 17

Table 2 Comparison of Diploid Chromosome Numbers of Trematodes in which Different Numbers have been Reported by Different Authors . . 90

LIST OF PLATES

Plate 1 Figures 1 through 30. 109

Plate 2 Figures 31 through 60 110

Plate 3 Figures 61 through 81 111

INTRODUCTION

The blood flukes of the family Schistosomatidae are the only group of digenetic trematodes in which sexual dimorphism exists in the final generation. This dimorphism offers interesting possibilities for studying reproduction in the earlier generations, i. e., the mother and daughter sporocyst generations. Before a study of the process of reproduction in the sporocyst generations could be undertaken, a knowledge of this process in the final generation where the sexes are separate would be very helpful. It is the purpose of this paper to describe gametogenesis in the final generation of Schistosomatium douthitti Cort as a basis for further study on the sporocyst generations.

This species was used because: its life cycle is known, Price (1931); it is relatively easy to maintain this parasite in the laboratory; and the intermediate host Lymnaea palustris Muller, as well as the small mammals used as final hosts are also easy to maintain in the laboratory. It was very fortunate that S. douthitti was used, because, unlike any of the other schistosomes in which spermatogenesis has been studied, the developing gametes remain attached throughout the spermatogonial and spermatocyte divisions. Thus the number of germ cells in a cluster aids in identifying the stage being studied. This permits the drawing of more definite conclusions

than has been possible with the other species of schistosomes studied.

Early workers studying natural snail infections of schistosomes found that when suitable mammalian hosts were exposed to cercariae from a single snail, the majority of the hosts were found to harbor only one sex of schistosome, either male or female. Occasionally, however, a mixed infection of both males and females was found. Such results have been reported by Faust and Meleney (1924), Faust (1927), Severinghaus (1928), Lee (1932), and several Japanese workers including Sugiura (1933). Cort (1921) mentions the work of Tanabe (1919), and Short (1952) mentions the work of Tanaka (1929). Both of these workers also found similar results. Later workers, Giovannola (1936), Vogel (1941), Short (1947, 1952), and others have shown that in various species of schistosomes, when a snail is exposed to a single miracidium, and the cercariae from that snail are introduced into a mammal, the adult schistosomes which then develop are always of the same sex. These facts led to the hypothesis that sex is determined at the time of fertilization of the egg which will develop into the miracidium.

On the other hand, Maldonado and Matienzo (1947) have reported finding the haploid number of chromosomes in the daughter sporocyst of Schistosoma mansoni. Also of interest here is the experiment of Short (1948), who, working with S. douthitti, was able to obtain miracidia

from eggs shed by hosts which contained only female worms. Some of these miracidia, when put into snails, developed and produced cercariae which, when put back into a final host yielded worms of both sexes. Out of the five positive snails which he obtained, four yielded only males, and one only females. The origin of the male component in this experiment remains problematic. Obviously, cytological studies in gametogenesis would be helpful in the solution of this problem.

Since the sex of the cercariae is apparently predetermined at some stage of reproduction in the snail, considerable study has been carried out by various workers including Cort (1921), Faust and Meleney (1924), Ikeda and Makino (1936), Vogel (1941), and El-Gindy (1951) who worked on S. douthitti, in an attempt to find morphological differences between the male and female cercariae, but with no conclusive results. Cort (1921) found cercariae of two sizes in Planorbis trivolvis and interpreted the difference as sexual dimorphism. However, Short (1952) indicates that "in the light of subsequent work on schistosomes, it seems probable that Cort was dealing with two species of cercariae, and I know of no proof that sexual dimorphism exists in the cercariae of schistosomes." Possibly such a dimorphism might be detectable when cytological studies of the germ cells are made. Studies in gametogenesis of the final generation would aid in indicating such a possibility.

The importance of the study of gametogenesis for its own sake should not be overlooked. Such a study offers interesting material for the study of taxonomy as well as aiding in an understanding of the process of gametogenesis itself, about which there is still some disagreement. It should also be mentioned here that the process of oogenesis has never been worked out in any of the schistosomes. This is due to the fact that most of the process does not take place in the parasite but rather in the tissues of the host.

Since the literature dealing with gametogenesis in the trematodes shows considerable disagreement in terminology, the writer has chosen to follow the terminology used by De Robertis et. al. (1948). In cases where this is not adequate, the terminology of Wilson (1925) is used.

The reader may feel that in the descriptions which follow, too much emphasis has been placed on the problem of distinguishing one stage from another similar one. Since other writers have not done this, this information is presented here in the hope that it may facilitate the working out of future studies of this nature.

HISTORICAL REVIEW

Relatively few studies have been made on the process of gametogenesis in trematodes, although the number is increasing steadily. For a review of the earlier works, the reader is referred to the paper of Cable (1931). Another contribution to the review of early studies of gametogenesis in trematodes is the paper of Anderson (1935), who summarized in tabular form the work done up to that time. However, contrary to what Anderson reported, oogenesis has not been worked out completely in the three species of schistosomes mentioned, although the very early stages are described for two of them.

Early workers concerned themselves mainly with oogenesis. However, Dingler (1910) worked out spermatogenesis in Dicrocoelium lanceatum, and von Kemnitz (1913) worked out both spermatogenesis and oogenesis. Except for the work on schistosomes, nothing was done along this line until the papers of Brooks (1930) and Woodhead (1931) renewed interest in the germ cell cycle of trematodes, which usually involved a study of gametogenesis in the final generation.

The papers published since Cable (1931) which deal with the germ cell cycle and also include a description of gametogenesis are: Chen (1937), Rees (1939), Churchill (unpublished thesis), Pieper (1953) and Van der Woude (1954). The main purpose of these papers was to work

out the germ cell cycle, but the descriptions given to the process of gametogenesis in the final generation are, in some cases, sketchy.

A number of workers have studied gametogenesis in trematodes in one phase or another without subsequent study of the reproductive process in the intermediate generations, their interest being mainly of a histological and taxonomic nature. Those published since the review by Cable (1931) are: Anderson (1933), Pennypacker (1936) and (1940), Markell (1943), Jones et. al. (1945), Willey and Koulish (1950) and Willey and Godman (1951). The works of Britt (1947) and Ciorida (1949) and (1950), which deal only with the chromosomes but do describe these in some of the stages of gametogenesis, should also be mentioned. None of the above lists, however, include any of the work done on schistosomes.

The first work done on gametogenesis of schistosomes is that of Lindner (1914), who worked on Schistosoma haematobium. Lindner's material came from two sources, man and cattle. He recognized the possibility that he might be dealing with two different species, but, since the pattern of gametogenesis appeared to be the same in both types, he did not attempt to differentiate between the two in his paper. Subsequent work has shown that the schistosome of cattle is a different species, S. bovis. In the primary spermatocyte, Lindner found 8 chromosomes arranged on the equatorial plate. Of these,

6 were large, peripherally located autosomes, and 2 were small, centrally located heterochromosomes which he concluded were probably sex chromosomes. He noticed that these 2 small heterochromosomes lagged behind during either the formation of the equatorial plate, the migration of the daughter chromosomes, or the anaphase. These chromosomes did not split at the early anaphase, but both migrated to one of the poles, thus producing two secondary spermatocytes, one of which had 6 chromosomes and the other 8. Thus the chromosome number for a male would be 6 ⁄ 8 = 14, and for a female 8 ⁄ 8 = 16. In the process of spermatogenesis the cells do not remain connected. Lindner did not report any work on oogenesis.

Faust and Meleney (1924) published a report of studies on schistosomiasis which includes a study of spermatogenesis in Schistosoma japonicum. They reported that there were 15 distinct chromosome elements prior to syndesis which takes place in a spermatogonial cell. After syndesis only 8 could be found, 7 of which were bivalents (tetrads) and the other one apparently a single diad containing the x members. In the anaphase which followed, the diad containing the x members reached the pole earlier than the other diads. This spermatogonial division resulted in the formation of two primary spermatocytes, one containing 8 bivalents, and the other 7. The next division, which was considered quantitative, resulted in the production of four secondary spermatocytes. Two

of these contained 8 chromosomes each, and the other two 7. Again the chromosome containing the x members is seen to precede the other chromosomes to the pole. Apparently secondary spermatocytes change directly into spermatids without dividing, and the latter form spermatozoa. Faust and Meleney report a slight tendency on the part of the secondary spermatocytes to remain together. No discussion of oogenesis is included in this report.

The next paper, published by Severinghaus (1928), also deals with S. japonicum. Severinghaus found, as did Faust and Meleney (1924), that the male is heterozygous. However, he found the diploid number to be 14 instead of 15. He reported that reduction takes place at the division of the primary spermatocyte whose metaphase plate contains 8 chromosomes of which two are x chromosomes and the other six are autosomes. Of the two resulting secondary spermatocytes, one contains 6 chromosomes and the other 8. This latter one receives the two x chromosomes which precede the autosomes to the pole of the anaphase spindle. The second maturation division results in the formation of 4 spermatids which change directly into spermatozoa. Severinghaus was able to find only the early maturation stages in the female. However, he was able to determine that the diploid number of chromosomes for the female is 16.

Ikeda and Makino (1936) also worked on S. japonicum.

These workers report results which are at variance with both Faust and Meleney (1924) and Severinghaus (1928). According to Ikeda and Makino the spermatogonium contains 16 chromosomes. The primary spermatocyte has 8 bivalents at the metaphase. There is no precession or succession of any chromosome, and all chromosomes behave the same in anaphase. All secondary spermatocytes contain eight chromosomes. The diploid chromosome number of the female is the same as the male, i. e., 16. From their study of the morphology of the chromosomes of both male and female worms, no x or y chromosomes could be detected.

The most recent paper dealing with gametogenesis in the schistosomes is that of Niyamasena (1940) who worked on Schistosoma mansoni. The results he obtained are essentially the same as those reported by Ikeda and Makino (1936), i. e., the spermatogonia have 16 chromosomes, the primary spermatocytes have 8 bivalents, and all the secondary spermatocytes have 8 diads. No mention is made of the behavior of the chromosomes during the anaphase of the first maturation division. Niyamasena described the first stages of oogenesis, but was unable to go beyond the diffuse stage found in the posterior part of the ovary.

MATERIALS AND METHODS

Since this study was begun late in the fall of 1953 when natural infections in the vicinity of Ann Arbor were difficult to obtain, material was secured from the Zoological Laboratory of the University of Pennsylvania, and from the Department of Bacteriology and Parasitology of the University of Chicago where *S. douthitti* is maintained in culture. The Pennsylvania source had recently obtained its material from Chicago, and therefore, all specimens can be considered to have come from the same laboratory, although the Chicago culture is a "mixed" one, part of the original source coming from Illinois and part from Michigan.

A series of preparations was made so that the ages of the parasites would be approximately 25, 35, 45, and 55 days. The ages given throughout this paper refer to the time interval between the penetration of the cercariae into the mice or hamsters and the time of fixation of the parasite. They do not include the age of the cercariae prior to penetration. The hosts were killed instantly by a sharp blow on the head in order to assure that the parasites would be free from any effects of anesthesia. The peritoneal cavity was immediately opened and the worms removed. Some of the worms were removed by teasing them out of the mesenteric veinules by the use of fine needles. This however, was time consuming, and it

was not always possible to get the entire worm out. Most of the worms were removed by the better and more rapid method of cutting out the veinule or the portion of the intestine harboring the parasites, and fixing the worms with the surrounding tissue. This method assured rapid fixation and less handling of the worms. It also provided material for study of the maturation stages of the oocyte, which take place in the eggs in the tissue surrounding the worms. As far as can be determined, such a method was not used by other workers who studied gametogenesis in schistosomes.

Four different groups of parasites were prepared whose ages were 24, 35, 45, and 55 days. Those 24 days old came from a male hamster (Cricetulus griseus). It was killed at about 10:00 a.m., and approximately 60 male and 6 female worms were obtained from the mesenteric veins. No worms were found in the liver. Parasites 35 days old were obtained from a male albino mouse (Mus musculus) which was killed at about 9:30 a.m. Approximately 60 worms were removed, of which about 25 were males and 35 females. Most of these came from the mesenteric veins, although one pair was found in the spleen, and 6 females were found in the liver. The parasites 45 days old came from a male hamster killed at 10:00 a.m. An estimated 64 worms were removed. The ratio of females to males was about 2 to 3. No worms were found in the liver or the spleen, but one pair was found in a vein

coming from the spleen. The 55-day old group was obtained from a female mouse killed at about 2:30 p.m.; 48 worms were removed, of which more than half were males. One worm, a female, was found in the liver, and 8 worms were found in the veins coming from the spleen. The rest of the worms came from the mesenteric veins. The majority of the worms found were in copula.

All worms and tissues were fixed in Bouin's, cleared in benzene, and then left overnight at 45°C. in a solution of benzene saturated with paraffin. They were then embedded in pure paraffin. This technique prevented excessive collapse of the eggs, which would have made observation very difficult. The worms were sectioned at various thicknesses ranging from 3 to 13μ, the majority being sectioned at 8μ. They were then put in iron alum at room temperature for 3 hours, and then in Heidenhain's hematoxylin for 18 hours, again at room temperature. Destaining time varied between 25 and 40 minutes. Tissues were counterstained using light green or congo red. Of the 132 worms sectioned, 53 were females, and 71 were males. Due to the smallness of the testes, no smear preparations were made.

The following methods of preparation seemed most satisfactory for the various phases of this study. The best thickness for the study of cellular detail was found to be between 5 and 8μ. Sections thinner than 5μ are difficult to work with because at that thickness many

of the cells appear on two sections, and the disadvantages due to errors which could be made in attempting reconstruction of chromosome pictures from two sections outweigh the advantage of clearer definition in thinner sections. For the study of the number and grouping of the male germ cells, sections 13μ in thickness were most useful, and possibly thicker ones would have been even better. Sections 6 to 8μ thick were best for the study of the maturation process in the eggs. Thicker sections are almost valueless because the yolk material stains so deeply that it becomes almost impossible to observe any details. The length of time for destaining which yielded the best results was 30 or 35 minutes. Light green counterstain was generally found to be more satisfactory than congo red. This was especially true in the study of the oocytes, where the limits of the cells were much more distinct when the light green counterstain was used. The worms fixed while still in the tissue gave finer cytological detail than those which had been teased out of the tissue, indicating that delaying fixation in order to extract the worms is not justified. No particular age group was found to be superior to another for the study of gametogenesis. Worms in which the developing germ cells were not crowded were the easiest to study, and these occurred in all age groups, although more were found in the 24-day old worms.

THE MALE REPRODUCTIVE SYSTEM

Price (1931) described the general features of the male reproductive system of S. douthitti. Repetition of this seems unnecessary at this time. The writer's observations are in agreement with hers, except for the statement "Each follicle [of the testes] opens through a short vas efferens into a single median vas deferens." Upon examining longitudinally sectioned worms, it was found that this statement does not always hold true in my preparations. There is no vas efferens in the posterior follicles of the testes, but, as is shown in figure 78, the follicles communicate with each other through openings in the wall between them. The spermatozoa pass through these openings to reach the more anterior follicles which have a distinct vas efferens opening into the vas deferens.

The follicles of the testes are lined by what appears to be a very thin membrane, and are usually surrounded on the outside by a thin layer of condensed parenchymal fibers. These fibers, as well as all other parenchymal fibers in the parasite appear to be of the collagenous type and not of the elastic type. This was determined by comparing their staining qualities with those of the connective tissue layers in the wall of the blood vessels of the mammalian host. In some instances the testicular follicle lies just beneath the cuticle

of the parasite with no parenchymal fibers in between, and some of the cuticular spines project into the lumen of the follicle.

Inside the follicles are found the male germ cells which are in various stages of development. Some follicles contain practically every major stage of development. On the other hand, in one instance, two follicles were found side by side, one of which appeared full of spermatids and spermatozoa, while the other contained only spermatogonia and primary spermatocytes. Such a situation was the exception rather than the rule. Some of the follicles were found to be completely filled with developing germ cells tightly pressed against each other, while some others were more than half empty. The follicles on one worm tend to show the same degree of fulness, and it was also noticed that in some worms meiotic figures were much more frequent than in some others, indicating that meiosis was going on at a faster rate in some cases.

The facts mentioned above prompted the writer to try to determine if there is any correlation between the degree of compactness or the number of meiotic figures as compared to the age of the worm, the type of host, i. e., mouse or hamster, the sex of the host, whether the worm was in copula or single, or the time of day the host was killed. A summary of the results obtained is given in table 1. For this study, only worms which were

fixed in the veinules were used since it was only in these cases that it could be determined with certainty that the males were or were not in copula. No single males, i. e., males not in copula, are reported for groups B and D because none were found. The degree of fulness of the follicles is reported using arbitrary figures. If there was a relatively large amount of free space in the testes of a worm it was rated as 1; if there was a moderate amount of free space, a rating of 2 was given; and very little or no free space was rated as 3. The relative rate of meiosis was determined by counting the number of clusters of primary spermatocytes in which at least one of the cells showed a metaphase plate. This showed considerable variation from worm to worm. In one instance, a worm 45 days old which was not in copula showed only 1 such group. On the other hand, a worm 55 days old which was in copula showed 11 such groups.

Examination of table 1 reveals that in the specimens examined the testes tended to become fuller with age. Also there seems to be a slight tendency on the part of the worms in copula to have fuller testicular follicles than the single worms. Other factors, i. e., host, sex of host, and time of day of killing of host, do not appear to have any effect on the fulness of the testes. Since only 39 specimens were rated, such conclusions can by no means be definite. By comparing the results obtained by counting the number of groups of primary

TABLE 1

COMPARISON OF THE DEGREE OF FULNESS OF THE TESTICULAR FOLLICLES AND THE RELATIVE RATE OF MEIOSIS WITH OTHER FACTORS LISTED IN THIS TABLE*

Group	Age of parasites in days	Mammalian host**	Sex of host	Time of day when host killed	Average degree of fulness of follicles***			Average number of groups of primary spermatocytes at metaphase		
					Single	In copula	Average for group	Single	In copula	Average for group
A	24	H	♂	10:00 a.m.	1.5 (6)	1.8 (4)	1.6 (10)	4.8 (6)	6.2 (4)	5.4 (10)
B	35	M	♂	9:30 a.m.		2.0 (10)	2.0 (10)		5.5 (10)	5.5 (10)
C	45	H	♂	10:00 a.m.	1.6 (5)	2.6 (5)	2.1 (10)	4.0 (5)	4.5 (5)	4.2 (10)
D	55	M	♀	2:30 p.m.		2.6 (9)	2.6 (9)		8.0 (9)	8.0 (9)

* Numbers in parenthesis indicate number of specimens examined

** M = Mouse: Mus musculus, H = Hamster: Cricetulus griseus

*** For explanation of values see text

spermatocytes at the metaphase, it is obvious that the various factors studied do not account for the large range, i. e., 1-11, mentioned above. However, group D shows a definitely higher number of meiotic figures than the other groups. This high number does not appear to be related to age since group C has the lowest average. Unfortunately, group D is different from the other groups in two factors, i. e., time of day when the host was killed, and the fact that the host was a female. Also there was a delay of about two hours before the worms were extracted from the host for this group, but the tissues were kept warm and moist until removal was possible. It appears that some other factor is responsible for the wide range of numbers of primary spermatocytes at the metaphase, or possibly several factors working together produce this variation. Obviously, further studies are necessary before any definite conclusions can be drawn. Some phases of this study will be taken up in the discussion.

SPERMATOGENESIS

In undertaking the study of spermatogenesis, all the male specimens prepared were examined, and from these, those best suited for the particular phase of study under question were selected and studied more thoroughly. It was noticed that the size of various corresponding stages varied from worm to worm. In cases where the testes are very crowded the developing germ cells are usually small, and vice versa. Never-the-less, as will be seen below, there is considerable variation in the size of corresponding stages within the same worm. In order to present figures which would be adequate for use in comparing the size of the various developing stages, all measurements given in the section on spermatogenesis which deal with the size of the cell, nucleus, and nucleolus, are taken from a single specimen. In some cases, therefore, only a few stages could be measured to give an average figure. The specimen used was a 45-day old male which was not in copula. This specimen was used because it had a comparative abundance of all stages, and also because the cells in the testes were not crowded. This makes identification of the stages relatively easy and the cells have a more normal shape when they are free from pressure from other cells. Since the cells are quite irregular in shape the average diameter was calculated by taking an average of the longest and shortest "diameter." This was not

done for the nuclei or nucleoli which are usually spherical.

Primordial cells and primary spermatogonia.--These two types of cells will be considered together because no adequate morphological basis for distinguishing between them was found. This matter will be considered further in the discussion. In this section, the writer will refer to both types of cells as primary spermatogonia.

In each follicle of the testes, there can usually be found attached to the wall one or two groups of small cells (Fig. 1). The subsequent history of these cells indicates that these groups are formed, at least in part, of primary spermatogonia. Within these groups, other more advanced stages of spermatogenesis are often found. In only one instance was a primary spermatogonium found free from other cells. It was, however, still attached to the wall of the testes. This cell and other primary spermatogonia can easily be distinguished from other cells which are apparently in the wall of the testes. The former clearly project into the lumen of the testicular follicle, while the latter have sides that taper off gradually, blending into the follicular membrane (Fig. 78).

A typical resting primary spermatogonium is usually smaller than the other cells in the testes. The average diameter of 13 cells was found to be 6.2μ (range, 5.3-9.0). The cell membrane, which is sometimes very diffi-

cult to discern, shows that the cells are highly irregular in shape due to pressure from surrounding cells. The small amount of cytoplasm present appears to be a hyaline mass, throughout which is dispersed a fine non-basophylic reticulum. The nucleus tends to be spherical, and contains fine chromatin filaments which appear in the form of a net-like structure distributed throughout its contents. Whether or not these fine filaments are continuous could not be determined. Many small dark granules appear in the nucleus, probably indicating where two filaments cross each other. The diameter of the nucleus of 16 primary spermatogonia averaged 4.9μ (range, 4.0-6.9). Usually a small homogenous spherical nucleolus which stains deeply with hematoxylin can be distinguished in each nucleus. The nucleolus is eccentrically located, sometimes found against the nuclear membrane where it forms a flattened body as shown in figure 6c. At times a structure is encountered which might be interpreted as a second nucleolus, but it might very well be the point of crossing of two chromatin filaments. The average diameter of 11 nucleoli of primary spermatogonia was found to be .8μ.

The division of the primary spermatogonia is a normal mitotic one. By studying the anaphase it could be determined that the resulting cells are usually equal in size, although in two instances it was found that one cell appeared distinctly smaller than the other. It

should be mentioned here that in studying sectioned material one does not have a very adequate means of judging depth, and two cells which appear unequal in size when only two dimensions are considered might have equal volumes when a third dimension is taken into account.

The first indication of mitosis is a peripheral migration of the chromatin in the nucleus, leaving a clear area in the center. This is followed by a gradual shortening and condensing of the chromonemata, presenting a picture similar to that shown in figure 2. At this stage the chromonemata, which form part of the chromosomes, are located close to the nuclear membrane. In some instances they seem to show a double nature, possibly indicating that duplication has already taken place (Fig. 2). At this stage the nucleolus is somewhat larger and less basophylic, but still homogeneous. It disappears soon after this. The chromosomes shorten and their double nature is no longer apparent. The nuclear membrane now disappears and the chromosomes soon migrate to form the equatorial plate (Fig. 3) for a very brief period of time. This latter conclusion is based on the fact that very few such stages were found. By this time, considerable growth of the cell has taken place. The average size of the three metaphase stages of primary spermatogonia which could definitely be identified as such in the specimen used for measurements was found to be 8.6μ (range, 8.1-8.8). The peripheral portion of the cell still shows

an acidophylic reticulum which appears less condensed than that found in the cytoplasm of the resting primary spermatogonium. The central portion of the cell is clear except for the structures of the mitotic figure.

The achromatic figure is already formed at this stage. There are no astral rays, and no centrioles appear to be present. Occasionally there was seen at one end of the spindle an elongated, darkened area which might be interpreted as a centriole, but it was never found at both ends of the spindle. The condensation of the achromatic fibers at the poles of the spindle also forms a darkened area, but no structure that could definitely be interpreted as a centriole was found.

During the anaphase the chromosomes split and the daughter chromosomes migrate to the opposite poles. The anaphase is the most rapid of all the mitotic stages and therefore the least frequent stage to be observed in fixed material. In some instances (Fig. 4) there is distinct evidence that some of the chromosomal elements precede the rest. It should be noted, however, that in the case of the spindle of a spermatogonium shown in figure 4, more chromatin is preceding on the left side than on the right. The first stages of the telophase are characterized by the chromosomes forming a crescent shaped mass (similar to that shown in figure 5) at the opposite ends of the cell. The chromosomes gradually elongate, forming fine chromatin filaments which appear as a net-

work. The nucleolus and nuclear membrane reappear while cytokinesis takes place forming two cells which are now called secondary spermatogonia. The later stages of the telophase of the primary spermatogonia are similar to those shown in figure 6, a, b, and c.

Secondary spermatogonia.--The secondary spermatogonia remain attached to each other, but are generally found free in the lumen of the testicular follicle. This makes their identification relatively easy if it can be determined with certainty that they are not attached to other cells in neighboring sections. The cells of the pair are usually nearly spherical, but flattened on the surface where they are in contact with each other. At the interphase, the secondary spermatogonia are quite a bit larger than the primary spermatogonia. The average diameter of 9 such cells was found to be 8.2μ (range, 7.4-8.9), the average diameter of their nuclei was 6.3μ (range, 5.3-6.8), and the average diameter of 7 of their nucleoli .6μ.

All the main stages of mitosis were followed in the secondary spermatogonia. The process goes on simultaneously in both cells of a pair, and appears to be exactly the same as that described for the primary spermatogonium. No lateral view of an early anaphase could be found so that no indication of precession of a chromosome was observed. However, later anaphases show that the daughter chromosomes do not all migrate at the same time. The

average diameter of 4 secondary spermatogonia at the metaphase was found to be 9.2μ (range, 7.5-9.7), which is somewhat larger than the 8.6μ reported for the metaphase of the primary spermatogonia. The metaphase plates do not always form at right angles to the axis of the pair, so that the resulting cluster of cells is often highly irregular in shape.

Tertiary spermatogonia.--The simultaneous division of the two secondary spermatogonia results in the formation of 4 tertiary spermatogonia. Like the secondary spermatogonia, they remain attached to each other. The tertiary spermatogonia are the largest germ cells in the testes. At interphase the average diameter of 8 of these was found to be 8.6μ (range, 8.0-9.1); their nuclei were found to have an average diameter of 6.2μ (range, 5.7-6.7); and the nucleoli of 7 of these were found to have an average diameter of .9μ. The 4 tertiary spermatogonia undergo a further division to produce 8 cells which, as will be seen later, are primary spermatocytes. All the main stages in the process of division were followed and the same mitotic pattern described for the primary spermatogonia is also found here. The average diameter of 13 cells at the anaphase was 8.4μ (range, 6.4-11.3). This is slightly below that found for the interphase, indicating that probably little or no growth takes place between these two stages. Because no adequate view of an early anaphase was found, no precession of any parti-

cular portion of chromatin was observed, although, as in the case of the secondary spermatogonia, middle anaphases show that all the chromosomes do not migrate at the same time. The direction of the division of the tertiary spermatogonia is very irregular. However, it results in the formation of a cluster of 8 elongated primary spermatocytes which are uniformly distributed into a ball-shaped mass. A section through one of these masses is shown in figure 6. The cells in this figure are in various stages of late telophase and interphase, showing that synchronization is close but not absolute.

First meiotic division.--The interphase of the primary spermatocyte appears frequently in sections of the testes, indicating that it is a stage of relatively long duration. A typical primary spermatocyte at interphase appears wedge-shaped in longitudinal section. The pointed end of the cell is directed toward the center of the cluster of 8 primary spermatocytes while the opposite end which is rounded forms the outside of the cluster. In portions of the testes which are not crowded, the primary spermatocytes of one cluster are not in contact with each other, but are united at the center of the cluster by what appears to be a cytoplasmic mass. This mass appears to be extracellular, since sometimes there seems to be a membrane separating it from the attached cells. The presence of fibrilar structures in the central mass as well as in the cytoplasm of the attached

cells makes it difficult to determine this with certainty.

During interphase the spherical nucleus of the primary spermatocyte (Fig. 7) is very similar to that of the spermatogonial cells at the same stage. The chromatin is in the form of fine filaments, forming a network which is diffused throughout the nucleus. Granules appear on this network, some of them possibly indicating where two filaments cross each other. There is only one nucleolus present, and this is usually eccentrically placed, and occasionally appears less basophylic than the surrounding chromatin. Average measurements for primary spermatocytes during interphase are as follows: Diameter of 16 cells, 8.6μ (range, 7.9-9.2); diameter of 16 nuclei, 6.2μ (range, 5.8-6.7); diameter of 6 nucleoli, .9μ.

The prophase of the first meiotic division is an extended process, which permits syndesis and crossing over of the chromosomal elements. The first indication of this process is the preleptotene stage which is similar to the early prophase of mitosis and consists in a peripheral migration of the central portion of the chromatin network. This leaves a clear area in the center of the nucleus (Fig. 8). The uniform peripheral arrangement of the chromatin permits the differentiation of this stage from the late telophase of the tertiary spermatogonia which are also arranged in clusters of 8 cells.

The next stage, the leptonema, begins by a condensa-

tion of the chromonemata. In some cases the chromonemal filaments appear to be paired. The filaments which are still peripherally located begin to orient themselves so that their free ends converge toward the same region of the cell. Figure 9 shows the beginning of such a process, and figure 8 shows that the nucleolus is located on one of the pairs of filaments. At this stage, orientation is not sufficiently distinct to permit counting of the filaments which are very fine. Their thickness is estimated to be in the neighborhood of .1μ. In the later stages of the leptonema the orientation of the filaments becomes much more conspicuous, forming a typical bouquet (Fig. 10). This clearly distinguishes the late leptotene stage from the late prophase of mitosis. The direction in which the bouquet orients itself seems to be independent of the position of the cell in the cluster of primary spermatocytes. The free ends of the filaments are all found in the same region of the cell; the nucleolus is also usually found in this region. An exception is shown in figure 10 where the nucleolus is clearly located in the middle portion of one of the loops. This seems to indicate that the nucleolus has little to do with the orientation of the filaments, but rather that the nucleolus is usually located at the end of a filament and therefore is usually associated with the ends of the other filaments, see figures 15, 17, and 18. In a few instances it was possible to ascertain that the loops

have a double nature (Fig. 10), as was also found in some cases in the early leptonema. This indicates a case of preleptotene splitting of the chromonema. The split parts will separate at the beginning of the anaphase of the second meiotic division. Sometimes the loops at this stage also show the beginning of the formation of the chromomeres which take the form of darkened granules along the filaments. The number of leptotene loops could not be determined with certainty, because they are crowded toward one side of the cell. However, in a few instances counts were made and figures between 12 and 14 were obtained, indicating that probably the diploid number is present. In the later stages of the leptonema the nuclear membrane disappears.

The next stage which, in fixed material, occurs much more frequently than the leptonema, is the zygonema where syndesis takes place. The beginning of this stage is marked by a shortening and thickening of the loops, and the chromomeres become more conspicuous (Fig. 11). The number is still in the neighborhood of the diploid chromosome number. Contraction proceeds further and the filaments form a relatively compact mass located on one side of the cell. This mass, viewed in cross section, often appears crescent shaped (Fig. 12). It is in this contracted phase that syndesis takes place. The loops of the homologous chromosomes pair up with each other. A polar view of an early stage amphinema in the condensed

phase is shown in figure 13, where it can be seen that parts of some of the loops have paired up and that in pairing the chromomeres of one filament lie next to the chromomeres of its mate. This contracted stage is followed by a gradual loosening up of the loops (Fig. 14) which are now composed of paired leptotene filaments. The uniting of the leptotene loops by parasynapsis during the zygotene stage results in a pseudoreduction, and the haploid number is found throughout the rest of the process of spermatogenesis. Further loosening up of the loops leads to the next stage called pachynema.

As in the case of the leptonema, the loops of the pachynema are polarized. Their free ends are directed toward the same region of the cell, forming a typical bouquet stage. The nucleolus is located at the base of the bouquet (Fig. 15). The pachytene loops are thicker than those found in the leptotene stage, and vary in thickness between .3 and .5μ. Their double nature is conspicuous in many instances, although each pachytene loop is composed of 4 chromonemal filaments, since, as described above, each leptotene filament is composed of 2 of these chromonemal filaments. The most striking difference between the leptotene and pachytene stage is that in the latter case the number of loops is about half the number found in the former. Counts to determine the number of loops were made in a number of pachytene stages by first making a sketch and then counting

the number of loops drawn. Usually 7 loops were found. Sometimes only 6 were found, but in two instances there were 8. It is difficult to determine whether a very small loop is present near the base of the bouquet or whether the ends of the larger loops crossing each other give the appearance of another loop. Never-the-less, the number is about half of what was obtained for the leptotene stage. Figure 16 shows a cross section through a polar view of a pachytene bouquet. One or two of the smaller loops are not represented here.

The next stage, the diplotene, is characterized by a tendency on the part of the united filaments to separate from each other, and to shorten and thicken conspicuously. Figure 17 shows the beginning of that process. The polarization of what used to be the pachytene loop is still clearly evident. At a later stage (Fig. 18) there is no evidence of polarization left, and the chromatin units which will eventually be the bivalents are found distributed throughout the cell. The individual filaments of a diplotene pair can be seen crossing each other at frequent intervals. These points are the chiasmata which are supposed to be the foci for the exchange of chromosomal parts in the process of crossing over. The nucleolus is still present and closely associated with one of the pairs of filaments (Fig. 18). Shortening proceeds further and terminalization takes place reducing the number of chiasmata (Fig. 19). The filaments are much

thicker now and the nucleolus has disappeared. In a later stage (Fig. 20) the bivalent elements appear shorter and rotation has taken place separating the paired components (filaments) farther apart. Heteropyknosis is very conspicuous, and it is also evident that terminalization has proceeded further. Shortening, rotation, and terminalization, continue almost until diakynesis is reached (Fig. 21).

In S. douthitti diakynesis is a very brief stage (judged on the basis that few such stages were found) in which condensation of the chromosomes is slightly greater than found at the metaphase (Fig. 21). At this stage the chromatids begin to arrange themselves so as to form a flat plate which will be the metaphase plate. Some of the chromosomes show a double nature, indicating that at this stage they are formed of at least 2 chromatids.

Increase in size of the chromosomes and their migration to the equatorial plate brings about the metaphase which is the most conspicuous of all the stages found in the testes. The bivalents (chromosomes) are short and rod-shaped, usually clumped together making identification of the individuals impossible. The arrangement of the bivalents is highly variable from cell to cell. There is a general tendency for the smaller ones to be found in the center of the group while the larger ones are peripherally located. Six of the bivalents appear normal, but it could not be determined with certainty

if what appears to be a seventh bivalent is actually one or two. In figures 22 and 23 it seems like one, making a total of 7 bivalents. Evidence to support this is also found in figure 19 where there seem to be 7 distinct diplotene pairs. On the other hand, in figures 24 and 25, the bivalent in question appears to be composed of two bivalents or possibly one bivalent and a single diad. Possibly two of the bivalents are attached to each other at times while being separate at other times, or one of the bivalents may split early in beginning its anaphase, making the counting uncertain. In some instances where there seemed to be 8 bivalents, one of them was not exactly on the metaphase plate. However, in diakynesis (Fig. 21) there seem to be at least 8 distinct chromosomal elements.

The length of the bivalents varies between approximately .9 and 1.5μ. Their width is about .9μ. Their double nature is clearly visible in some instances (Fig. 27). If they are formed of 4 chromatids at this stage it is not yet apparent. The cells at this stage are about the same size as those found in the interphase. The average diameter of 14 such cells was found to be 8.5μ (range, 6.9-9.8). During this stage the achromatic figure is formed. Except for the fact that it is bigger, it is essentially the same as that described for the primary spermatogonium. No centrioles are seen.

At the beginning of the anaphase, the bivalents show a peculiar behavior by clumping and forming spherical

chromatin masses (Figs. 28 and 30). Very rarely could the full complement of at least 7 separate bivalents be accounted for at this stage. There is precession of one of the diads at this stage. Figure 28 shows this precession occurring only on one side of the spindle. Figure 29, which is a polar view of an early anaphase, also shows that a diad behaves in a peculiar manner. The double nature of these diads is clearly evident in both of these figures. When later stages of the anaphase are examined, it is not so evident that precession takes place only on one side of the spindle. As anaphase progresses a few interzonal fibers appear between the separated halves of the bivalents.

The chromosomes in early telophase form a compact crescent shaped mass which gradually loosens up into chromatin filaments (Fig. 31 a and b). This reconstruction is accompanied by the formation of the nuclear membrane. While the chromatin is still concentrated on one side of the nucleus, it is obvious that in some instances at least there is rotation of the nucleus, because the pole of concentration of chromatin is not always opposite another cell, and is sometimes opposite the outer surface of the cluster. While the chromatin becomes evenly distributed throughout the nucleus, cytokinesis takes place, resulting in the formation of a spherical cluster of 16 cells which are radially arranged. When the cluster is viewed in cross section it appears like a rosette

(Fig. 31). The elongated cells are united together in the center of the cluster. The nucleus increases considerably in size between the late telophase and the interphase (Fig. 31).

Synchronization of the meiotic stages among the cells of a cluster is not as close in the first meiotic division as it is in the spermatogonial divisions. By comparing, it was found that a moderate amount of variation in stage could exist between the cells of a cluster, but that variation was never greater than one of the main stages of mitosis; i. e., prophase, metaphase, etc.

Second meiotic division.--During interphase, the secondary spermatocytes are quite similar to the primary spermatocytes of the same stage. The most conspicuous difference is the apparent absence of a nucleolus. Numerous granules are present in the chromatin network as described for the primary spermatocyte, and if a nucleolus is present, its size does not permit it to be differentiated from the granules appearing in the chromatin network. These granules vary in size between .2 and .4μ. Average measurements for 10 secondary spermatocytes at the interphase are as follows: Length of cells, 9.5μ (range, 8.0-10.7); width of cells, 6.1μ (range, 4.8-8.2); diameter of nucleus, 4.2μ (range, 3.8-4.7); this last figure shows that there is considerable decrease in size of the nucleus as compared to the 6.2μ for the average diameter of the nucleus of the primary spermatocyte.

The prophase of the secondary spermatocyte is a very rapid stage and very few such stages were found. As is the case in the primary spermatocyte, the first indications are a peripheral migration of the chromatin network leaving a clear area in the center of the cell (Fig. 32). This uniform distribution of the chromatin permits differentiation of this stage from the late telophase of the primary spermatocyte where the chromatin is localized on one side of the nucleus (Fig. 31a). As prophase advances the chromatin filaments condense and begin to form the chromosomes in a manner similar to that described for the primary spermatogonia except that here only the haploid number of chromosomes is present. They soon migrate to form the metaphase plate where they tend to be clumped together making identification and counting uncertain. In a few instances (Fig. 35) it was possible to ascertain the nature of the chromatids which appear as small rods whose length is about 1μ and whose width is about .5μ. Average measurements for 11 secondary spermatocytes during metaphase are as follows: Length, 7.9μ (range, 7.5-10.8); width, 6.3μ (range, 4.5-7.5). The average length of these cells is 1.6μ shorter than those measured at the interphase. However, since the width is .2μ greater, it may well be that when larger numbers of cells are considered, the volume of both types of cells is about equal. An increase in width would be expected prior to a division which takes place in the transverse direction

of the cell. During anaphase the chromosomes tend to migrate in groups (Fig. 34). If there is precession of a chromosome in the anaphase of the secondary spermatocytes it is not very conspicuous. In some instances an achromatic figure is clearly visible. The interzonal fibers are especially conspicuous. No centrioles are visible. The telophase follows the same pattern as that described for the primary spermatocytes resulting in a cluster of 32 elongated spermatids forming a somewhat spherical mass. The stages of telophase are shown in figure 35 a-d.

Spermiogenesis.--Prior to spermiogenesis, the spermatids go through a resting period corresponding to the interphase of the previous divisions (Fig. 35e). Many such stages are encountered, signifying that the stage is of relatively long duration. As in the case of the primary and secondary spermatocytes, the cells at this stage are elongated with a blunt and a pointed end. Their pointed ends are united at the center of the cluster, but unlike the primary and secondary spermatocytes, their sides are also in contact with each other, leaving only the rounded ends exposed to the surroundings of the cluster (Fig. 35). The nucleus is spherical, and, as in the case of the secondary spermatocytes at this stage, no nucleolus is present unless it is so small that it cannot be distinguished from the granules appearing in the chromatin network (Fig. 35e). The cells at this stage are smaller than the secondary spermatocytes

at the interphase. The nuclei are the smallest to be found in the testes. Average measurements for 15 spermatids prior to spermiogenesis are as follows: Length, 7.9μ (range, 6.5-9.0); width, 4.5μ (range, 3.8-5.6); diameter of the nucleus, 3.6μ (range, 3.0-4.0).

The first indication of spermiogenesis is a gradual condensation and peripheral migration of the chromatin. This is accompanied by an elongation of the nucleus. At the same time the chromatin becomes less basophylic and does not stain as readily with hematoxylin. This makes it more difficult to discern from the surrounding cytoplasm. The end of the nucleus which is directed toward the outside of the cluster of spermatids elongates further and becomes pointed so that the entire nucleus appears pear-shaped (Fig. 36). At this stage the spermatogonia begin to show signs of shrinking, and the entire cluster is smaller. The cell membranes between the spermatogonia are not as distinct now. The chromatin condenses further and a few large vacuoles appear in its midst. This condensation is accompanied by further elongation of the chromatin body (Fig. 37). Corresponding stages of spermatids do not always show the same number of vacuoles surrounded by chromatin. Elongation now proceeds at both ends of the chromatin body, forming a spindle-shaped body (Fig. 38). The volume of the spermatids has contracted further, so that the diameter of the entire cluster is only about 2/3 of its original size. The shrink-

ing also causes some of the cells to separate from each other at their sides but they remain attached to each other in the center of the cluster. Further elongation of the chromatin body is usually accompanied by a slight curling of the end directed toward the center of the cluster. Vacuoles are still conspicuous in these elongated bodies, and in this state they leave the cytoplasm of the spermatid as spermatozoa (Fig. 39). Since only a very few clusters were found with some of the spermatozoa inside while the rest were already out, it can be concluded that the spermatozoa leave rapidly and at approximately the same time. While the spermatozoa are in the lumen of the testicular follicle, the process of elongation continues, vacuolization disappears, and the basophylic part of the spermatozoa becomes a long, narrow filament whose maximum width is about .3μ (Fig. 40). The average length of the chromatin part of 11 such spermatozoa found in the testicular follicles was found to be 16.5μ (range, 15.0-18.0). Free spermatozoa are found scattered throughout the testes and usually a large number of them are found in the seminal vesicle. In this latter organ the spermatozoa are usually much more contorted than those found in the testes, and when viewed in cross section, each spermatozoon appears to be surrounded by a distinct layer which stains faintly with light green (Fig. 42). Also in the seminal vesicle, some of the spermatozoa appear to have a very fine tail

in which no chromatin is found (Fig. 41). This tail appears somewhat shorter than the chromatin portion described above, and could not be observed in the testes due to the crowded conditions in that organ.

When the spermatids change into spermatozoa, the latter leave residual masses with no chromatin. These masses shrink further and can occasionally be seen as a group of spherical masses of cytoplasm along the edge of the follicles (Fig. 43). Since such groups are not any more numerous in the older specimens than in the young, it is obvious that they disintegrate.

THE FEMALE REPRODUCTIVE SYSTEM

Price (1931) and El-Gindy (1951) described parts of the female reproductive system of S. douthitti. Since the writer's observations are at variance with those given by both of these authors, the female reproductive system will be described as it was found in the present study. Differences will be considered in the discussion.

The ovary is an elongated, slightly spiral-shaped organ found in the posterior part of the anterior third of the body. As was found in the case of the testes, it is limited by a very thin membrane around which there is a slight condensation of the parenchymal fibers. The ovary opens posteriorly into a narrow oviduct which soon bends on itself and begins to curve toward the anterior part of the worm (Fig. 81). Soon after this bend the oviduct is joined by the seminal receptacle, which is a large sac projecting posteriorly into the parenchyma. It is lined by a thin membrane and usually a large number of spermatozoa can be found in it. Occasionally spermatozoa are also found in the next portion of the oviduct which is considerably larger than the previous portion. Here it is joined by Laurer's canal which at first is as large as the oviduct at this region, but narrows down abruptly and becomes a small duct which proceeds posteriorly for a short distance, then turns dorsally where it continues until it opens inconspicuously,

with no signs of a papilla, on the dorsal surface of the female (Fig. 81).

After the oviduct is joined by Laurer's canal it turns and proceeds anteriorly at the side of the ovary. After it has passed the ovary, it narrows down and continues until it reaches the ootype. The latter organ is surrounded by elongated cells forming the Mehlis' gland. These cells appear brick red in preparations counterstained with congo red, and when the above stain is used, they are the only structures which appear red in either the male or female worms. The vitelline duct opens into the ootype just before its bulbous portion (molding chamber) is reached. Against the inside of the wall of this "molding chamber" are found a number of cuboidal cells with spherical nuclei. At times the lumen of this "molding chamber" is lined by a thin membrane attached to the inner ends of the cells described above (Fig. 80). The bulbous portion of the ootype is followed by a short passage at the end of which is found a valve which opens into the uterus (Fig. 80). The uterus proceeds anteriorly as a wide tube, often distended with eggs. It opens to the outside through a small genital pore just posterior to the acetabulum. The number of eggs in the uterus varies considerably in different worms and does not appear to be correlated with the age of the worms, which were, in this case, between 24 and 55 days old. The number of eggs in 5 different worms was esti-

mated by counting the number of eggs in several representative sections and multiplying the average by the total number of sections in which the uterus was located. Results varied between 15 and 137 eggs per worm. The average was found to be 69.

The greater part of the posterior 2/3 of the body of the female consists of vitelline follicles in which can be found large numbers of developing vitelline cells. The follicles are connected to the vitelline reservoir, which lies to the left of the seminal receptacle and the ovary. This vitelline reservoir contains over 100 fully developed vitelline cells. These are large cells whose approximate diameter is around 15μ. The nucleus of these cells contains large granular chromatin masses united by fine filaments. The nucleolus is large and spherical, and only its peripheral portion stains deeply with hematoxylin. Usually this peripheral portion is much thicker on one side. The central portion appears homogenous. In the peripheral portion of the cytoplasm of the vitelline cells are found several dozen small, somewhat irregular granules which stain deeply with hematoxylin. The diameter of these granules varies between .2 and 1.5μ. As will be seen below, these granules go into the formation of the shell of the eggs. The vitelline cells leave the anterior end of the vitelline reservoir by way of a small duct which is connected to the ootype at the point described above (Fig. 81).

OOGENESIS

As mentioned earlier, most of the process of oogenesis takes place in the eggs which are already in the tissues of the host. The early stages take place in the ovary and uterus of the female. For these early stages, all measurements which deal with the size of the cells, nucleus, and nucleolus were taken from a single specimen, so that comparisons would be more significant. Such a procedure, of course, is not possible for the later stages found in the eggs in the tissue, since there is no way of telling from which parasite the eggs came. The specimen used for the measurements of the early stages was a 45-day old female in copula which had a good representation of the various stages to be measured. Since the cells are quite irregular in shape, the diameter of each cell was measured the same way as the male germ cells, i. e., by taking an average of the longest and shortest "diameter." This was not necessary for the nuclei and nucleoli because these are usually spherical.

Primordial germ cells and oogonia.--As in the case of the male, no morphological difference was found which would permit the differentiation of a primordial cell from a gonial cell. Both types of cells will be designated here by the term oogonia.

The ovary is a solid mass of developing germ cells tightly packed against each other. The oogonia are found

on the outer edge of the anterior part of the ovary and also in small groups along the outer edge of the middle portion. The cells in the central and posterior part of the ovary are almost all primary oocytes. Occasionally one or two oogonia can be found mixed in with them. This is especially true in the anterior part of the organ. The ovaries of mature worms at various ages (24, 35, 45, and 55 days) were compared and very little difference could be seen between them except that there appeared to be slightly fewer oogonia in the older specimens.

The morphology of the oogonia during interphase is similar to that of the spermatogonia at the same stage. They are sometimes more irregular in shape due to the greater pressure from surrounding cells, and occasionally one of the cells along the edge of the ovary is conspicuously flattened. The cytoplasm, nucleus, chromatin, and nucleolus appear the same as found in the primary spermatogonia (Fig. 44). Measurements of 10 oogonia during interphase are as follows: Average diameter of cell, 6.1μ (range, 5.0-7.1); average diameter of nucleus, 5.2μ (range, 4.0-6.0); average diameter of nucleolus, .8μ. Some of these cells are much larger than others and it may be that some of them are primary spermatocytes, as will be seen below.

Division of the oogonia is a normal mitotic one, and apparently continues throughout the life of the worm. All the main stages of mitosis were found in various

specimens, and the process appears to be the same as that described for the primary spermatogonia, except that no precession of any chromatin material was observed.

Since the primary oocytes leave the ovary one by one, it seems logical to conclude that, unlike the primary spermatogonia, the dividing oogonia do not remain attached to each other. On two occasions two dividing oogonia were found side by side and in the same stage (Fig. 45), but never were 4 oogonia found together in or near the same stage of division. This latter fact seems to indicate that the three gonial divisions found in the male do not have a similar counterpart in the female. Also supporting this idea is the fact that the number of mitotic figures in the ovary is very small as compared to that found in the testes. This last conclusion is subject to analysis of the timing factor which cannot be done in fixed material.

First meiotic division.--The division of the oogonia by mitosis produces primary oocytes which during the interphase cannot be readily distinguished from the oogonia. It appears that the larger cells are the primary oocytes. This statement is based on the fact that the cells which are known to be primary oocytes, i. e., those which are in the leptotene, zygotene, or pachytene stage, are large, and their size readily distinguishes them from the oogonia usually found in the peripheral portion of the anterior part of the ovary. It cannot be stated

that if a cell is large it is a primary oocyte. However, since the early prophase of a primary oocyte is large, it can be concluded that some of the large cells at interphase are probably primary oocytes. All parts of these large cells appear similar to those of the oogonia except that the nucleolus is usually less basophylic.

The early stages of prophase in the primary oocyte proceed in the same manner as was described for the primary spermatocyte, and the reader is referred to the section dealing with the first meiotic division of spermatogenesis for details of the process. The double nature of the leptotene filaments indicates that preleptotene splitting, is also evident here (Fig. 46c). There is also further evidence to support the fact that the condensed zygotene stage (Fig. 47) is intermediate between the two loop stages, i. e., leptotene (Fig. 46) and pachytene (Fig. 48). As one views these various stages in the ovary, there can be seen a general tendency for the leptotene stages to be located peripherally; and zygotene stages are found more centrally, while the pachytene are found in the middle and posterior part of the ovary. The position of these various types of cells varies considerably in different worms.

Following the pachytene stage, the primary oocyte usually deviates from the pattern followed by the primary spermatocyte and forms a diffuse stage. The ends of the loops which were concentrated at one side of the cell

move away from each other and the bouquet stage disappears. This is followed by a reformation of the nuclear membrane. The chromatin filaments which were the pachytene loops come to lie in the peripheral portion of the nucleus. Some of the filaments are distinctly paired at this stage. Later they are found as very fine filaments distributed throughout the nucleus. The nucleolus at this stage is much enlarged and strongly basophylic, and is usually eccentrically placed. The narrow layer of cytoplasm around the nucleus shows a fibrillar structure. Unlike what has been reported for other trematodes, no darkly staining "nutritive granules" are found in the cytoplasm, neither are there any signs of growth at this stage (Fig. 49).

Usually by the time the diffuse stage is reached the primary oocyte is near the posterior end of the ovary, ready to leave by way of the oviduct. Cells at this stage are more spherical than in the anterior portion of the ovary, probably because the pressure from surrounding cells is lessened. Average measurements for 10 oocytes in the diffuse stage at the posterior end of the ovary are as follows: Diameter of the cell, 8.6μ (range, 8.0-9.0); diameter of the nucleus, 7.0μ (range, 6.5-7.5); diameter of nucleolus, 1.7μ. Although these cells are not very much larger than the oogonial cells, the diameter of the nucleolus is more than twice as long.

In the younger worms (24-35 days old) the oocytes

in the posterior half of the ovary may all be in the leptotene stage. Often a few are in the diffuse stage and are located near the region where the ovary is attached to the oviduct. In older worms (45-55 days old) practically all the oocytes in the posterior half of the ovary may be in the diffuse stage. Considerable variation exists within the same age group.

As the oocyte proceeds down the oviduct it is fertilized. This supposedly takes place in the enlarged portion of the oviduct (Fig. 81). No instance of an oocyte in the oviduct was found, but oocytes were found in the ootype and these had been fertilized.

When the oocyte enters the ootype it is usually surrounded by about a dozen vitelline cells coming from the vitelline reservoir. These cells and the zygote enter the bulbous portion (molding chamber) of the ootype, and, judging from the number of such situations found, remain there for a while. The zygote is found in the posterior region of this group of cells. In some instances the darkly staining granules of shell material are still in the peripheral part of the vitelline cells. This stage is considered to occur before another one in which almost all the granules of shell material have migrated away from the vitelline cells (Fig. 79). These can be found as lighter staining granules forming part of the newly formed shell. In this state the egg moves into the uterus. From the varied shapes the eggs show

while in the anterior part of the uterus it is obvious that the shells are still very soft and pliable. They stain bluish with hematoxylin stain, and the granules which came from the vitelline cells can be seen as slightly darker blue spots scattered through the lighter matrix of the shell. Some of these granules which still remain in the vitelline cells continue to move out toward the shell so that by the time the eggs have reached the middle part of the uterus, very few such granules are left behind. A few remain in the vitelline cells throughout the process of oogenesis, and, as will be seen below, are not always easily distinguished from the polar bodies.

The quickest way to differentiate between the oocyte and the vitelline cells which are in the uterine eggs is to examine the nucleolus. As previously stated, the nucleolus of the oocyte is strongly basophylic throughout, while in the vitelline cells only the peripheral portion of the nucleolus stains deeply with hematoxylin. The nucleus of the oocyte is larger than that of the vitelline cells, but the entire oocyte is smaller than a vitelline cell. This latter fact cannot be readily used as a means of distinguishing these two types of cells because the cell membranes do not stain readily and are difficult to discern among the various inclusions found in the eggs.

In some instances where the shell of the egg has been chipped away from the contents of the egg, there

appears to be a thin membrane surrounding the vitelline cells and the oocyte. This membrane normally lies just beneath the shell of the egg.

The stage at which the oocyte is found while in the uterine eggs varies in different worms. Sometimes all the oocytes may be in the diffuse stage, but usually at least some are in the pachytene stage. This is especially true of the eggs found in the posterior part of the uterus. In one instance all the oocytes in the posterior part of the ovary as well as all those which could be seen in the uterine eggs were in the pachytene stage. It could not be determined if these oocytes would eventually go through the diffuse stage or if they would change directly to the diplonema, because the rest of the stages take place in the tissue of the host where it is not possible to determine from which worm a particular group of eggs came.

Usually a large filamentous spermatozoon which stains black with hematoxylin can be found in the cytoplasm of the oocyte. In a few instances it was found to be missing. In two instances a basophylic bilobed mass appeared to be attached near one end of the spermatozoon (Fig. 50). The estimated length of 10 spermatozoa found in the oocytes of uterine eggs averaged 13.4μ (range, 10.5-17.0).

The eggs leaving the uterus of the female can be found in the surrounding tissue. They are usually in groups, often distending the veinules of the hepatic

portal system to a considerable degree. Frequently a number of white blood cells are found associated with them.

In order to find the various meiotic stages of the oocyte, over 10,000 eggs were examined. Of these, not more than 200 showed meiotic stages. Most of the eggs were too old, and were already showing signs of embryonic development. Quite a number were too young, and the oocyte was still in the diffuse stage. Examination of over 10,000 eggs was a relatively easy task after it was found that the color of the shell could be used as an indicator of the stage of development of the oocyte. Eggs in which the oocyte is still in the diffuse stage have a shell which stains bluish with hematoxylin, while eggs in which embryonic development is taking place have a tan colored shell. It is in eggs whose shell color is intermediate between blue and tan that one may find some of the meiotic stages. Often the color of the shell of all the eggs in one group will be the same, and the entire group can be discarded as unsatisfactory for the study of meiosis.

Conditions under which the eggs must be examined are not ideal. Sometimes the somewhat opaque shell covers that portion of the egg where the oocyte is located. The shells chip, and bits of them can be found all over the slides. Also the cytoplasm of the vitelline cells stains dark with the hematoxylin and makes observation

of details through them difficult. However, by examining a fairly large number of specimens it was possible to follow the sequence of the main stages of meiosis in the oocyte.

The diffuse stage lasts several times as long as the entire remaining portion of the process of oogenesis. This conclusion is based on the frequency of occurrence of various stages. The first indication of the continuation of oogenesis after the diffuse stage is a recondensation of the chromatin filaments which are now in the early diplotene stage (Fig. 51). The diplonema proceeds the same way as was described in the case of spermatogenesis. Figure 52 shows some of the chiasmata on the diplotene filaments. It can also be seen that by this stage the chromatin of the spermatozoa takes the form of a nearly round body which is less compact than was found in earlier stages. The nucleolus is now less basophylic; only the peripheral portion stains dark, while the nucleolus of the vitelline cells stains black at this stage. This is just the reverse of the situation described for the oocyte and vitelline cells in the uterine eggs. Further contraction of the diplotene chromosomes brings on diakynesis (Fig. 53) which can be distinguished from the metaphase by the fact that the chromosomes are smaller and do not form a plate.

During the metaphase, the appearance and size of the bivalents are similar to those found in the primary

spermatocytes. Their double nature is also clearly evident in some instances (Fig. 54). Counts made on several plates show that the haploid number is most likely 7. Once there appeared to be only 6, while in two cases there appeared to be 8, but in both of these latter instances one of the "bivalents" was not on the plate, and this might indicate that separation had already taken place and what was counted as two bivalents actually was only a pair of diads. Six of the bivalents are large and approximately the same size, while one is conspicuously smaller, but clearly appears to be double (Fig. 55).

The spermatozoon at this stage can be very irregular in shape (Fig. 55). Often it appears condensed, but sometimes it is quite diffuse. It usually lies at one side of the metaphase plate, but occasionally it is mixed in with the chromosomes and can hardly be distinguished from them. The average diameter of the chromatin mass representing the spermatozoon as found in 13 primary oocytes at the metaphase was found to be 1.9μ (range, 1.2-2.7).

During this stage, the achromatic figure is formed. No centrioles or asters were found. The oocyte is still nearly spherical, but often the side on which the spermatozoon is found bulges out a little. The average diameter of 17 primary oocytes at the metaphase was found to be 8.4μ (range, 6.8-10.5). This figure indicates that no growth has taken place since it left the ovary, where,

as stated above, the diameter of the oocyte in the diffuse stage is 8.6μ.

At the beginning of the anaphase, some of the cells show the peculiar behavior described in the case of the primary spermatocyte, i. e., the chromatin condenses down into a few spherical masses before migration begins (Fig. 56). As anaphase proceeds the diads begin to move away from each other and interzonal fibers appear between them. No precession of a particular diad was observed, but it should be noted that not as many anaphases could be studied in the case of oogenesis as in spermatogenesis. The cells elongate in the direction of the spindle, and the end at which the polar body is to be formed is narrower than the other (Fig. 57).

The polar body which is formed during telophase is a spherical, compact mass of chromatin (Fig. 58), which usually separates from the oocyte and is found among the vitelline cells. There is a clear area around it but this is not interpreted as being cytoplasm since no limiting membrane is visible, and since other foreign bodies such as bits of shell located among the vitelline cells, also show a clear area around them. The average diameter of 9 first polar bodies found during various stages of the second maturation division was found to be 2.1μ (range, 1.5-2.7).

The small polar bodies can usually be distinguished from the large granules of shell material which are also

of the same size, and which occasionally lag behind in the vitelline material, by the fact that the former appear as intensely black spherical masses, while the latter appear as irregularly stained masses which often have an irregular shape.

Second meiotic division.--There appears to be no true interphase between the first and second meiotic divisions. The diads which remained in the primary oocyte lose their compactness and become filamentous (Fig. 58), but apparently do not form the fine filaments normally found during interphase. The nuclear membrane does not re-form, neither does a nucleolus appear. At this stage the chromatin from the male also becomes less compact.

Re-condensation of the chromatin of the secondary oocyte forms the chromosomes which during metaphase are elongated filamentous structures (Fig. 59). By this stage the secondary oocyte shows signs of growth. The average diameter of 5 secondary oocytes during the metaphase was found to be 10.4μ (range, 9.0-11.9) as compared to the 8.4μ found for the primary oocytes. The chromatin mass from the spermatozoon has also increased in size. Four of these averaged 2.6μ in diameter (range, 2.2-2.7) as compared to 1.9μ for the same structure during the metaphase of the primary spermatocyte.

Anaphase (Fig. 60) proceeds after the formation of the achromatic figure in which no centrioles are found.

As was found in spermatogenesis, this is obviously a reltively rapid stage, for out of the approximately 200 stages of meiosis in the eggs examined, no late stages of anaphase or early telophase were found. The second polar body is usually given off, although on several occasions when later stages were examined it was still in the cytoplasm of the zygote. Figure 61 represents a late telophase stage of a secondary oocyte in which the chromatin has not yet diffused to form the pronuclei. The chromatin which originally came from the spermatozoon now has a filamentous appearance. Reconstruction follows; a nucleolus and nuclear membrane appear with each of the groups of chromatin filaments, and a fine chromatin network is formed which is found distributed throughout what are now called the pronuclei.

The second polar body is similar to the first and cannot be differentiated from it. The size of the two polar bodies was compared by measuring those in eggs where their position permitted distinguishing them. If in an egg with 2 polar bodies, one was out among the vitelline cells, while the other was still in the cytoplasm of the zygote, or in the process of leaving, or still attached to the outside of the zygote, the former was considered to be the first polar body and the latter the second. Ten such eggs were found, and the average diameter obtained for the first polar body is 2.0μ (range, 1.4-2.4), while that of the second is 1.7μ (range, 1.3-2.0. The

2.0μ average diameter of the first polar body at this stage compared favorably with the 2.1μ obtained as an average diameter of 13 first polar bodies found before the second polar body was formed.

Occasionally during both the first and second meiotic divisions, the disintegrating nucleus of a vitelline cell can be seen. Usually the nucleolus has disappeared and the chromatin stains very faintly with hematoxylin. Since cell outlines are very difficult to discern, it was not possible to determine if the cell membrane disintegrates at this time.

A study was made to determine if similar meiotic stages in the eggs would tend to be found more in one region of a veinule than in another, and if such an arrangement could be used as an aid in determining the proper sequence of some of the stages. Each of the sections of a veinule which was distended with eggs was numbered in order. There were about 40 sections. The stages found in each section were recorded in their respective groups, given their section number, and an average of the section number for each group was taken. The results obtained showed that no particular section of a veinule had a significant predominance of a specific stage. It was just about as common to find a primary oocyte metaphase at one end of the group of eggs in the veinule as at the other.

In a larger veinule where there was a larger num-

ber of eggs, it was found that development in the eggs found in the center of the mass was very retarded as compared to those near the wall of the veinule. While the oocytes of some of the eggs in the center had not yet undergone meiosis, most of the outer ones were well advanced in embryonic development. Possibly close association with host tissue aids in development.

Pronuclei.--The pronucleated stage is the most common one to be found among the zygotes. The two pronuclei formed during the telophase of the second meiotic division lie at opposite ends of the now slightly elongate zygote. They are unequal in size, the male pronucleus being the smaller (Fig. 62). Twelve of these stages were measured, and the following average dimensions were obtained: Length of zygote, 12.9μ (range, 11.5-15.7); width of zygote, 9.5μ (range, 8.0-11.2); diameter of male pronucleus, 5.3μ (range, 3.7-7.0); diameter of nucleolus of male pronucleus, 1.0μ; diameter of female pronucleus, 7.3μ (range, 6.1-7.9); diameter of nucleolus of female pronucleus, 1.5μ.

At this stage the zygote tends to be located more toward the center of the egg than previously. The male pronucleus increases in size and eventually both pronuclei are of about the same size (Fig. 63). Six of these stages were measured, and the following average figures obtained: Length of zygote, 12.9μ (rance, 11.2-14.5); width of zygote, 9.9μ (range, 7.0-11.2); diameter of pronuclei,

6.5μ (range, 5.8-7.0); diameter of nucleolus, 1.4μ. The above sets of figures show that little or no growth of the zygote takes place between the metaphase of the second meiotic division and the stage where both pronuclei are of the same size. Since in the writer's preparations few specimens could be measured, the fact that the average size of the pronuclei in the second set of figures was smaller than was found for the female pronucleus in the earlier stage cannot be considered as significant, although the possibility that one pronucleus grows at the expense of the other is not to be overlooked. Once in a while in a zygote in the pronucleated stage, there can be seen in the cytoplasm a small mass of basophylic material which is usually elongate. As a rule this structure is absent, and it is concluded that it is not constant.

In some of the eggs in which the zygote is in the pronucleated stage, what appears to be the first polar body can sometimes be seen as an elongated and sometimes bilobed structure (Fig. 64). It was never seen to divide completely. Some of the polar bodies disintegrate while the zygote is in the pronucleated stage. Out of 35 of these stages examined, 21 showed 2 polar bodies, 13 had only 1, and in 1 case no polar bodies could be found.

KARYOGAMY AND CLEAVAGE

The first indication of karyogamy is a breakdown of the nuclear membranes surrounding the two pronuclei (Fig. 64). The nucleoli are still conspicuous. This stage can be distinguished from the telophase of the second meiotic division because here the chromatin masses representing the pronuclei are about equal in size.

No stage intermediate between this and the metaphase plate of the first cleavage division was found, and it is concluded that in S. douthitti, as in some other trematodes, there is no resting fusion nucleus. The chromosomes found during the metaphase and early anaphase of the first cleavage division are usually short rods. This division appears to be a normal mitotic one, and in early anaphase the tetraploid number of chromosomes is present. This would give each daughter cell a diploid number of chromosomes. In figure 65b, one end of the spindle shows two small granules which appear as centrioles, but none were found at the other end, and it cannot be stated that centrioles are present at this division. During later cleavage divisions they do not appear.

The first cleavage division is unequal, one cell being slightly smaller than the other. Two eggs were found with these cells at interphase. The chromatin, cytoplasm and nucleoli (Fig. 66) are similar to that described for an oogonium. In one of the specimens, the

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larger cell has two large nucleoli. Average measurements of the two specimens found are as follows: Diameter of large cell, 11.4μ (range, 10.4-12.4; diameter of large nucleus, 7.8μ (range, 7.5-8.0); diameter of nucleolus in large cell, 1.7μ; diameter of small cell, 8.6μ (range, 8.2-9.0); diameter of small nucleus, 7.0μ (range, 6.5-7.5); diameter of nucleoli in small cell, 1.8μ. These figures indicate that considerable growth has taken place between this stage and the late pronucleated stage. Surrounding the two-celled embryo, a few vitelline cells can still be seen.

Further development continues while the eggs are still in the veinules, but before the embryo has very many cells, the eggs move into the connective tissues of the host. Here they can usually be seen in groups. Now and then in older infections several eggs can be seen together being walled off by concentric layers of connective tissue. More often they are seen near the intestine, and by the time they reach the muscularis layer of the intestine, fully developed miracidia can be found in them. The eggs have enlarged by this time and their diameter is about 1½ times that of eggs found in the veinules.

THE MITOTIC CHROMOSOMES

During the division of the spermatogonial and oogonial cells, it is possible to study the chromosomes as they appear in ordinary mitosis. The best polar view of a metaphase plate found in the testes is shown in figure 67. It can be seen that the chromosomes tend to group themselves in the center of the cell, making counting difficult. By comparing this plate with other metaphase plates, it was found that the shape, and especially the position of the chromosomes is variable (compare with Fig. 3), but, as in the case of the meiotic metaphase plate, the larger chromosomes tend to be located peripherally, with the smaller ones in the center.

Usually there are 2 v-shaped chromosomes with a distinct centromere located near the middle of the chromosome. Sometimes one of these chromosomes appears longer than the other. Usually there is also a curved pair of chromosomes with no apparent centromere. A third pair which appears straight has a constriction in the middle. This constriction is considered to be a secondary constriction, and not the centromere, because whenever this chromosome could be distinguished it appeared straight. Since this pair could not always be identified, the possibility that one of these chromosomes represents 2 should not be overlooked. A fourth pair in which no centromere is apparent, is characterized by being slightly curved,

especially at one end. This pair also could not always be identified. In a fifth pair, the chromosomes appear as small, nearly round dots. The rest of the chromosomes are short rods of different lengths. There appear to be 4 of these, yet, as can be seen in the middle of figure 67, it is possible that a fifth one is present. Whether this is actually a distinct chromosome or whether it represents half of one which divides precociously could not be determined. Other metaphase plates studied present similar and other problems. Never-the-less, when all factors are considered, the diploid number for the male seems to be between 14 and 16, and is probably 15.

Dividing oogonia are much rarer than dividing spermatogonia. It is not uncommon to search through all the sections of the ovary of a female and not find a single cell undergoing mitosis. Polar views of the metaphase are, of course, even more scarce. Figure 68 represents such a view, and it can readily be seen that the shape of some of the chromosomes follows the same pattern as the spermatogonial cells. The number appears to be 14.

On two occasions, while studying young eggs, a peculiar situation was found where the oocytes at metaphase showed the elongate chromosomes of mitosis, but their number was haploid as in meiosis. Near the oocyte could be found a dark spherical mass of chromatin similar to a polar body. A search of adjoining sections revealed that no spermatozoon or male pronucleated stage was present.

This is interpreted as a case of parthenogenesis which is known to occur in S. douthitti. The chromosomes are large and far apart (Figs. 69 and 70). In each case 7 distinct chromosomes are present, and their characteristic shape permits associating them with the chromosome pairs found in the gonial divisions. This indicates that the chromosome number of at least some of the secondary oocytes is 7. At the lower right edge of the oocyte shown in figure 70 there is a mass of basophylic material which might be interpreted to be an eighth chromosome. However, since this mass is a little lower down in the focus than the rest of the metaphase plate, and its size is subnormal for a chromosome, it is concluded that it probably represents a non-chromosomal inclusion. If it does represent a chromosome, this would indicate that the diploid number for the female is 15.

Cells dividing in the early embryonic stages of the miracidia were studied in an attempt to determine their nature and number of chromosomes. These cells are large and the chromosomes tend to be far apart. In 3 instances the number could be determined with reasonable certainty, and in each instance 14 were counted. The shape of the chromosomes (Fig. 71) is characteristic of that described for the gonial cells, but in some instances no evidence of the two v-shaped chromosomes could be found. The matter of chromosome numbers will be considered later in the discussion.

MISCELLANEOUS OBSERVATIONS

Cells of unknown function.--In the testes there are often found cells whose morphology differentiates them from the normal germ cells. In one of these types of cells, pyknosis appears to be going on (Figs. 72 and 73). The cytoplasm of these cells appears to be of the same fibrilar nature as that of the germ cells. These cells are usually located on the edge of the testicular follicle, but sometimes are also found in the middle. They do not seem to occur any more frequently in the older worms than in the young ones.

In what might be a second type of cell, the nucleus is small, spherical, and compact (Fig. 74). Possibly this latter type of cell is an earlier stage of the pyknotic cells described above. In one instance, more than half of the cells in a testicular follicle were of this second type, but usually they are quite rare. Since the main substance to leave the testes is chromatin in the form of spermatozoa, that substance might be produced by the above two types of cells.

Another type of cell (Fig. 75) is very rare, and is larger than the above types of cells. It has a large nucleus which is very similar to the nucleus of a vitelline cell. The nucleolus shows a dark peripheral layer somewhat thicker on one side, and the chromatin in the nucleus is in the form of large masses located on a net-

work of fine filaments. Unlike the vitelline cell, there is little cytoplasm surrounding the nucleus. Other authors have reported seeing similar cells in the testes of other trematodes, and various theories are presented with reference to their function.

A fourth type of body found in the testes cannot really be called a cell, as no nucleus is present. It is a large round body in which no chromatin can be seen. The peripheral portion is densely fibrilar, while the central portion appears to be composed of large anastomosing vacuoles, which contain sparsely scattered fibers (Fig. 76). The relationship of these to the residual body left by the spermatozoa will be considered in the section on discussion.

In the ovary there can also be seen a few cells that do not appear to be germ cells. These are apparently all of one type, and are found scattered among the primary oocytes. The most striking feature of these cells is the presence of a large nucleus which usually forms a solid black mass when stained with hematoxylin (Fig. 77). These cells can be distinguished from the zygotene stage of the primary oocyte, because the nucleus is larger, more compact, and more spherical than the mass of chromatin forming the zygonema. Furthermore, in these cells the cytoplasm stains dark and appears homogenous. Intermediate stages which might indicate the origin and fate of these cells have not been found.

Other miscellaneous observations.--During the course of this study, polygamy was found in two instances. Each of the two males in question had two females in its gynecophoric canal. Another male was found which had another male clasped in its gynecophoric canal. One male was found in which no traces of a testis could be found, although all the other males had well developed testes.

While examining the eggs in the tissue, two instances of twins were found, in both of which the individuals were approximately at the metaphase of the primary oocyte. Also, while examining the vitelline cells, one instance was found where a spermatozoon had penetrated one of them.

DISCUSSION

Effect of various factors on the rate of spermatogenesis.--Examination of table 1 indicates a possibility that the presence of female tissue, either a female parasite or a female host, may have some influence on the rate of spermatogenesis. In groups A and C, the highest rates were obtained for the worms in copula, and the highest rate of all was obtained in group D, in which the host was a female. Since no single worms could be studied for groups B and D, comparison of the rate of these with females in copula was not possible.

To the writer's knowledge, the idea presented above has no support in the literature, but a number of authors have shown that the presence of one sex of a schistosome can influence the other. In S. japonicum, Severinghaus (1928) showed that the females of this species fail to grow normally unless the males are present, reaching less than 1/5 the body length of females which are in copula. Never-the-less, if such an underdeveloped female comes into copula with a male, it then proceeds to grow normally even if 128 days old. In the case of the males Severinghaus found little variation in development with respect to the presence or absence of females. Severinghaus makes no mention of the sex of the host used. Sagawa, Ogi, and Sumikoshi (1928), Lee (1932), and Ikeda and Makino (1936), who all worked on S. japonicum, also

found that the female does not develop normally unless the male is present, and that, contrary to the statement of Severinghaus (1928), the male does not develop normally unless the female is present. Moore, Yolles, and Meleney (1949), and Maldonado and Herrera (1949) also made similar observations while working with S. mansoni, and Brumpt (1936a) has reported that the female of S. bovis does not mature unless the males are present.

Such a situation is not common to all the schistosomes. Brumpt (1936b) has shown that in the case of Bilharziella polonica the females develop normally in the absence of males, and Short (1947, 1948, and 1952) has shown that in S. douthitti the females not only develop normally without the males, but also produce eggs in which viable miracidia develop.

Several workers have studied the effect of sex hormones on parasites. Of special interest here is the work of Beck and Chandler (1950) on Hymenolepis diminuta. These workers found that in single worm infections, the rate of egg production reaches a higher level in male rat hosts than in females. Castration of the male host greatly lowers egg production, which can be restored by the injection of either testosterone or progesterone. Moore, Yolles, and Meleney (1954), who conducted experiments on S. mansoni, found that the administration of 5 mg. of testosterone propionate to mice, which harbored unisexual females, did not affect the development of these

very much, but in some cases these females developed extra ovarian tissue and the production of abnormal eggs was increased. The sex of the host apparently had no effect on either the development of the ovary or the length of the abnormally short worms. The intraperitoneal injection of desiccated powdered males into the hosts failed to produce any change in the development of the females.

The experiments listed above seem to indicate that sex hormones have some influence on parasites and their egg production. A hormonal influence might account for the higher number of meiotic figures which was obtained in the case of males in groups A and C which were in copula and group D which had the only female host of the group (Table 1). Since in the case of S. mansoni Moore, Yolles, and Meleney (1954) found that testosterone stimulated the production of extra ovarian tissue and abnormal eggs, it seems plausible that sex hormones from a female schistosome (if female schistosomes produce sex hormones) or a female host might stimulate spermatogenesis in a male. Such a hypothesis, of course, warrants further experimental data.

It must not be overlooked that the host for group D (Table 1) was killed at a different time of the day than the other groups, and there might be a period of the day when meiosis is most active.

Grouping of male germ cells.--Lindner (1914), work-

ing on S. haematobium, Severinghaus (1928) working on S. japonicum, and Niyamasena (1940) working on S. mansoni, have shown that in the schistosomes of man the dividing germ cells of the male do not remain attached to each other. This is in contrast to the situation found by the writer in S. douthitti, where the germ cells remain attached to each other through the 3 spermatogonial and 2 meiotic divisions, finally forming a cluster of 32 spermatids. In this respect S. douthitti is similar to the monoecious digenetic trematodes where grouping of the male germ cells has been reported by most workers who have studied spermatogenesis. Britt (1947) found this to be the case in each of 35 species studied. In Pneumonoeces medioplexus and P. similiplexus Pennypacker (1936) and (1940) does not state whether the dividing germ cells remain attached to each other. Woodhead (1931) found that in Bucephalus elegans the clusters of spermatids were not always composed of 32 cells, but rather of 16 or multiples thereof.

Spermiogenesis and the residual body.--Most workers who have studied the process of spermiogenesis in trematodes have found, with minor variations, that the early stages are similar to those described above for S. douthitti, i. e., that the nucleus assumes a pear-shaped form with the pointed end directed toward the outside of the cluster of spermatids. On the other hand, Cable (1931) found that in Cryptocotyle lingua the pointed end of

the pear-shaped nucleus was directed toward the center of the cluster. Woodhead (1931), Markell (1943), and Willey and Koulish (1950), who worked on Bucephalus elegans, Probilotrema californiense, and Gorgoderina attenuata respectively, all found that in the early stages of metamorphosis, the nucleus becomes crescent-shaped instead of pear-shaped. It is apparent that species vary in respect to this latter factor.

Regarding the nature of mature spermatozoa, the writer's observations are more in agreement with those of Dingler (1910) and Severinghaus (1928) than those of other workers who have described the spermatozoon of trematodes as being only an elongated mass of chromatin. Dingler described a thin filamentous cytoplasmic tail in the spermatozoa of Dicrocoelium lanceatum. Severinghaus, who worked on S. japonicum, described a fine flagellum at the posterior end of the spermatozoa which does not stain with hematoxylin. The present writer has found evidence to indicate that in S. douthitti the spermatozoa have a fine tail, and also a thin layer of cytoplasm surrounding the elongated chromatin mass.

The filiform shape of the spermatozoa of S. douthitti resembles closely that described for the hermaphroditic digenetic trematodes, and is unlike the shortened comma-shaped form found in the dioecious schistosomes of man, as described by Lindner (1914) in S. haematobium, Faust and Meleney (1924) and Severinghaus (1928) in S. japonicum,

and Niyamasena (1940) in S. mansoni.

The residual mass left when the spermatozoa leave the spermatids is described by Dingler (1910) in Dicrocoelium lanceatum, Anderson (1935) in Proterometra macrostoma, and by Rees (1939) in Parorchis acanthus, as being a compact spherical mass of cytoplasm. On the other hand Markell (1943), who worked on Probilotrema californiense, finds it difficult to understand how the disintegrating spermatids could form a spherical, compact residual mass. The present writer agrees with this latter opinion, since evidence was found to indicate that the remainders of the spermatids left behind by the spermatozoa do not fuse (Fig. 43). In a few instances the writer has also seen large spherical vacuolated cytoplasmic masses of undetermined origin in S. douthitti (Fig. 76). Markell (1943) also found these in Probilotrema californiense, and stated that they originate from vacuolated cells which first appear in the peripheral layer of the testes. He states further that as these cells increase in size their nuclei disappear and become scattered throughout the testes. Further evidence to indicate that these masses are not the residual bodies left by the spermatozoa comes from the fact that in S. douthitti too few of the spherical masses were found as compared to the number of degenerating clusters of "spermatids" (Fig. 43). This conclusion, of course, is subject to analysis of the timing factor.

The female reproductive system.--In the preparations examined for this study, the female reproductive system was found to be different in several ways from that found by Price (1931). The major differences are that in the present study a large vitelline reservoir was found (Fig. 81), a valve was found at the entrance of the uterus (Fig. 80), and a Laurer's canal was also found (Fig. 81). Price reported this latter structure as "not observed," and Tanabe (1923) did not mention it in describing the new genus Schistosomatium. El-Gindy (1951) described a Laurer's canal for S. douthitti, but his figure 41 shows the canal to be a short straight tube of uniform width opening directly into the dorsal end of the seminal receptacle which is opposite to the end opening into the oviduct. This is quite different from the long curved tube which enlarges abruptly before it unites with the oviduct, as found in the three specimens in which the canal was traced in my preparations. It is conceivable that this is a case of individual difference. Severinghaus (1928) found considerable variation in the relationship of the seminal receptacle to the oviduct while comparing various specimens of S. japonicum. In some instances, it was a simple enlargement of the oviduct, while in others it formed a pocket separated from the oviduct by a small duct.

Growth of the oocyte as related to nutrition granules.--Most of the workers describing oogenesis in the

trematodes have reported that the oocyte increases considerably in size prior to leaving the ovary. This increase in size is accompanied by the appearance of basophylic masses in the cytoplasm of the oocyte. Willey and Godman (1951) studied these granules in Zygocotyle lunata and found that they were Feulgen negative. Most authors consider these granules to be related to nutrition in one form or another.

In the schistosomes, both the process of formation of the granules and the increases in the size of the oocyte appear to be reduced or missing. According to his figures, Severinghaus (1928) shows little growth in the oocyte of S. japonicum prior to leaving the ovary. He did not mention any nutrition granules in the cytoplasm, but it may be that the dots which appear in the cytoplasm of the oocyte shown in his figure 15 represent this condition.

In S. douthitti there is little or no growth of the oocyte before it leaves the ovary, and no basophylic granules are found in the oocyte. This seems to show that the increase in size of the oocytes and the appearance of the basophylic granules in them are related to each other. For further consideration of these granules in the oocyte the reader is referred to the papers of Pennypacker (1940) and Willey and Godman (1951).

Locale of oogenesis.--To the best of the writer's knowledge, whenever oogenesis has been described in the

final generation of the trematodes, it has been found to take place in the ovary and uterus of the parasite. In the schistosomes of man oogenesis has not been observed in the uterus. Severinghaus (1928) was unable to find any stages of oogenesis beyond the diffuse stage in the uterus of S. japonicum although it may contain as many as 300 eggs. He suggested that further stages in development take place in the tissues of the host. Niyamasena (1940) was also unable to find any of the advanced stages of oogenesis in S. mansoni, which has few (1 to 4) eggs in its uterus at a time. Short (1952) found that in S. douthitti no development beyond the early stages of prophase takes place in the uterine eggs. Price (1931), while studying the same species, states that the eggs are "entirely undeveloped when deposited." The present writer's observations are in agreement with those of the above authors, and it has been shown here that the later stages of oogenesis take place in the tissues of the host.

It might well be that such a situation is common to all the schistosomes. The significance of this is not understood. Possibly the oocyte requires nourishment from the host tissues. In S. douthitti the oocyte does not increase in size while in the ovary. The greatest increase in size takes place between the first maturation division and the pronucleated stage, while the eggs are in the tissues of the host. Since the eggs contain a number of vitelline cells the need for nourishment is

probably not great, although, as pointed out above, the eggs in the tissue of the host increase considerably in size while moving toward the lumen of the intestine, and the eggs which are closest to the host tissue undergo maturation earlier than those which are separated from it by other eggs.

Differentiation of primordial cells from more advanced stages.--The problem of distinguishing the primordial cells from the primary spermatogonia has received some attention by various authors working on the trematodes. Lindner (1914) working on S. haematobium, and Severinghaus (1928) working on S. japonicum, both described the primordial cells as being elongated cells flattened against the wall of the testes. Niyamasena (1940) also mentions the presence of a layer of flattened cells against the wall of the testes in S. japonicum, but concludes that they might just as well be flattened spermatogonia. Cable (1931), Woodhead (1931), Anderson (1935), Markell (1943), and Willey and Godman (1951), who worked respectively on Cryptocotyle lingua, Bucephalus elegans, Proterometra macrostoma, Probilotrema californiense, and Zygocotyle lunata; all indicate that a primary spermatogonium can be distinguished from a primordial cell by the fact that the former is a lone cell which has migrated away from a group of the latter. Some of these authors also indicate a larger size for the primary spermatogonium. The writer does not feel that such criteria can always

be used as a means of separating these two types of germ cells in S. douthitti because, obviously, too few single cells (primary spermatogonia) were found to account for the large number of developing germ cells of a more advanced stage. Also, in S. douthitti advanced stages of spermatogenesis are found among groups of "primordial" cells indicating that no separation has taken place. Size is also inadequate as a means of distinction because it is evident that growth takes place prior to, or during cell division. The statement made by the writer to the effect that the primordial cells are morphologically similar to the primary spermatogonia also has support from various authors, including Anderson (1935), Markell (1943), and Niyamasena (1940), who worked on Proterometra macrostoma, Probilotrema californiense, and S. mansoni respectively.

In the female the problem of distinguishing the primordial cells from the oogonia is more difficult than in the male, because in the ovary the cells are closely crowded together, and it is not possible to determine whether a cell is separate from another. Most authors do not mention the primordial cells in the female. Anderson (1935), working on Proterometra macrostoma, and Markell (1943), working on Probilotrema californiense, do mention them, but find that they cannot be distinguished from neighboring cells. The present writer's observations are in agreement with this.

Centrioles.--The presence of centrioles during the process of gametogenesis in the schistosomes does not appear to be a very constant factor. Lindner (1914) found in S. haematobium two small dots that might be interpreted as centrioles. He did not find centrioles in any other instance. Faust and Meleney (1924) illustrate centrioles in the first maturation division of spermatogenesis in S. japonicum. No mention of them is made in the text, and centrioles are not illustrated in any other figures. Severinghaus (1928), who also worked on S. japonicum, found centrioles in the spermatogonial cells but nowhere else.

Centrioles have been reported in some of the stages of gametogenesis in hermaphroditic trematodes by Rees (1939) in Parorchis acanthus, Markell (1943) in Probilotrema californiense, Willey and Koulish (1950) in Gorgoderina attenuata, Willey and Godman (1951) in Zygocotyle lunata. Chen (1937) found that centrioles are present throughout most of the stages of spermatogenesis in Paragonimus kellicotti, dividing at each pole in every division up to that of the secondary spermatocyte where they remain single. Anderson (1935) found in Proterometra macrostoma that it is possible to make out a centriole apparatus in a few of the dividing primary oocytes.

In S. douthitti no centrioles were found in any of the divisions except the one questionable case described for the first cleavage. Possibly in this species and

in other trematodes the centrioles are sub-microscopic at times and cannot be seen.

It could be conjectured that the bilobed structure found at the end of the spermatozoon (Fig. 50) and the possible centrioles found during the first cleavage division represent the same structure. Such a supposition would fit into the classical interpretation of the origin and function of the centrioles during fertilization and cleavage.

Prophase of the first meiotic division.--The descriptions of the extended prophase of the primary spermatocytes and oocytes in trematodes as given by various authors are in many instances very brief. In instances where these descriptions are sufficient to permit recognition of the stage in question, the terminology is often at variance with conventional practice. Also, there seems to be no common agreement as to what sequence the various stages follow. A consideration of some of the similarities and differences between the findings of other authors and those found in the present study may be helpful in clarifying this process.

In S. haematobium Lindner (1914) described more or less the same process for the prophase of the primary spermatocyte as has been found for S. douthitti, the main difference being that the zygotene stage is not a very condensed stage as compared to what was found in the present study. Severinghaus (1928), while describ-

ing spermatogenesis in S. japonicum, mentions the spireme condensing down directly into the leptotene loops. While studying the ovary of the same species Severinghaus found that all the cells of that organ fall into 2 groups, the oogonial cells and cells in the resting condition. No intermediate stages were found between these two types of cells. Severinghaus states:

> The most striking feature is the total absence of stages transitional between the cells first described and these resting cells. The one type lies by the side of the other in a sharp line of demarcation. It seems almost incredible that transformation from the one type into the other so widely different should take place so rapidly that no record of the process is obtainable; yet the fact remains that no transitional stages have been found in the hundreds of serial sections of ovaries which have been examined.

It may be that the resting condition described above represents the diffuse stage found in S. douthitti, but since no further stages of oogenesis are described by Severinghaus, it cannot be stated definitely whether this resting condition represents a pre- or post-synaptic stage. Possibly there is a time of the day when transition between these two stages takes place more frequently than another, and studying worms fixed at different times would reveal these stages.

The observations of Niyamasena (1940) on S. mansoni apparently differ from those found for S. douthitti in the present study. Niyamasena mentions first the leptotene, then the pachytene stage. He then mentions another stage where the filaments are pressed together without

order in the nuclear membrane. This latter stage possibly represents the zygotene stage which in the present writer's opinion takes place between the leptotene and pachytene stages.

In a number of cases where the details of the prophase of the primary spermatocyte have been studied in the hermaphroditic trematodes, no compact zygotene stage is reported. This condition has been found by Anderson (1935) in Proterometra macrostoma, Markell (1943) in Probilotrema californiense, and Van der Woude (1954) in Megalodiscus temperatus. Apparently species of trematodes differ in their method of synapsis.

Cable (1931) described a condensed stage in Cryptocotyle lingua, but thought that it probably occurs just before metaphase, and from it the metaphase chromosomes arise. He found further evidence for this in the fact that such a stage was seen in the eggs prior to the metaphase of the primary oocyte, while the loop stages are found in the ovary. The present writer has found some metaphase plates in the primary oocytes of S. douthitti in which all the chromosomes formed a solid mass, but since they were in the eggs they could not represent the condensed zygotene stage found in the ovary. It appears, from the above facts, that probably there is no condensed zygotene stage in Cryptocotyle lingua, and this species belongs with those listed above in which such is the case.

Chen (1937), in a study of Paragonimus kellicotti,

appears to indicate that syndesis takes place before loop formation in the primary spermatocyte. She also finds that in the oocyte the loops are formed before and after the resting diffuse stage, and that these loops are attached to the nucleolus, but become free before the diplotene stage. Also varying considerably from what appears to be the normal sequence of prophase are the observations of Rees (1939), who in studying Parorchis acanthus, does not report the presence of any loops and finds that in the primary oocyte the leptotene chromosomes are "short oval bodies which during zygotene come together in pairs." She also reports a similar situation for the zygotene stage of the female which takes place in the egg. In reference to this Pennypacker (1940) states:

> Rees's figures of oocytes (figs. 39 to 46 inclusive) cannot be classified as "zygotene," according to Wilson's use of this term. Their location in the uterus, rather than in the ovary, and their place in the sequence of stages, coming after the growth of the oocyte, and the dispersion of the chromatin throughout the nucleus, confirm my interpretation of them as being later stages than the zygotene. It may be that there occurs in this trematode the same condition of the separation of the chromatids in late prophase as I have described in two species of turbellarians, and that Rees has mistaken it for the zygotene stage.

The present writer agrees with Pennypacker and it appears that Rees is dealing with some phase of the diakynesis stage and has overlooked the less obvious loop stages. It might be conceivable that synapsis takes place after the chromonema condense down, but such a radical devia-

tion from the normal method of synapsis warrants further investigation.

A few workers have reported a condensed stage for the zygonema in the hermaphroditic trematodes. Pennypacker (1940), while describing gametogenesis in Pneumonoeces similiplexus, mentions that both the male and female germ cells "possess contraction figures at the time of synapsis, with the chromatin mass towards one side of the nucleus." She does not illustrate these stages, but the description given fits closely what has been found in S. douthitti. Willey and Godman (1951) also found in Zygocotyle lunata a tendency for condensation during synapsis.

Cases of preleptotene splitting as found in S. douthitti have been reported in a few instances in the literature. To the writer's knowledge, the only reference to this to be found in the literature dealing with the trematodes is that of Cable (1931), who, in describing the leptotene stage of Cryptocotyle lingua, states that the "threads have a double appearance before and after they break up to form loops." Since the anaphase chromosomes, when studied with special techniques, show that they are composed of double chromonemata, and since the anaphase chromosomes of the tertiary spermatogonia as well as the leptotene loops are diploid in number, it only seems reasonable that the leptotene filaments should appear to have a double nature.

Reduction and equation divisions.--Most of the papers dealing with the process of meiosis in the trematodes state that the first meiotic division is reductional, while the second is equational. No morphological differentiation between the maternal and paternal chromosomes has been pointed out in the monoecious digenetic trematodes. According to Carothers (1926), such differentiation is necessary in order to determine which division is reductional, i. e., when the homologous chromosomes separate from each other.

In the dioecious trematodes, where some workers have described differences between the maternal and paternal chromosomes, there has been some basis for claiming that one division is reductional and the other equational. However, as pointed out by De Robertis (1948), in view of the concept of crossing over, no pair of chromatids can be designated as either paternal or maternal by the time the bivalents are formed. Although in S. douthitti the apparent precession of a diad during the first meiotic division toward only one end of the spindle would indicate that that division is reductional, it cannot be stated as such in view of the concept of crossing over.

Chromosome numbers.--Although the diploid chromosome number appears to be 14 for the female and 14 or 15 for the male, the fact that no definite conclusion could be reached might in itself be significant.

It should first be emphasized that observational

conditions are not ideal. The cells are small and the chromosomes tend to clump together. This apparently is not limited to S. douthitti. Severinghaus (1928), while describing mitosis in the spermatogonial cells of S. japonicum, states that:

> The chromosomes condense rapidly into a compact and deeply staining mass, and in this condition go on to the spindle. Such metaphase spindles are not uncommon.... The compact mass of chromosomes lies in the middle of a spindle of small size.

This statement and the observations of the writer appear to contradict Schrader (1953) who states that "the metaphase chromosomes do not come in contact with each other." Comparison of techniques as well as the timing factor might clear up this disagreement.

A possible solution to the problem of counting chromosomes in these small cells, might be by using a technique similar to that described by Hughes (1952), which consists in taking cultured cells and placing them in a hypotonic solution. This causes expansion of the cells and of the metaphase plate when such is present. By rapidly fixing and staining such cells a metaphase plate which is large and where the chromosomes are far apart is obtained.

Variation in the chromosome numbers of a single species has been observed by many workers both in the germ cells and in the somatic cells. One of the most striking examples of this is described by McClung (1917) in the orthopteran Hesperotettix viridis. He found,

while studying the primary spermatocytes of this species, that the chromatids group themselves in various ways so that the apparent number of bivalents varies from 9 to 12, and that they form six different combinations at the metaphase. He found that a particular combination is constant for all the germ cells of an individual. It could not be determined that a similar situation is the case in S. douthitti. However, the facts described above indicate that it might be a possibility, and might explain why sometimes 6, 7, or 8 bivalents were found at the metaphase of the primary spermatocytes and oocytes.

Further evidence that the grouping of chromosomes occurs at times in S. douthitti is seen in figure 10, where the nucleolus is found in the middle of one of the leptotene loops. The position of the nucleolus on the chromonema to which it is attached has been found to be constant in Drosophila, according to White (1948), and as found in this study, the nucleolus is usually found at one end of the leptotene loops. Finding it in the middle of one of the loops would indicate that the end where the nucleolus is usually found united with another loop forming a larger one with the nucleolus in the center.

As described earlier, it was possible to determine the chromosome number in three embryonic cells of the miracidium in S. douthitti, and in each case the number 14 was obtained. Since the embryology of the miracidium

has not been worked out and it cannot be stated whether the above cells represent germ cells or somatic cells, the counts made lose significance in the light of some of the older, as well as recent, cytologocal studies.

Timonen and Therman (1950), while studying human endometrial cells, found the chromosome number varying from 4 to approximately 100 per cell, with most of the cells having around 20 to 30 chromosomes. Hsu and Pomerat (1953) describe the work of Tanaka (1951) (in Japanese), who found that the number of chromosomes in the somatic cells of the rat, Rattus norvegicus, varied from 36 to 49. Variation was greatest in the cells of the bone marrow. Tanaka did not find any variation in chromosome numbers in the cells of the testes. Hsu and Pomerat (1953) studied the somatic cells of the mouse, Mus musculus; the guinea pig, Cavia cobya; and the dog, Canis familiaris; in tissue culture, and found that in addition to haploid and polyploid cells, the chromosome numbers of the somatic cells varied in each species. The greatest variation was found in the dog which ranged from 72 to 82 chromosomes.

The studies mentioned above may explain why at times the writer was unable to find the two v-shaped chromosomes in some of the embryonic cells in the miracidia. Also these studies, if verified, may change our concept of the constancy of the number of chromosomes and may indicate that counts made on somatic cells are not im-

TABLE 2

COMPARISON OF DIPLOID CHROMOSOME NUMBERS OF TERMATODES IN WHICH DIFFERENT NUMBERS HAVE BEEN REPORTED BY DIFFERENT AUTHORS

Species	Diploid chromosome number	Observed by
Fasciola hepatica	8-16	Schubman (1905)
" "	6-8*	Henneguy (1906)
" "	10	Dehorne (1911)
" "	12	Schellenberg (1911)
Gyrodactylus elegans	8	von Janicki (1903)
" "	8	Kathariner (1904)
" "	12	Gille (1914)
Megalodiscus temperatus	16**	Cary (1909)
" "	18	Van der Woude (1954)
Polystoma integerrimum	8	Goldschmidt (1902)
" "	20	Halkin (1902)
" "	20	Minouchi (1936)
Schistosoma japonicum	♂ 15 ♀ 16	Faust and Meleney (1924)
" "	♂ 14 ♀ 16	Severinghaus (1928)
" "	♂ 16 ♀ 16	Ikeda and Makino (1936)
Zoogonus mirus	10	Goldschmidt (1905)
" "	22-26	Schreiner, A., and Schreiner, K. E. (1908)
" "	12	Gregoire (1909)
" "	12	Wasserman (1913)

* Only haploid number reported
** According to Churchill (1950), Cary was dealing with a different species in the snail where the count was made.

portant in describing chromosome numbers.

In several instances where different workers have counted chromosomes in the same trematodes, the number reported has been different (Table 2). It is possible that in some cases the discrepancy arises because at times the chromosomes may group themselves together. Although most of the investigators who counted chromosomes counted them in the germ cells where Hsu and Pomerat (1953) report that Tanaka (1951) found no variation in number, the work of McClung (1917) and others shows that there can be variation in the pattern of association of the chromosomes in the germ cells. Wilson (1925) summarizes a discussion of changes in chromosome numbers by stating:

> All this sustains the conclusion, that the number of chromosomes is of relatively minor importance. What is essential is the material of which they are composed. Their number represents no more than a particular configuration assumed by these materials in the process of mitosis and meiosis; it is in the phrase of Fick (though in a very different sense from his) a tactical formation of the nuclear constituents, and one that may change from species to species or even within certain limits from individual to individual, without necessarily producing any other visible disturbance of heredity or development. In view of all this, the surprising and significant fact is the fidelity with which within the species the number and relative size of the chromosomes adhere to the type.

SUMMARY

1. In the course of spermatogenesis in S. douthitti the dividing germ cells remain attached to each other, finally resulting in a cluster of 32 spermatids. There are 3 mitotic spermatogonial divisions. During the prophase of the first meiotic division pseudoreduction takes place by parasynapsis while the chromatin is condensed into a compact mass. The metaphase which follows has 7 or 8 bivalents. During the anaphase there appears to be precession of one of the diads on only one side of the spindle. Following spermiogenesis the spermatozoa leave the greater part of the cytoplasm of the spermatids behind. The spermatozoon is long and filiform and possesses a fine cytoplasmic tail.

2. Oogenesis begins in the ovary where it proceeds through to the pachytene stage. Further stages in oogenesis take place while the eggs are in the hepatic portal veinules of the host. In oogenesis the process of meiosis follows the same general pattern as found in spermatogenesis except that no precession was observed. The number of bivalents appears to be 7. Two polar bodies are given off; the first one does not divide, but occasionally can be seen as a bilobed structure. When the pronuclei are first formed that of the male is smaller. Later the two are equal in size, and their fusion takes place during the metaphase of the first cleavage division

which is unequal, one of the cells being a little smaller than the other. No centrioles are seen in any of the stages of gametogenesis.

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EXPLANATION OF PLATES

Figures 1-77 are drawn at the same scale. The scale is shown on figures 1, 31, and 61. Figures 78-80 are drawn to the scale shown on figure 78. The scale shown for figure 81 applies to that figure only. The fibrilar structure of the cytoplasm is shown only in figures 3, 7, 26, 49, 66, 76, and 77.

Abbreviations

lc	Laurer's canal
mg	Mehlis' gland
o	oocyte
oo	ootype
ov	ovary
ovd	oviduct
pb	polar body
sr	seminal receptacle
t	testis
u	uterus
va	valve between ootype and uterus
vc	vitelline cell
vr	vitelline reservoir
♂	chromatin from spermatozoon in oocyte

Plate 1

Fig. 1. Group of primary spermatogonia, interphase;

located along membrane of testicular follicle.

Fig. 2. Optical section through a tertiary spermatogonium, early prophase.

Fig. 3. Primary spermatogonium, polar view of metaphase.

Fig. 4. Primary spermatogonium, lateral view of early anaphase.

Fig. 5. Tertiary spermatogonia, early telophase.

Fig. 6. Section through a cluster of tertiary spermatogonia, late telophase, and primary spermatocytes, interphase; a-c represents various stages in the process of reconstruction of the nucleus.

Fig. 7. Primary spermatocyte, interphase.

Figs. 8, 9. Primary spermatocytes, early leptonema.

Fig. 10. Primary spermatocyte, leptonema.

Fig. 11. Primary spermatocyte, late leptonema.

Fig. 12. Primary spermatocyte, zygonema.

Fig. 13. Optical section through primary spermatocyte, zygonema.

Fig. 14. Primary spermatocyte, early pachynema.

Fig. 15. Primary spermatocyte, pachynema.

Fig. 16. Optical section through a primary spermatocyte, pachynema. Section perpendicular to view shown in figure 15.

Fig. 17. Primary spermatocyte, late pachynema.

Fig. 18. Primary spermatocyte, early diplonema.

Fig. 19. Primary spermatocyte, diplonema.

Fig. 20. Primary spermatocyte, late diplonema.

Fig. 21. Primary spermatocyte, diakynesis.

Figs. 22-27. Primary spermatocytes, polar view of metaphase.

Fig. 28. Primary spermatocytes, lateral view of early anaphase.

Fig. 29. Primary spermatocyte, polar view of early anaphase.

Fig. 30. Primary spermatocyte, lateral view of anaphase.

Plate 2

Fig. 31. Section through a cluster of primary spermatocytes during late telophase, and secondary spermatocytes during interphase; a-c represents successive stages in the process of reconstruction of the nucleus.

Fig. 32. Optical section through a secondary spermatocyte, early prophase.

Fig. 33. Secondary spermatocytes, polar view of metaphase.

Fig. 34. Secondary spermatocytes, lateral view of anaphase.

Fig. 35. Section through a cluster of secondary spermatocytes during late telophase and spermatids during interphase; a-e represents successive stages in the process of reconstruction of the nucleus.

Figs. 36-39. Successive stages in the process of spermiogenesis.

Fig. 40. Chromatin portion of a spermatozoon found free in the testes.

Fig. 41. Spermatozoon with tail touching wall of seminal receptacle.

Fig. 42. Cross section of a few groups of spermatozoa in seminal vesicle.

Fig. 43. Cluster of residual masses remaining after spermatozoa have left spermatids.

Fig. 44. Oogonia, interphase, along membrane surrounding ovary.

Fig. 45. Oogonia, lateral view of metaphase.

Fig. 46. Group of primary oocytes along membrane surrounding ovary, showing (a) optical section through early leptonema, (b) probable primary oocyte during interphase, and (c) leptonema.

Fig. 47. Primary oocyte, zygonema.

Fig. 48. Primary oocyte, pachynema.

Fig. 49. Primary oocyte, diffuse stage, found in ovary just prior to entering oviduct.

Fig. 50. Primary oocyte, pachynema, found in posterior part of uterus.

Fig. 51. Primary oocyte, early diplonema.

Fig. 52. Primary oocyte, late diplonema.

Fig. 53. Primary oocyte, diakynesis.

Figs. 54, 55. Primary oocytes, polar view of metaphase.

Fig. 56. Primary oocyte, lateral view of early anaphase.

Fig. 57. Primary oocyte, lateral view of late anaphase.

Fig. 58 a, b. Sections of a secondary oocyte "interphase", and first polar body.

Fig. 59 a-c. Sections of a secondary oocyte metaphase, and first polar body.

Fig. 60 a, b. Sections of a secondary oocyte anaphase, and first polar body.

Plate 3

Fig. 61. Secondary oocyte "telophase" with two polar bodies.

Fig. 62. Zygote showing pronuclei of unequal size. Only one polar body present.

Fig. 63. Zygote showing pronuclei of equal size, and two polar bodies.

Fig. 64 a, b. Sections of Zygote and two polar bodies. The chromatin from the two pronuclei is beginning to condense in preparation for karyogamy and cleavage.

Fig. 65 a, b. Sections of zygote showing lateral view of metaphase of first cleavage division.

Fig. 66. Embryo in two cell stage.

Fig. 67. Tertiary spermatogonium, polar view of metaphase.

Fig. 68. Oogonium, polar view of metaphase.

Figs. 69, 70. Secondary oocytes in parthenogenetic (?)

eggs, polar view of metaphase.

Fig. 71. Early cleavage division, polar view of metaphase.

Figs. 72-76. Cells of undetermined function found in the testes.

Fig. 77. Cell of undetermined function found in the ovary.

Fig. 78. Part of a section of a male showing a testicular follicle opening into another.

Fig. 79. Section through the "molding chamber" of the ootype with an egg in it.

Fig. 80. Section showing detail of valve found between ootype and uterus.

Fig. 81. Outline of female reproductive complex; reconstructed from several sections.

PLATE I

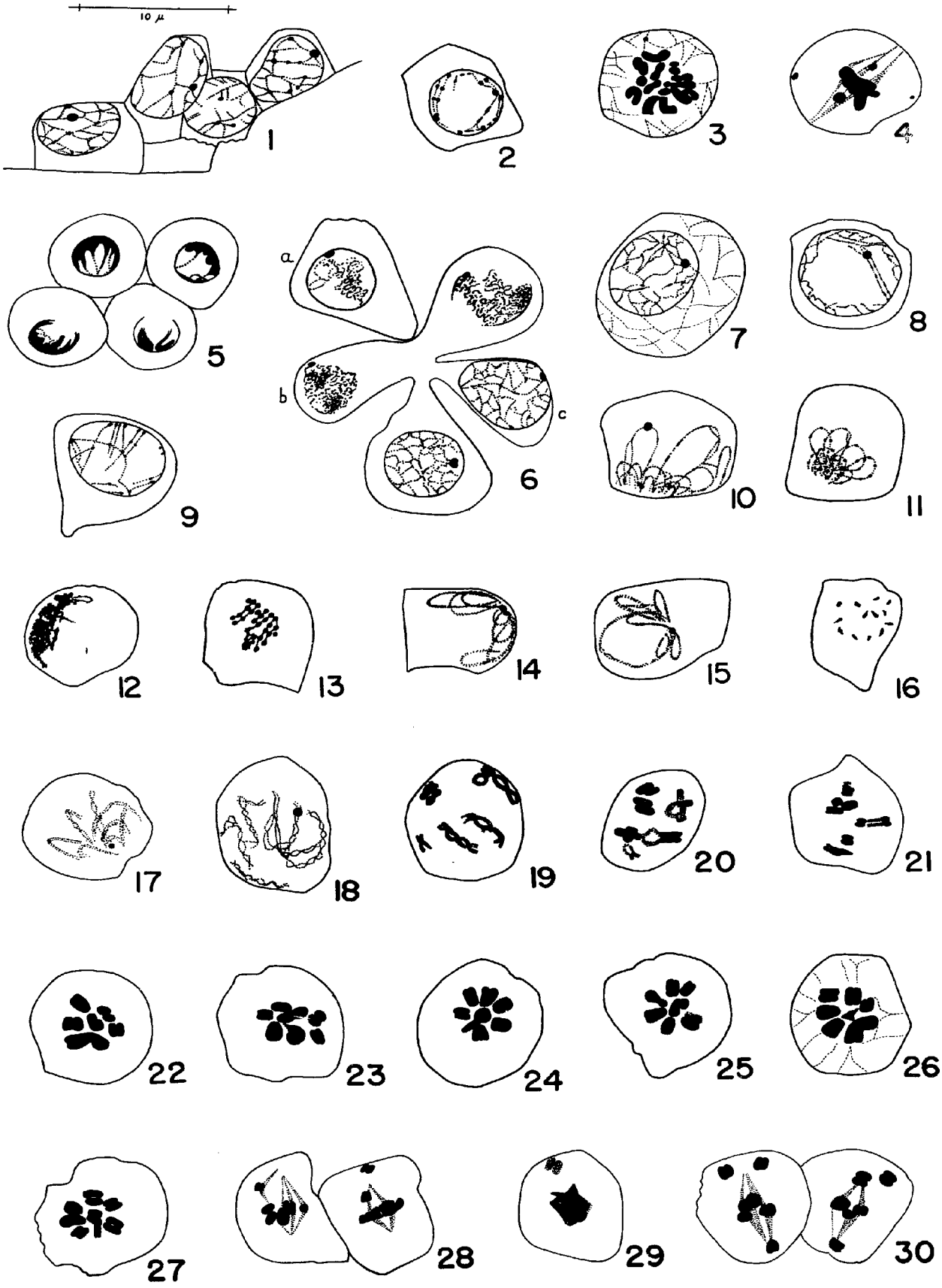

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PLATE 2

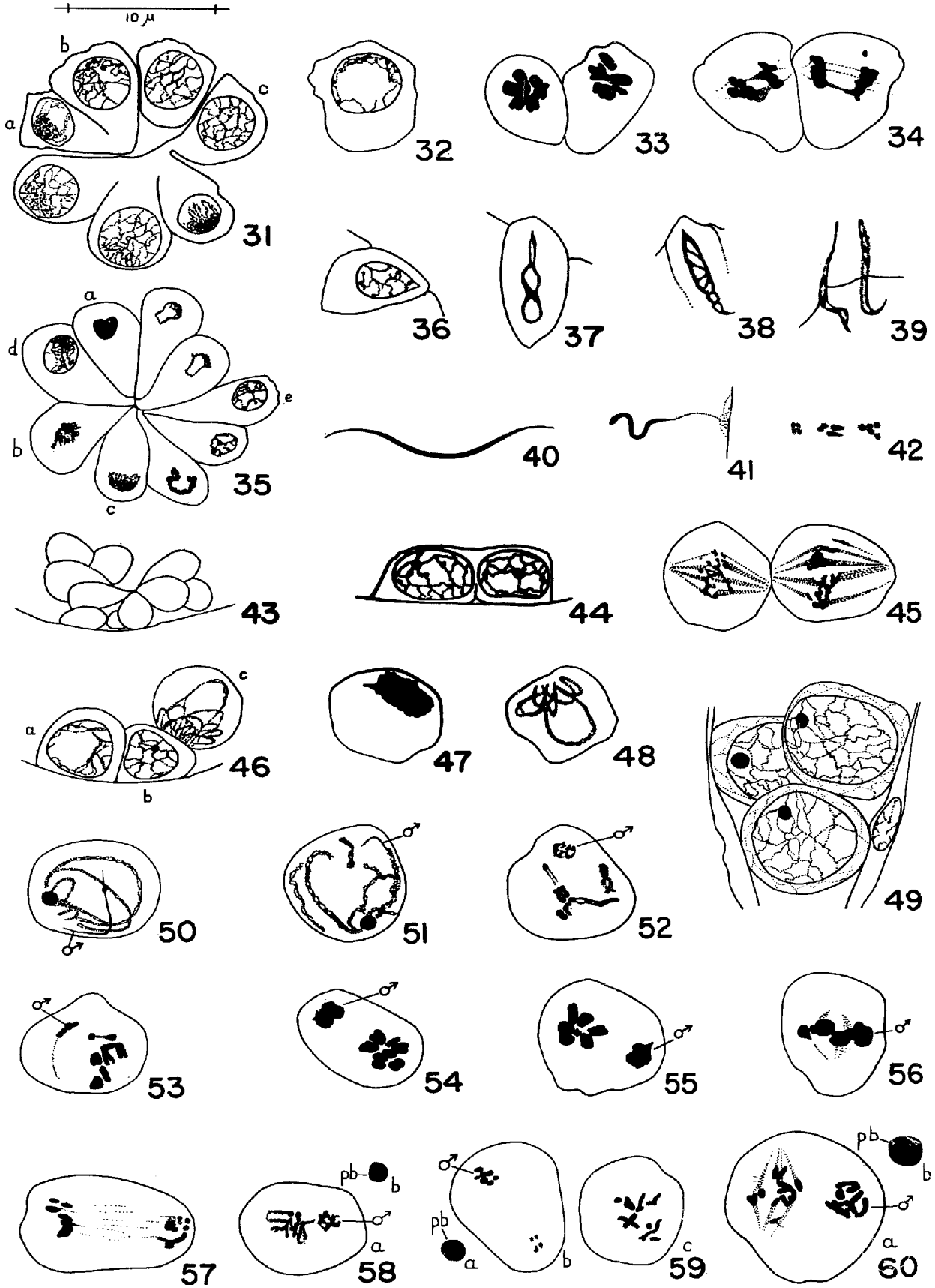
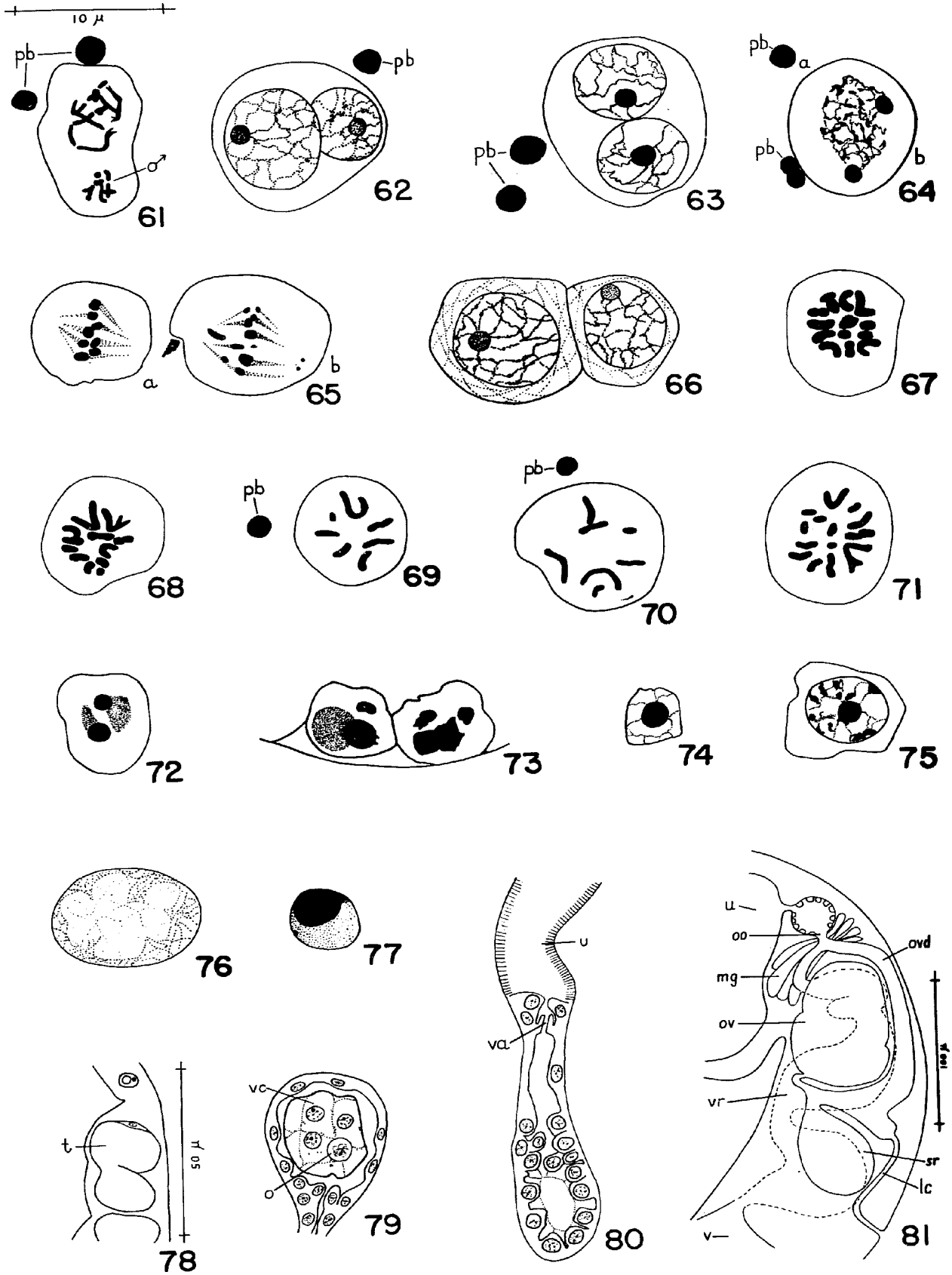

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