

PARASITOLOGICAL REVIEWS

Germinal Development in the Sporocysts and Rediae of the Digenetic Trematodes¹

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In the vertebrate-dwelling adults of the digenetic trematodes spermatogenesis and oogenesis are not fundamentally different from these processes in other metazoan groups. The fertilized ovum in the egg develops directly into the miracidium which contains in its body cavity a varying number of germinal cells. After entering the molluscan intermediate host the miracidium metamorphoses into the primary germinal sac, the mother sporocyst. In its body cavity the germinal cells multiply and develop into secondary germinal sacs which may be either rediae or daughter sporocysts. There may be one, two, or in a few cases even more generations of rediae, but only one generation of daughter sporocysts is present in those groups that have this type of secondary germinal sacs. In each generation there is multiplication of germinal cells in the body cavity of the germinal sacs. However, the number of embryos produced varies greatly in different trematode groups.

Ever since Steenstrup in 1842 presented the theory of alternation of generations for the life cycle of the digenetic trematodes, the method of reproduction in the germinal sacs has been a subject of controversy. Steenstrup's view that this reproduction is an asexual process of internal budding was soon discarded by most workers because of the finding of specific reproductive cells. Grobben in 1882 first proposed the hypothesis that the alternation of generations in the digenetic trematodes is a heterogeny, and that the reproductive cells in the germinal sacs are true parthenogenetic ova. Later, several workers described and figured the for-

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mation of polar bodies by these reproductive cells. Brooks (1930) was unable to find polar bodies and pointed out that there was no uniformity in the descriptions of the maturation processes by the different workers. Also, he showed that the structures designated as polar bodies could be otherwise interpreted. Most recent workers on the germ cell cycle have not reported the finding of polar bodies. Woodhead (1931) described a complicated type of sexual reproduction in the sporocysts of the Bucephalidae, and postulated that the life cycle of the digenetic trematodes is an alternation of three polymorphic adults in each of which the reproduction is bisexual. His observations have not been confirmed, and his views have not been accepted by other workers in the field.

The view that reproduction in the germinal sacs of the digenetic trematodes represents a "germinal lineage" was first proposed by Leuckart in 1879. This hypothesis was revived by Dollfus in 1919 and has been supported by most of the recent studies of the germ cell cycle in this group. According to this view, the reproductive cells in sporocysts and rediae can be traced back directly to the fertilized ovum. These cells of the germinal line, which Cort, Ameel, and Van der Woude (1948) called germinal cells rather than "germ cells", undergo no reduction divisions, remain separate from the somatic cells during the development of the germinal sacs, and are never localized in reproductive glands. Therefore, the multiplication of these germinal cells in the body cavities of the germinal sacs is really a polyembryony of the original zygote (fertilized ovum). In the development of the miracidium, rediae and daughter sporocysts, numerous somatic cells are split off from the cells of the germinal line to form the tissues of the germinal sacs. The germinal cells and the embryos that develop from them become localized in the body cavities of these sacs. According to the germinal lineage hypothesis the only cells in all the stages of the life cycle of the digenetic trematodes that have the reduced or haploid number of chromosomes are the spermatozoa and mature ova which have gone through reduction divisions in the reproductive organs of the adult.²

The germ cell cycle has been followed throughout all the stages of the life cycle for four trematode species in cytological studies of sections of fixed material. These studies were made on *Paragonimus kellicotti* of the family Troglotrematidae by Chen (1937), on an echinostome species,

² For a more detailed discussion of the older literature on the methods of reproduction in the germinal sacs of the digenetic trematodes the reader is referred to articles by Brooks (1930), Cable (1934) and Cort (1944).

Parorchis acanthus by Rees (1939 and 1940), on an amphistome, *Megalodiscus temporatus* by Van der Woude (1954), and on a spirorchiid by Pieper (1953). In these researches and in those on a few other species it has been demonstrated that the germinal cells in the sporocysts and rediae have the diploid number of chromosomes, and in no cases have reduction divisions been found. Other workers have also traced the cells of the germinal line in sectioned material of certain stages of the germinal sacs, viz., Ishii (1934) in the miracidium of *Fasciolopsis buski*; Mattes (1936) in the mother sporocyst of *Dicrocoelium dendriticum*; and Cable (1934) in the daughter rediae of a heterophyid, *Cryptocotyle lingua*. Also, in some of their recent studies on the mechanisms by which the germinal cells multiply in the germinal sacs, Cort and his co-workers have been able to trace the cells of the germinal line in living material supplemented by sections in the early stages of the mother and daughter sporocysts of a plagiurchioid (Cort, Ameel, and Van der Woude, 1952), of strigeoid species (Cort, Ameel, and Van der Woude, 1951; Van der Woude, Cort, and Ameel, 1953) and of spirorchiids and schistosomes (Cort, Ameel, and Van der Woude, 1953 and unpublished studies). The evidence from these studies that the multiplication of the germinal cells in the germinal sacs is a germinal lineage will be considered in this review and in addition the information that has been obtained on the mechanisms of multiplication of the germinal cells in different trematode groups will be summarized.

STRUCTURE OF GERMINAL CELLS AND SOME GENERAL FEATURES OF GERMINAL DEVELOPMENT

The germinal cells (cells of the germinal line) in the germinal sacs of the digenetic trematodes are remarkably uniform in structure in different groups. They have a large nucleus surrounded by a limited amount of cytoplasm. The chromatin is concentrated in one or two large nucleoli and on strands extending from the nucleoli to a granular zone just inside of the nuclear membrane. Figure 1 shows germinal cells in a section through the germinal mass and a cercarial embryo in a young daughter sporocyst of *Lechrorchis primus*. When germinal cells are not tightly packed together in a morula-like mass as shown in Figure 2 strands can usually be seen extending from their cytoplasm connecting them with each other and with the inner layer of the body cavity of the germinal sacs (Fig. 3). Germinal cells that are not closely packed together are frequently elongate and somewhat irregular in shape, as in branched mother sporocysts (Fig. 4). In fact, sometimes when freed in normal

saline they may elongate, assume varied shapes, and actually show ameboid movement. They vary considerably in size since those that are dividing rapidly are smaller. The larger ones may have a diameter of 12 to 14 μ . In the developing embryos of daughter sporocysts and rediae germinal cells can be readily distinguished from somatic cells (Figs. 1 and 3). Figures 5 and 11 show difference in size of germinal cells in germinal masses of a strigeoid. In these sections the germinal cells in the small embryos of the mass are smaller than the single germinal cells. Figure 6 shows the difference in size between germinal cells attached to the wall of the mother sporocyst of *Schistosomatium douthitti* and those in a small daughter sporocyst embryo.

The germinal material in fully developed miracidia is located in a body cavity in the posterior half of the body. Only germinal cells may be present, but in some groups there is already a precocious development into embryos. The number of the germinal elements in miracidia varies greatly in different trematode groups and sometimes in different species in the same group. In some cases only one is present which develops precociously into a redia (Rees, 1940; Van der Woude, 1954). In some of the Plagiorchioidea the miracidium has only four germinal cells (Talbot, 1933; Mattes, 1936). In some groups there may be somewhat larger numbers of germinal elements some of which have already developed into embryos, as in the miracidium of the sheep liver fluke. In the schistosomes, the miracidium may contain numerous germinal cells either in a compact group (Ameel, Van der Woude, and Cort, 1953) or held separately on strands in the body cavity (Price, 1931). After the metamorphosis of the miracidium into the mother sporocyst the germinal cells in most cases continue to multiply and to develop into embryos. There is a great variation in different trematode groups in the extent of multiplication of germinal cells in the mother sporocyst, and in the mechanisms involved in this multiplication.

The early embryos ("germ balls") of daughter sporocysts, rediae and cercariae in the various groups of the digenetic trematodes are strikingly alike (Figs. 1, 5, 6, 7, and 8). They are surrounded by a thin membrane enclosing a number of somatic cells in the midst of which are a few germinal cells. As the embryos of daughter sporocysts grow larger the somatic cells form the wall and the germinal cells become enclosed in the primitive body cavity (Fig. 9). In young redial embryos the intestine soon develops and the germinal cells become localized in a morula-like group in the primitive body cavity just back of its tip (Fig. 10).

The mechanisms by which the germinal cells multiply in the germinal sacs and the number of embryos that are produced varies greatly in different groups of the digenetic trematodes. Very characteristic in certain groups is the presence of persistent centers of multiplication of germinal cells in the germinal sacs either attached to the wall (Figs. 13, 14, 15, 23, 28) or floating freely in the body cavity (Figs. 35, 36, 42). These centers consist of germinal cells and very young embryos attached to each other in a group (Figs. 1, 5 and 11). The breaking off of the largest embryos from these groups which have been designated as germinal masses (Cort, Ameel, and Van der Woude, 1948) produces the embryos in the body cavities of the germinal sacs. In some cases the germinal masses persist throughout the life of the germinal sacs and produce enormous numbers of embryos by a constant division of their germinal cells. The multiplication of the germinal cells is usually more limited in mother sporocysts and redia-producing rediae than in the germinal sacs that produce cercariae since the number of the progeny of these stages that can develop is limited by the space and food available in the organs of the intermediate hosts that are parasitized. The reproductive capacity of these stages is, however, very much greater than the numbers of cercaria-producing germinal sacs that are actually able to develop in the intermediate host. The reproductive potential varies greatly in the germinal sacs of different trematode groups, and a surprising number of different mechanisms have been evolved to increase the number of individuals that are produced. With a few exceptions the type of germinal development in closely related species is alike. There also seems to be a tendency in highly specialized groups to develop a higher reproductive potential with greatly increased production of cercariae.

GERMINAL DEVELOPMENT IN DIFFERENT GROUPS OF THE DIGENETIC TREMATODES

Order Fasciolatoidea (Szidat 1936)

Szidat (1936) pointed out the similarity between the larval stages of the Paramphistomidae and the Notocotylidae and placed them in the suborder Paramphistomata under the order Fasciolatoidea. Later he established the suborder Echinostomata in this order to include the Echinostomatidae, Psilostomidae and Fasciolidae (Szidat, 1939). These revisions based on similarities in the pattern of the life cycle and the structure of the larval stages seem to us to be an important step in the building of a more natural classification for the digenetic trematodes.

The order Fasciolatoidea, and especially the family Paramphistomidae appear to be the most primitive of all the trematode groups. The mother sporocysts which are small simple sacs are much less specialized than the large complex mother sporocysts of certain other groups. The secondary germinal sacs are rediae which usually have well developed digestive systems and locomotor appendages. It seems probable, as suggested by Brooks (1930), that the simpler sac-like rediae and daughter sporocysts of other groups are derived from this type of secondary germinal sac by gradual modifications produced by parasitic life, and the necessity of producing larger numbers of embryos. The cercariae of this order are large with well developed adult characters, and those of the amphistomes and notocotylids have eyespots and extensive pigmentation. It can be suggested that such cercariae are more primitive than the smaller types with highly developed larval characters found in many other groups. No second intermediate hosts are utilized by the amphistomes and the notocotylids, and in the psilostomes and echinostomes the second intermediate host relations, when present, are of a primitive type, since the cercariae have no specialized structures for penetration and the metacercariae with only a few exceptions do not feed or grow. It can be suggested that the acquiring of second intermediate hosts in the evolution of the digenetic trematodes was a secondary adaptation for more effective transfer to the final host, which has led to a variety of specializations in the cercaria and metacercaria stages.

Another feature which suggests that the order Fasciolatoidea is a primitive group is the surprising lack of specificity in the intermediate host relations, and the almost complete lack of specificity in the choice of second intermediate hosts. The family Paramphistomidae may perhaps be considered to be the most primitive and least specialized of all the trematode groups for which we have information on the life cycles. Szidat (1939) pointed out as evidence of the great age of this family that it has representatives in all classes of vertebrates, and suggested that the Notocotylidae were derived directly from it. It is of special interest in this connection that the number of individuals produced in the germinal sacs of the Paramphistomidae is very small compared with most other trematodes and that the mechanism of multiplication of the germinal cells in the germinal sacs is very simple.

Family Paramphistomidae: Van der Woude (1951, 1954) made a very detailed study of the germ cell cycle of the frog amphistome, *Megalodiscus temporatus*, using both living material and sections. She followed the cells of the germinal line throughout all the stages of the life cycle of

this trematode. Her results are entirely in accord with the germinal lineage hypothesis. She showed that the germinal cells in the germinal sacs have the diploid number of chromosomes, which is 18 in this species, and found no evidence of reduction divisions. Very characteristic of the germ cell cycle of *M. temporatus* is the absence of a true persistent germinal mass in the redia. When a distinct body cavity is formed in the redial embryos the germinal cells are in a cluster and those at the anterior end are the first to produce embryos while those at the posterior end continue to divide. By the time that the pharynx and intestine of the rediae are well developed the division of the germinal cells is completed and only embryos of the next generation are present.

The absence of a true germinal mass and a limited multiplication of the germinal cells were also described by Cort, Ameel, and Van der Woude (1948) for the rediae of the turtle amphistome, *Allassostomum parvum*. In this species the mother and daughter rediae have a group of germinal cells attached at the posterior end of the body cavity. Embryos develop from germinal cells detached from the anterior end of this group (Fig. 12). In older rediae these groups of germinal cells appear to be soon used up by the formation of embryos, indicating that as in *M. temporatus* the multiplication of the germinal cells is limited to an early stage in the development.

It may be suggested that this type of germinal development in the rediae is characteristic of the amphistomes. The groups of germinal cells are not persistent centers of multiplication and, therefore, cannot be considered as true germinal masses, and only a comparatively small number of embryos are produced since the multiplication of the germinal cells is limited. Observations on the family Paramphistomidae suggest that in general the number of embryos produced in the germinal sacs is small. In specific instances it may be very small. Bennett (1936) estimated that in *Cotylophoron cotylophorum* only about 225 cercariae were produced from a single fertilized ovum, and Takahashi (1928, cited from Bennett, 1936) gave the number for *Paramphistomum cervi* as only about 180. The mechanism of germinal development just described for representatives of the Paramphistomidae in which there is a limited multiplication of germinal cells and no persistent germinal masses may perhaps be considered as a very primitive type. The extension of the period of multiplication of germinal cells and the development of a germinal mass at the posterior end of the body cavity of the redia is one method of increasing the number of embryos produced.

Suborder Echinostomata: Several investigators have traced the cells of

the germinal line more or less completely throughout the development of the miracidium of representatives of this suborder. The most detailed of these studies is that of Ishii (1934) on the miracidium of *Fasciolopsis buski*. He was able to show that the germinal cells that come to lie on the body cavity of the miracidium are the direct descendents of the fertilized ovum.

Rees (1940) traced the cells of the germinal line throughout the development of the germinal sacs of an echinostome, *Parorchis acanthus*. She found no reduction divisions, and demonstrated that the germinal cells in the rediae have the diploid number of chromosomes which in this species is 22.

Cort, Ameel, and Van der Woude (1948, 1949) studied the mechanism of germinal development in the rediae of one psilostome and five echinostome species. In both first and second generation rediae they found centers of multiplication of germinal cells (germinal masses) (Figs. 13, 14 and 15). These masses are irregular in shape and are tightly attached at the posterior end of the body cavity. They consist of a small number of germinal cells, and a varying number of small embryos. The largest embryos are exactly like the smallest ones free in the body cavity. It is evident that the free embryos are produced by the breaking off of the largest embryos from the germinal mass. These germinal masses are the only centers of multiplication of germinal cells in the rediae, and no germinal cells were ever found in any other location. The presence of germinal cells in the germinal masses of old rediae indicates that they are able to multiply and produce new embryos throughout the whole life of the rediae.

Evidence on the method of development of the germinal masses in the suborder Echinostomata is available from the study of very small redial embryos of an echinostome and a psilostome species (Cort, Ameel, and Van der Woude, 1949). In the smallest embryos in which the digestive system is definitely outlined the germinal material consists of a morula-like group of germinal cells in the primitive body cavity (Fig. 16). These cells are evidently derived from divisions of the single cell of the germinal line that in rediae of this group comes to lie just back of the intestine after the completion of the production of the somatic cells. In larger redial embryos in which the body cavity is considerably extended and free embryos are present the developing germinal mass may still be composed only of germinal cells (Fig. 17). In later stages of redial embryos and young free rediae the germinal masses become more complex and

irregular in shape due to the adherence of a number of embryos and the number of germinal cells in them appears to be reduced (Fig. 14). They appear to have their greatest development in young mature rediae from which daughter rediae or cercariae are just beginning to escape and still persist in most of the very oldest rediae that were examined. In the suborder Echinostomata the development of these persistent centers of multiplication of germinal cells in the first generation rediae provides for sufficient numbers of second generation rediae to fill completely the digestive gland of even the largest intermediate hosts. In daughter rediae this mechanism provides for the production of the large numbers of cercariae which escape daily over the whole mature life of the infection.

It seems probable that multiplication by germinal masses is characteristic of the rediae of the whole suborder Echinostomata. However, Rees (1940) did not find them in the rediae of *Parorchis acanthus*. In this species the number of embryos produced in the rediae is very small and the multiplication of germinal cells is limited to early stages of their development. There would appear, therefore, to be no need for the development of persistent centers of multiplication of germinal cells. Whether this represents a primitive condition for the group or a secondary modification is a matter for speculation.

Our knowledge on the numbers of individuals produced in the germinal sacs of members of the Echinostomata is rather limited. Cort, Ameel, and Van der Woude (1948) made counts of daughter rediae in mature infections of *Echinostoma revolutum*. In seven large specimens of *Helisoma trivolvis* (about 25 mm in shell diameter) there were from 558 to 3960 with an average of 1724; in intermediate hosts less than half this size the counts were from 175 to 540 with an average of 360. Wisniewski (1937) reported this same relation to size of the intermediate host in counts of daughter rediae of *Parafasciolopsis fasciolaemorpha*, a species belonging to the family Fasciolidae. In five infected snails 12 to 16 mm in shell diameter the number of daughter rediae varied from 670 to 1800 with an average of 1058; while in six larger snails, which varied from 22 to 32 mm in shell diameter, the counts were from 1700 to 8000 with an average of 4217. These are illustrations of what appears to be a general relation in most digenetic trematodes, viz., that the number of secondary germinal sacs that develop in infected snails is determined by the space and food available and not by the reproductive potential.

Estimates of the total numbers of cercariae that can be produced from a single fertilized ovum in species of the suborder Echinostomata are at

best only rough guesses. Cort (1944) suggested that in certain echinostome species the number might be well over 25,000. This seems a very conservative figure since in infections containing 500 mature second generation rediae only 100 cercariae would need to be produced during the life of each redia to bring the figure to 50,000. Cort (1922) found for an echinostome species for which counts were made over a period of a few days, that the number of cercariae escaping each day varied from a few hundred to almost a thousand. More complete daily counts over longer periods, which ranged from 12,000 to 100,000 in different infected snails, were made by Wisniewski (1937) for *Parafasciolopsis fasciolae-morpha*. It seems evident, therefore, that the development of germinal masses in the rediae of this group has served to produce much larger numbers of embryos in the germinal sacs than the simpler mechanism that is characteristic of the amphistomes.

Family Heterophyidae

Information on germinal development in the family Heterophyidae is available from the studies of Cable (1934) of sections of young rediae of *Cryptocotyle lingua*, and from those of Ameel, Cort, and Van der Woude (1950) on living material of mother and daughter rediae of *Euryhelmis monorchis*.

Cable traced the germinal cells in very small redial embryos back to small cells which he called "primordial germ-cells" which are located among strands of connective tissue in the region of the future body cavity. At later stages he found typical germinal cells with nuclei 7 to 8 μ in diameter (*loc. cit.* Pl. 32, Figs. 1 to 4). These cells increase in number in older embryos by equal division and come to lie in a distinct group just back of the posterior end of the intestine (*loc. cit.* Pl. 34, Fig. 19). They have the diploid number of chromosomes which in this species is 12. The first embryos develop from the cells at the anterior end of this group, and as more embryos are produced they are packed tightly in the body cavity in a graded series with the largest at the anterior end. Cable concluded that his observations on the germinal development in the rediae of this species supported the germinal lineage hypothesis since there was no evidence of reduction divisions.

In their studies on *Euryhelmis monorchis*, Ameel, Cort, and Van der Woude (1950) found the same type of development in both first and second generation rediae. In the smallest redial embryos they examined, about 60 μ in diameter, a morula-like group of germinal cells was present

in the primitive body cavity. In older redial embryos the body cavity is packed with cercarial embryos in a graded series with the smallest embryos and the remaining germinal cells at its posterior tip (Figs. 18 and 19). In older immature rediae the number of germinal cells is reduced and in most of the mature and old daughter rediae all the germinal cells have developed into embryos. No true germinal masses are present in the rediae of this species.

The method of multiplication of the germinal cells in these two heterophyid species, which is probably characteristic of this family, is like that of the amphistomes in which there are no germinal masses. The heterophyids also resemble the amphistomes in that the cercariae are very large, complete their development in the tissues of the snail host, and in most species have eyespots. For these reasons it was suggested that the members of the family Heterophyidae are rather primitive and may have been an offshoot from the amphistome group (Ameel, Cort, and Van der Woude, 1950). They are, however, much more specialized than the amphistomes since they have evolved a second intermediate host relationship in which the cercariae are modified for penetration into fish and amphibians and the metacercariae undergo considerable development.

*Other Groups in Which the Secondary Germinal sacs are
rediae*

We will discuss next the germinal development in representatives of three families in which the secondary germinal sacs are rediae in which germinal masses resembling those of the Echinostomata are present, viz., *Paragonimus kellicotti* of the family Troglotrematidae (Ameel, Cort, and Van der Woude, 1951), *Halipegus eccentricus* of the family Hemiuridae (Ameel, Cort, and Van der Woude, 1949), and *Triganodistomum mutabile* of the family Lissorchiidae (Cort, Ameel, and Van der Woude, 1950a). None of these families appears to have any close relationship to the members of the order Fasciolatoidea.

Paragonimus kellicotti: Chen (1937) followed the cells of the germinal line in sectioned material throughout all the stages of development of *Paragonimus kellicotti*. She could find no evidence of reduction divisions and determined that these cells have the diploid number of chromosomes which in this species is 16. Her studies gave very significant support to the germinal lineage hypothesis.

Ameel, Cort, and Van der Woude (1951) also traced the cells of the

germinal line in the development of the germinal sacs of *P. kellicotti* in living material supplemented by sections of critical stages. Their studies confirmed the observations of Chen. In the miracidium of this species there are from 4 to 9 germinal cells in the body cavity. In the early development of the mother sporocyst this number increases slightly before any of the germinal cells develop into embryos. As the mother sporocyst grows in size its body cavity becomes filled with developing embryos and the few germinal cells that remain are attached at its posterior end along with a few small embryos (Fig. 8). Soon after the first mother redia has escaped all the germinal cells have developed into redial embryos. These authors followed, in the living material, the germinal cells in the earliest stages of development of the first generation rediae of *P. kellicotti*. The "germ ball" stage, like that of other species, consists of a number of somatic cells surrounding a few germinal cells enclosed by a thin membrane (Fig. 20). By the time the outline of the digestive system can be first made out, a group of a few germinal cells is localized in the primitive body cavity (Figs. 2 and 21). At slightly later stages when the first of them has begun to form embryos they have increased to about 12 to 16 (Fig. 22). In later stages the body cavity becomes filled with embryos and only a few germinal cells are left attached at its posterior end. No germinal cells remain by the time the second generation rediae have begun to escape. In both the mother sporocyst and first generation redia the period of multiplication of germinal cells is limited and no germinal masses are produced. It was estimated that on the average about 25 embryos develop in a mother sporocyst and about 30 in each of the first generation rediae. While a small number of second generation rediae is sufficient to fill the digestive gland of the small snail that serves as the intermediate host of *P. kellicotti*, it is evident that much larger numbers of embryos must be produced by the second generation rediae, since numerous cercariae escape each day during the life of the infection. This much greater production of embryos is made possible in the daughter rediae by the development of a well-defined germinal mass (Fig. 23), which is still present and producing embryos in old rediae. This is the first case in which it has been clearly demonstrated that the mechanism of germinal development differs in the first and second generation rediae of the same species.

Halipegus eccentricus: The germinal development in the germinal sacs of *Halipegus eccentricus*, which belongs to a very highly specialized family, the Hemiuridae, was worked out by Ameel, Cort, and Van der Woude

(1949). In this species the most characteristic feature is the presence of a large germinal mass in the mother sporocyst. Also characteristic is a considerable multiplication of germinal cells and a rapid development of embryos in both mother sporocysts and rediae before germinal masses are formed.

The miracidium of *H. eccentricus* has a group of about 12 or more germinal cells in its body cavity. In early stages of the mother sporocyst up to about 5 days of age there is little if any change in the germinal material (*loc. cit.* Figs. 1 and 2). In somewhat larger mother sporocysts the much enlarged body cavity is almost filled with germinal material consisting of both germinal cells and small embryos which are attached in groups to the body wall (Fig. 24). In older stages free embryos are present, the groups of germinal material attached along the sides of the body cavity are reduced in size and later disappear, and a germinal mass attached at the posterior end is clearly defined. The large germinal masses persist in both mature and old almost empty mothers and in some of the oldest mothers they are extraordinarily large and complex (*loc. cit.* Fig. 9).

The germinal development in the rediae of *H. eccentricus* is like that in the mother sporocysts. In the smallest redial embryos there is a morula-like group of germinal cells in the primitive body cavity (Fig. 26). These germinal cells multiply very rapidly and in larger redial embryos are located in several groups along the wall of the body cavity (Fig. 27). In the largest rediae still inside the mother sporocyst and in those that have just escaped, numbers of the germinal cells have developed into embryos which are still in the groups of germinal material attached along the body cavity. In rediae, after escape from the mother sporocyst, embryos are free in the body cavity and in later stages a germinal mass at the posterior end of the redia becomes clearly defined; all the rest of the germinal material consists of numerous free embryos all at about the same stage of development (Fig. 28).

The germinal masses of the mother sporocysts and rediae of *H. eccentricus* are much larger and more complex than any described for the Echinostomata. The early rapid division of the germinal cells before the germinal mass is formed produces large numbers of embryos in the young mature germinal sacs that are in about the same stage of development. It appears that in the mother sporocyst stage these early embryos produced before the germinal mass is formed would be enough to fill the digestive gland of the snail host; in three naturally infected snails the

redial counts were only 71, 107 and 147. Possibly, therefore, the development of such a large germinal mass in the mother sporocyst of this species is unnecessary to produce the needed embryos, and the presence of such germinal masses in almost empty mother sporocysts suggests that they possibly have lost their reproductive function.

In the young mature rediae the early multiplication of germinal cells produces large numbers of cercarial embryos of about the same size. This would provide for the early escape of large numbers of cercariae, and the large germinal masses in the rediae would provide for later production of cercariae. In the few natural infections of *H. eccentricus* that were studied very large numbers of cercarial embryos were present in the rediae, a conservative estimate being from 30,000 to 40,000. The number of cercariae produced during the reproductive life of such an infection would be many times this number. The type of germinal development in *H. eccentricus* is of particular interest since the mother sporocyst is large and has a large germinal mass, and both stages show an early rapid multiplication of germinal cells before the germinal mass develops.

Triganodistomum mutabile: Cort, Ameel, and Van der Woude (1950a) studied the germinal development in the rediae of *Triganodistomum mutabile*, a representative of the family Lissorchiidae. Wallace (1941) suggested the relationship of this family to the Plagiorchioidea. Germinal masses are present in both immature and mature mother and daughter rediae of this species. They are very large and complex and are somewhat flattened. They are very discrete structures, surrounded by a definite membrane, and are strongly attached at the posterior end of the body cavity (Cort, Ameel and Van der Woude, 1950a, Figs. 1 to 6). In mature rediae the diameter of the germinal mass is much greater in proportion to the length of the redia than is the case with other rediae in which germinal masses have been observed (Fig. 29). These large germinal masses seem well adapted for the production of large numbers of embryos. The daughter rediae of this species are small and contain only a few cercarial embryos which grow to large size compared with the size of the rediae. They are produced in large numbers since redial counts from 18 naturally infected adult snails ranged from 273 to 3119 with an average of 1315. Although no counts have been made on mature infections, observations give the impression that very large numbers of cercariae are produced in infections of this species. It is of special interest that the germinal masses of *T. mutabile* are very discrete structures which

are similar to those in the daughter sporocysts of the Plagiorchioidea (cf. Fig. 29 and Fig. 35). This perhaps adds to the evidence that the Lissorchiidae are related to this group. It also indicates how the floating germinal masses in the daughter sporocysts of the Plagiorchioidea could have been derived from germinal masses attached at the posterior end of rediae.

Superfamily Plagiorchioidea

The superfamily Plagiorchioidea as defined by McMullen (1937) includes all species with true xiphidiocercariae. The family Dicrocoeliidae, in which the cercariae have been modified by adaptation to land snails as intermediate hosts, has also been included in this group. Information on the germinal development in the mother and daughter sporocysts has been published for a number of species belonging to four different families of this superfamily, viz., the Dicrocoeliidae (Mattes, 1936; Neuhaus, 1936; Denton, 1944, 1945; Maldonado, 1945; and Tang, 1950); the Plagiorchiidae (Cort and Olivier, 1943a; and Cort and Ameel, 1944); the Macroderoididae (Cort and Ameel, 1944); and the Reniferidae (Cort, Ameel, and Van der Woude, 1952). Unpublished observations by the authors of this review have also been made on several other species belonging to at least one other family. These studies show a remarkable type of mother sporocyst and germinal development entirely different from any known for other groups of the digenetic trematodes, which now can be considered as typical for the whole superfamily Plagiorchioidea.

The germ cell cycle in this superfamily is adapted for the production of enormous numbers of cercariae. The eggs are ingested by the intermediate host and the miracidia penetrate the wall of the digestive tract and metamorphose into small sac-like mother sporocysts. These develop into large complicated structures in which large numbers of germinal cells are produced by direct division without the formation of germinal masses. The cells of the wall of the mother sporocysts multiply enormously and by a variety of mechanisms come to surround the developing daughters with a permanent outer layer known as the paletot. In the early stages of the embryonic development of the daughter sporocysts there is a very limited multiplication of germinal cells and the formation of a germinal mass. This germinal mass which floats freely in the body cavity of the daughter sporocyst persists as its only center of multiplication of germinal cells and gives off a constant stream of cercarial embryos throughout its life.

Information is rather limited on the early stages of development of the mother sporocysts of the Plagiorchioidea. Mattes (1936) found that the miracidium of *Dicrocoelium dendriticum* penetrated into the digestive gland of the snail host and attached itself to the outside of one of the lobes to form a small sac containing four germinal cells. Maldonado (1945) also described the earliest stage of the mother sporocyst of *Platynosomum fastosum* as a small sac containing four germinal cells. The same type of the earliest stage of the mother sporocyst was described by Cort, Ameel, and Van der Woude (1952) for *Lechriorchis primus* (Fig. 30). Mattes (1936) stated that after a period of rest the mother sporocyst of *D. dendriticum* grows rapidly into an irregular mass filling the space between the lobes of the digestive gland. During this growth there is a very rapid multiplication of the germinal cells. In the development of the mother sporocyst of *L. primus* the small sac soon increases greatly in size with a rapid multiplication of the germinal cells and branches grow out into the digestive gland each crowded with multiplying germinal cells (Fig. 31 and Fig. 4). A very early stage of a mother sporocyst which was considered to belong to *Plagiorchis proximus* (Cort and Ameel, 1944) shows the beginning of the dichotomous branching that is characteristic of this species (Fig. 32). The earliest stage of the mother sporocyst of *Macroderoides typicus* described by Cort and Ameel (1944) in natural infections is composed of three small masses of string-like tubules radiating from a common center, each containing a series of small daughter-sporocyst embryos.

The mother sporocyst of *Plagiorchis muris*, which was described by Cort and Olivier (1943a) and Cort and Ameel (1944), has a very peculiar structure. In this species the mother sporocyst becomes divided by ingrowths of the wall into compartments, each containing germinal cells (Fig. 33). As the germinal cells increase in number and develop into embryos, further ingrowths of the wall divide the mother sporocyst into smaller and smaller compartments until each embryo is surrounded by a part of the wall which forms its outer layer or paletot. As the daughter sporocysts grow the mother which is attached to the anterior part of the intestine becomes a mass composed of large numbers of elongate daughter sporocysts, each surrounded and held together by cells of the wall of the mother (Cort and Olivier, 1943a, Fig. 2). Then when these daughters break away and migrate to the digestive gland of the snail the mother completely disappears since practically all the cells of its wall have been used in the formation of the paletots of the daughter sporocysts.

One of the most striking characteristics of the mother sporocyst of the

P. muris type is that all the daughters are at about the same stage of development. This appears to be brought about by all the germinal cells starting to develop into embryos at about the same time after the completion of a definite phase of multiplication. This is the only type of plagiorchoid mother sporocyst of those so far described that has a synchronous development of daughter sporocysts. In the others, germinal cells will still remain and be multiplying in some parts of the mother when in other locations daughter-sporocyst embryos are well along in development. The number of daughter sporocysts developed in the mother sporocysts of the Plagiorchioidea may be very large. It was estimated that in *P. muris* about 300 to 500 daughters are produced. In infections with *Alloglossidium corti* the number is much larger; in one case over 3000 were actually counted. In *Macroderoides typicus* the number appears to be even larger; in one infection in a large snail approximately 4000 were counted. It is evident, therefore, that in the mother sporocysts of the Plagiorchioidea there is a very considerable multiplication of germinal cells without the development of any germinal masses.

The early stages of development of the daughter sporocyst embryos are similar in all the species of the Plagiorchioidea that have been studied, although it has only been followed in detail in *Lechriorchis primus* (Cort, Ameel, and Van der Woude, 1952, Figs. 12-16). In the development of daughter-sporocyst embryos of this species, the early divisions of the germinal cell produce the soma cells that will form the sporocyst wall (Fig. 34). At a very early stage the wall and the body cavity become clearly defined. When about six germinal cells are present some of them begin to develop into cercarial embryos. The number of germinal elements produced by these early divisions is in most cases from 4 to 8, although rarely it reaches 9 or 10. One of these germinal cells develops into a germinal mass and the others into cercarial embryos. All further embryos come from the germinal mass which persists and gives off a constant stream of embryos throughout the life of the daughter sporocyst.

The favored site of development of the daughter sporocysts of the Plagiorchioidea is the digestive gland of the molluscan intermediate host. In those forms in which the mother sporocysts develop outside the digestive gland, as in *P. muris*, the daughters migrate actively from the mother at an early stage of development. Figure 35 shows an elongate daughter sporocyst of *P. muris* at the stage just before migration from the mother. In species in which the mother sporocyst penetrates into the digestive gland by branching, as those of *Plagiorchis proximus* and

Lechriorchis primus and in those imbedded in the gland as in the case of *Alloglossidium corti*, daughter sporocysts may develop to maturity without leaving the mother, but even in these forms some migrating stages are produced that actually leave the mother. Although the descriptions of the development of the mother sporocysts of the Dicrocoeliidae are incomplete, and sometimes rather confused, they indicate that in some cases daughter sporocysts migrate from the mother and that in others they complete their development without leaving the mother. In *Macroderoides typicus* the mother sporocyst develops attached to the bulb of the esophagus next to the stomach of the snail host. It is evident that the daughter sporocysts develop within the tubular branches of the mother and never migrate. In fact they form a mass that is in contact with the digestive gland of the snail without penetrating it. The arrangement in strings of the daughter sporocysts of this species can even be made out in mature infections (Fig. 36).

Also characteristic of the plagiorchoids is the method of growth of the daughter sporocysts in the digestive gland of the intermediate host during which they become very irregular in shape. This was first described by Cort and Olivier (1943a, Figs. 18-26) for *Plagiorchis muris* but was found later in other species.

The mechanism of germinal development in the superfamily Plagiorchioidea is one of the most effective that has evolved in any of the trematode groups. The large size and complexity of the mother sporocysts make possible the production of large numbers of daughters by direct division of the germinal cells. Also, the development of a single persistent center of multiplication in each daughter in the form of a floating germinal mass, which in some cases may be quite large and complicated, provides for the production of very large numbers of cercariae which escape each day during the mature life of the infection. It can be suggested that this type of germinal development evolved from that of the Echinostomata by an increase in the multiplication of the germinal cells of the mother sporocyst, made possible by its increased size and complexity, and by a change from the rather simple germinal mass found in each redia to the complicated floating germinal mass of the plagiorchoid daughter sporocyst.

Order Strigeatoidea

The establishing of the order Strigeatoidea by La Rue (1926) was the first step in the building of a natural classification of the digenetic trematodes on the basis of similarity in the structure of the larval stages. He

included in this order the strigeoids and schistosomes since their cercariae have forked tails and homologous excretory systems, and their miracidia have four flame cells. La Rue also made the tentative suggestion that the Bucephalidae should be placed in this order, although the number of flame cells in their miracidia could not be made out. When the life cycle of *Clinostomum marginatum* was worked out by Krull (1934) and Hunter and Hunter (1934, 1935) it was found that its cercaria has a forked tail and that there are four flame cells in its miracidium. It seemed evident, therefore, that this species should also be placed in the order Strigeatoidea. This was done by Faust (1939, p. 90) who considered that the suborder Strigeata La Rue, (1926) should contain the superfamilies Strigeoidea Railliet, 1919, Clinostomatoidea Dollfus, 1931, and Schistosomatoidea Stiles and Hassall, (1926). Allison (1943) went a step further and considered that *C. marginatum* should be placed in the suborder Clinostomata in the order Strigeatoidea along with the suborders Bucephalata, Strigeata, and Schistosomata. Under the suborder Bucephalata he included the Bucephalidae and the Brachylaemidae. This last family was included because he found that a species belonging to it, *Leucochloridiomorpha constantiae*, had a free-swimming, forked-tailed cercaria similar to those of the strigeoids. He, therefore, considered that the species of this group that developed in land snails and had lost their tails were descendents of forms with forked tails. In the order Strigeatoidea studies on germinal development in the germinal sacs have been made on the clinostomes, strigeoids, spirorchiids, schistosomes and bucephalids.

Clinostomum marginatum: Studies have been made on the germinal development in the rediae of this species by Cort, Ameel, and Van der Woude (1950b) using experimental infections in laboratory raised intermediate hosts. The mother sporocyst of *C. marginatum* produces only one redia which is followed by at least two more generations of rediae. The same type of germinal development is present in all three redial generations of this species. In very small redial embryos no germinal material is present except groups of two to five germinal cells in the body cavity back of the intestine (Cort, Ameel, and Van der Woude, 1950b, Figs. 3, 4, 7, 8 and 9). The cells in these groups adhere very closely together, and only occasionally are single germinal cells seen. In older rediae, these groups of germinal cells are mixed with developing embryos (Figs. 37 and 38) which are often in groups of two, three and even more. The germinal cells in the groups must be going through very rapid division since large numbers of embryos are produced.

The interpretation of the germinal development in *C. marginatum* is

that the germinal cell groups are dividing and continuously breaking up into new groups, and that some of the germinal cells in them are constantly developing into embryos, which either quickly separate from each other or adhere for a time in groups. Some of the germinal cells must retain the ability to continue dividing throughout most of the life of the rediae, because groups of germinal cells are still present in mature rediae which contain large numbers of well-developed rediae or cercariae and embryos in all stages of development (Fig. 37).

The mechanism of germinal development in *C. marginatum* was shown by Cort, Ameel, and Van der Woude (1950b) to be very effective in producing large numbers of embryos. Eleven naturally infected specimens of *Helisoma campanulatum* which had shell diameters of about one-half inch harbored from 1075 to 3131 rediae with an average of 2025. Redial counts for two naturally infected adults of *H. trivolvis*, which had shell diameters of about one inch, were 4002 and 4072. Large mature cercaria-producing rediae are about 1 mm in length and some contain as many as 300 cercariae and developing cercarial embryos. It is evident, therefore, that extraordinarily large numbers of cercariae are produced in infections of this species. One large infected snail might contain more than half a million cercarial embryos at one time (2000 rediae each containing 250 cercarial embryos), and during the course of such an infection many millions of cercariae would be produced.

Suborder Strigeata: Cort and Olivier (1941) studied the germinal development in the mother and daughter sporocysts of the strigeoids. In both these stages they found free germinal masses which serve as centers of multiplication of germinal cells (Figs. 5, 11, and 39). These germinal masses are discrete structures surrounded by a thin membrane. They are composed of a small number of germinal cells to which are attached small embryos, the largest of which are at the ends. Free embryos are formed by the splitting off from the germinal masses of the largest embryos attached at their ends. These end components of the germinal masses are exactly like the smallest embryos of daughter sporocysts and cercariae that are free in the body cavity of the mother and daughter sporocysts. It is apparent that the germinal cells in the germinal masses must continue to divide and develop into new embryos to take the place of those that are split off. The best evidence that such division does occur throughout the life of mother and daughter sporocysts comes from the fact that such large numbers of embryos are produced in them, and that germinal masses of old sporocysts still contain germinal cells. Since no

single germinal cells have ever been found in sporocysts after the germinal masses have been formed, it is evident that all the embryos that are produced come from the splitting off of their end components.

Cort, Ameel, and Van der Woude. (1951) studied the development of the germinal masses in the mother sporocyst of *Diplostomum flexicaudum*. The miracidium of this species has a group of about 8 germinal cells in its body cavity. After it has penetrated into the snail intermediate host and metamorphosed into a mother sporocyst there is some increase in the number of germinal cells. They then develop directly into germinal masses which early in their development appear as groups of 2 to 4 cells (Fig. 41). Soon, however, these cell groups change into typical germinal masses. Thus at an early stage in the development of strigeoid mother sporocysts, before any free embryos have split off, the body cavity contains only germinal masses (*loc. cit.* Figs. 4 and 5).

Van der Woude, Cort, and Ameel (1953) studied the development of the germinal masses in the daughter sporocysts of the strigeoids. The largest embryos still attached to a mother sporocyst germinal mass contain 6 to 8 germinal cells. After they break away their germinal cells continue to divide until 12 to 24 or even more are present in the body cavity. As the daughter-sporocyst embryos begin to elongate the body cavity becomes more clearly defined and the germinal cells develop into germinal elements which are groups of 2 to 5 cells (Fig. 41). These groups of germinal cells and those in young mother sporocysts resemble the germinal cell groups in the rediae of *Clinostomum marginatum* (cf. Figs. 37 and 38 with Figs. 40 and 41). In slightly older daughter sporocyst embryos each of these germinal elements becomes a typical germinal mass (Fig. 42). Thus in daughter sporocysts that are about ready to escape from the mother the body cavity contains only germinal masses. In such daughters there is a considerable variation in the size and number of components in the germinal masses. The largest masses may be fully developed while the smallest are much smaller and have only 4 or 5 small components (Fig. 42). This lag in the development of a part of the germinal masses is still found in older daughter sporocysts containing large numbers of cercarial embryos. However, in mature sporocysts, from which numerous cercariae are escaping, all the germinal masses are fully developed and appear to be producing embryos. In old infections there is usually a reduced number of germinal masses in the daughter sporocysts, and a reduction in the number of germinal cells in them.

The mechanism of germinal development in the germinal sacs of the

strigeoids is adapted for the production of very large numbers of cercariae over a long period of time. Cercariae are escaping in numbers from the oldest daughter sporocysts before the germinal masses of the mother sporocyst have completed the production of new daughter-sporocyst embryos. Also, the lack of synchronism in the development of the germinal masses of the daughter sporocysts increases considerably the length of the period during which they can continue to produce cercarial embryos.

Family Spirorchiiidae: The blood flukes of turtles of the family Spirorchiiidae are very much like the true schistosomes in the structure of their larval stages and in the pattern of their life cycles. Pieper (1953) has recently investigated the germ cell cycle of a representative of this family, *Spirorchis artericola*. Her studies show that the theory of germinal lineage with multiplication by simple polyembryony applies to this form. In the multiplication of the germinal cells in the germinal sacs she found no suggestion of maturation phenomena or polar body formation and no indication of the formation of germinal cells from the body wall. The germinal cells divide repeatedly and develop directly into embryos of daughter sporocysts and cercariae without the formation of germinal masses and in the cercarial embryos they multiply to form the genital primordium. The exact number of chromosomes in the germinal cells was not determined. However, the author stated that the comparison of the metaphase plate of dividing germinal cells in young sporocysts with dividing somatic cells in developing cercariae showed similarity in the number and position of the chromosomes.

Studies on the germinal development of representatives of this group which have not yet been published have been made by the authors of this review in experimental infections. Most of the material studied belonged to *Cercaria elephantis*, Cort, 1917 which is the commonest spirorchiid cercaria of the Douglas Lake region. The adult belonging to this species of cercaria is still uncertain since studies now in progress indicate that Wall (1941) was mistaken in the adult that he assigned to it. A few infections with another species which has not yet been determined were also included in the examinations.

The miracidium of *C. elephantis* has a group of about 12 germinal cells in its body cavity. Mother sporocysts about 0.1 mm in length have a thick wall, and still show some remnants of the glandular structures at the anterior end and the eyespots (Fig. 43). There has been a considerable multiplication of germinal cells and a few of them have already developed into two to four cell embryos. The mother sporocysts grow

rapidly in size, the germinal cells continue to multiply, and large numbers of embryos are soon produced. Mother sporocysts about 1 mm in length are elongate sacs with the body cavity filled with large numbers of small embryos, the largest of which are only 0.03 to 0.04 mm in diameter; in some cases as many as 100 are present at this stage; numbers of germinal cells are attached to the wall. One mother sporocyst about 3 mm in length contained about 300 embryos most of which were under 0.03 mm in diameter; germinal cells attached to the wall were numerous. Soon after this stage the daughter-sporocyst embryos elongate and grow rapidly in size and about two and one-half weeks after infection begin to escape from the mother. Germinal cells and very small embryos are still present attached to the wall in mother sporocysts 24 to 25 days old from which numbers of daughters have already escaped. Figure 44 shows a section through a part of such a mother in which the germinal material is held in a fibrous network, and germinal cells and very small embryos are still present. It was estimated that mother sporocysts grow to about 6 mm in length and may produce as many as 400 daughter-sporocyst embryos. By five to six weeks practically all the daughters have escaped from the mothers which are degenerating.

The early stages of daughter-sporocyst embryos ("germ balls") of the spirorchiids are like those of other trematodes (Fig. 44). Embryos about 0.1 mm in length are distinctly elongate and the body cavity is well defined containing about 12 to 16 germinal cells and a few small embryos. The cells of the inner layer of the body cavity form a plug at the anterior end extending for about one-fourth the body length. A section through a daughter-sporocyst embryo at this stage is shown in Figure 44. Larger daughter sporocyst embryos have the body cavity crowded with small embryos, and germinal cells can be seen attached to the body wall (Fig. 45). The anterior plug of body wall cells is still prominent at this stage and is still present in much larger daughters after their escape from the mother. Germinal cells can still be found along the body wall in daughter sporocysts that contain well developed cercarial embryos. The first cercariae escaped from our experimental infections in about five weeks. In the mature experimental infections the daughter sporocysts attained a length of 2 to 4 mm and were crowded with cercarial embryos that were very large compared with the size of the sporocysts.

The pattern of the germinal development in the Spirorchiidae is of special interest because of their relationship to the schistosomes. It is characterized in both the mother and daughter sporocysts by the con-

tinued division of germinal cells throughout the life of the germinal sacs. No germinal masses are present and the germinal cells are scattered along the inside of the wall of the body cavity. Very early in the development of both the mother and daughter sporocysts embryos begin to be formed which crowd the body cavity. In the early stages of both mother and daughter sporocysts the germinal elements are held in a fibrous network. As the embryos grow larger and become active they break down the network and move freely in the body cavity. While the persistence of germinal cells attached to the walls of the germinal sacs insures the production of embryos over a considerable period of time, cercarial production appears to be not nearly as great in the family Spirorchiidae as in the strigeoids, and the infections seem to be much more quickly exhausted.

Schistosomatidae: The mechanism of germinal development in the mother and daughter sporocysts of the schistosomes is fundamentally similar to that of the spirorchiids. Reports of investigations have been published on *Schistosomatium douthitti* (Cort, Ameel, and Olivier, 1944; Cort, Ameel, and Van der Woude, 1953), on *Trichobilharzia stagnicola* (Cort and Olivier, 1943b) and on *Schistosoma mansoni* (Olivier and Mao, 1949).

In their studies on *S. douthitti* Cort, Ameel, and Olivier (1944) showed that in the early stages of the development of the mother sporocysts the wall grows much more rapidly than the germinal material, producing inflated sacs in which the germinal cells are distributed along the inner surface (loc. cit. Figs. 2 and 3). Multiplication of germinal cells is rapid, the number being estimated as 150 to 200 in mothers about 4 days old. After about a week the germinal cells begin to develop into daughter-sporocyst embryos. Apparently almost all of the multiplication of germinal cells is completed before any of them start to develop into embryos. The daughter-sporocyst embryos remain attached to the wall of the inflated, sausage-shaped mothers until they reach a considerable size and are distinctly elongate. After the daughter sporocysts are freed from the wall they grow rapidly and later escape from the birth pore. At all stages in the development of the mothers, even those in which many daughters are ready to escape, the embryos fill only a part of the body cavity. The development of the embryos in the mother is quite synchronous, so that the daughter sporocysts in an infection are about the same age.

The early development of the daughter sporocysts of *S. douthitti* was

checked by Cort, Ameel, and Van der Woude (1953) in experimental infections and certain errors in the previous study corrected. The "germ ball" stages of this species are like those of other trematodes. In embryos about 0.1 mm long, as in the spirorchiids, the body cavity is well defined, a few embryos are already present, and there is a distinct plug of body-wall cells at the anterior end (Fig. 46). In later stages of the development of the daughter-sporocysts the pattern is the same as in the spirorchiids with a great increase in length and the production of large numbers of embryos.

The authors of this review have made a study of germinal development in the mother and daughter sporocysts of *Trichobilharzia stagnicolae* in experimental infections in small laboratory-raised juveniles of *Stagnicola emarginata angulata* which has not yet been published. Germinal development in the mother and daughter sporocysts resembles very closely that in the spirorchiids. The germinal cells in the miracidium of *T. stagnicolae* are in an elongate compact mass extending from just back of the large central nerve mass almost to the posterior end of the body (Ameel, Van der Woude, and Cort, 1953, Fig. 3). In eight specimens their number varies from 21 to 30 with an average of 22. In very young mother sporocysts about 2 to 3 days old there has been a considerable multiplication of germinal cells but no embryos are yet present. Embryos begin to form soon after this, as shown in Figure 3. As in the spirorchiids the mother sporocysts grow rapidly into elongate sacs that are crowded with small daughter-sporocyst embryos and have numerous germinal cells attached to the inside of the wall. In a mother, 0.26 by 0.06 mm, there was a total of about 60 germinal elements more than half of which were small embryos. In mothers about 1 mm in length the number of embryos has greatly increased, the largest being still in the germ-ball stage. Numerous germinal cells and small embryos are present attached to all parts of the inside of the wall of the body cavity. Growth is very rapid and the largest mother sporocysts in 7 to 8 day old infections reach a length of 2 to 3 mm. One, 3 mm in length and 0.07 to 0.08 mm in width at its widest portions, contained about 20 compact groups of daughter sporocyst embryos which were separated from each other by constrictions. The largest embryos in this mother were somewhat elongate with a size of about 0.07 by 0.05 mm; most of them, however, were still small "germ balls." The wall was still quite thick and numerous germinal cells and small embryos were attached to it. In older mother sporocysts there are active daughters 0.3 to 0.4 mm in length moving freely in the body

cavity. Their activity has broken up the larger masses of germinal elements into smaller irregular groups of various sizes, some of which are composed only of embryos and others of embryos and a few germinal cells. Daughter sporocysts begin to migrate from the mothers into the digestive gland of the snail in less than two weeks after infection.

Small daughter-sporocyst embryos of *T. stagnicolae* are almost exactly like those of *C. elephantis* and *S. douthitti*. One that had a size of 0.086 by 0.045 mm had 16 to 20 germinal elements in its body cavity, three of which were 2 to 4 cell embryos. Further development is also similar to those species. In daughter sporocysts in 15 to 17 day old experimental infections the embryos are tightly packed together and small embryos and a few germinal cells are wedged between the larger cercarial embryos, none of which yet shows division into body and tail. Numerous germinal cells, small embryos, and even small groups of germinal material are attached to all parts of the inside of the body wall. In most of the daughter sporocysts in 19 to 20 day old infections the embryos are still tightly packed in the body cavity. Some of them are of about uniform thickness and are filled for their whole length with closely packed cercarial embryos of different sizes. In others, the germinal material is in a series of compact groups that are separated from each other by constrictions. Very small embryos and a few germinal cells are wedged between the larger embryos. In sporocysts at this stage and even in older ones containing numbers of fully developed active cercariae, germinal cells, small embryos and small groups of germinal elements are attached to the wall at different levels. Where active cercariae are present they are free in the body cavity and free groups of germinal material of varying sizes are also present. Cercariae were ready to escape from experimental infections of *T. stagnicolae* in about 21 days.

Cort and Olivier (1943b) described the mother sporocysts of *T. stagnicolae* obtained from fully grown adult snails as large inflated sacs with very thin walls in which the germinal material filled only a small part of the body cavity and was either suspended on a network of fibers or floated free in the body cavity. Groups of germinal elements, either consisting only of embryos or of varying numbers of embryos and germinal cells (*loc. cit.* Figs. 8 and 8a) were held by fibrous strands in the younger mother, and in the older ones floated freely mixed with elongate daughter sporocysts. They also described the daughter sporocysts in the digestive gland of the snail intermediate host as growing into large thin walled sacs which contained groups of germinal elements like those

in the mothers. They considered these groups of germinal elements in both the mother and daughter sporocysts to be true "germ masses" similar to those of the strigeoids.

In comparing these early studies with the recent work on the spirorchiids and schistosomes it is evident that Cort and Olivier (1943b) were mistaken in their interpretation of the groups of germinal material in the mother and daughter sporocysts as germinal masses. They are obviously not centers of multiplication of germinal cells but merely embryos and germinal cells that have temporarily stuck together in groups. Most of them are obviously fragments of larger groups broken up by the activity of well developed daughter sporocysts and cercariae. Some are probably groups that developed at later stages from germinal cells of the body wall in which the germinal elements have not yet been separated.

The differences that were noted in size of the sporocysts and arrangement of the germinal elements in the two descriptions of *T. stagnicolae* seem to be due to the differences in size of the snail hosts. Cort and Olivier (1943b) were dealing with natural infections that had entered practically full grown snails in the late fall and early spring and had continued their growth in the spring and early summer in sexually mature snails that are many times larger than the laboratory raised juveniles in which the experimental infections used for the later studies had developed. Under the more favorable conditions for development in the large snails with much more space and food available, it appears that the increase in size of the mother and daughter sporocysts is much more rapid compared with the development of the germinal material than in the small snails that were experimentally infected.

The authors of this review have also made studies of the germinal development in the mother and daughter sporocysts of another schistosome, *Trichobilharzia physellae* in experimental infections. Since the data from these studies have not yet been analyzed only a few of the results will be included. The pattern of germinal development in this species is essentially the same as in the spirorchiids and other schistosomes that have been studied. The mother sporocysts grow very rapidly and produce large numbers of germinal cells before many embryos are formed (Fig. 47). In the early development of the daughter sporocysts the anterior plug of germinal cells is less prominent than in *S. douthitti* and *T. stagnicolae* (Fig. 47). Later development shows only minor differences from these species (Fig. 48).

It can be seen that germinal development in the mother and daughter

sporocysts of representatives of the Spirorchiidae and Schistosomatidae is quite different from that of strigeoids. The germinal cells divide rapidly, are scattered along the whole wall of the body cavity, and no true germinal masses are formed. Embryos develop very early in all the germinal sacs, and continue to be formed throughout most of their life by germinal cells attached to all parts of the inner wall. An exception to this is found in the mother sporocyst of *S. douthitti*, in which the germinal cells go through a considerable period of multiplication before any of them form embryos and the daughter-sporocyst embryos develop almost synchronously. Finally the point should be stressed that when development of the germinal sacs occurs in very small snails, as in experimental infections in laboratory raised juveniles, the arrangement of the germinal material may be considerably altered by the crowded conditions under which the germinal sacs develop as compared with development in larger snails.

Bucephalidae. Woodhead (1931) made a study of the germ cell cycle in three species of the Bucephalidae. He reported the finding of maturation phenomena and the formation of germ cells in the germinal sacs of these forms and presented the hypothesis that the life cycle in this group is an alternation of three polymorphic adults in each of which reproduction is bisexual. This author also claimed that he found evidence that supported this hypothesis in studies on members of the Brachylaemidae which have never been published (Woodhead, 1932 and 1950). In fact, in his early paper (Woodhead, 1931) and in a number of reports and demonstrations to scientific societies he has argued that his hypothesis applies to the whole group of digenetic trematodes. The data from the large number of studies presented in this review support the theory of germinal lineage, and show clearly the fallacy of Woodhead's views when applied to the whole trematode group. Also in a recent study of the life cycle of a representative of the Bucephalidae, Kniskern (1952) was unable to confirm Woodhead's earlier reports on the germ cell cycle in this group, and reported the finding of germinal masses. In view of these differences of opinion it is hoped that further studies will soon be made on the germ cell cycle of the Bucephalidae and Brachylaemidae.

CONCLUSIONS

In the opinion of the authors of this review the evidence is now practically conclusive that reproduction in the germinal sacs of the digenetic trematodes is a germinal lineage, in which multiplication is merely a

polyembryony of the fertilized ovum. This view is supported not only by the instances in which it has been demonstrated that the germinal cells have the diploid number of chromosomes, but also by the numerous studies in which they have been traced and their relations determined both in sectioned and living material. Of special interest also are the different mechanisms that have evolved in different groups to increase the number of embryos produced in the germinal sacs. The study of these methods of multiplication of germinal cells has given some hints on the relationship of different groups, and more complete studies should be helpful in building a more natural classification.

Such investigations have certain pitfalls. Much confusion has resulted from investigations in which only sections of the germinal sacs have been used. On the other hand the use of only living material may also foster misinterpretations. While the greatest progress can be made by using experimental infections in laboratory raised juvenile snails, it must be noted that such infections may lead to misinterpretations due to the crowding of the stages as they develop in very small intermediate hosts. The most informative results, therefore, can be obtained by the study of the same species in both experimental and natural infections, and the checking of the observations on living material by numerous sections of critical stages. Much more work is needed before we really will have an adequate understanding of the germinal development in the stages of the digenetic trematodes in the intermediate host, and it is hoped that workers on life cycles in this group will not continue to neglect this phase of development.

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PLATES

PLATE I

FIG. 1. Section through one end of an immature daughter sporocyst of *Lechriorchis primus* (Cort, Ameel and Van der Woude, 1952, Fig. 26).

FIG. 2. Longitudinal section of a mother redial embryo of *Paragonimus kellicotti* (Ameel, Cort and Van der Woude, 1951, Fig. 21).

FIG. 3. Section through the anterior part of a 5- to 6-day-old mother sporocyst of *Trichobilharzia stagnicolae*.

FIG. 4. Section showing branches of a mother sporocyst of *Lechriorchis primus* in the connective tissue outside of the intestinal wall of the snail intermediate host (Cort, Ameel and Van der Woude, 1952, Fig. 8).

FIG. 5. Section of a germinal mass from a mother sporocyst of *Cercaria modicella* (Van der Woude, Cort and Ameel, 1953, Fig. 2).

FIG. 6. Section through a small portion of the body wall of a mother sporocyst of *Schistosomatium douthitti* (Cort, Ameel and Van der Woude, 1953, Fig. 7).

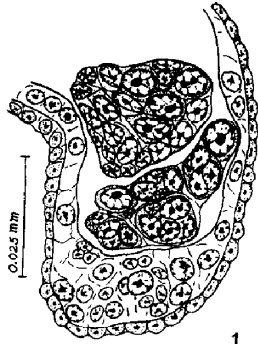
FIG. 7. Section of a very young daughter sporocyst embryo of *Lechriorchis primus* (Cort, Ameel and Van der Woude, 1952, Fig. 15).

FIG. 8. Section through the posterior end of an almost mature mother sporocyst of *Paragonimus kellicotti* (Ameel, Cort and Van der Woude, 1951, Fig. 15).

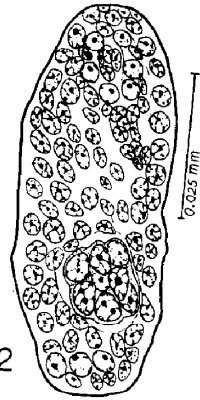
FIG. 9. Section of a very young daughter sporocyst embryo of *Diplostomum flexicaudum* (Van der Woude, Cort and Ameel, 1953, Fig. 7).

FIG. 10. Young mother redial embryo of *Paragonimus kellicotti* (Ameel, Cort and Van der Woude, 1951, Fig. 19).

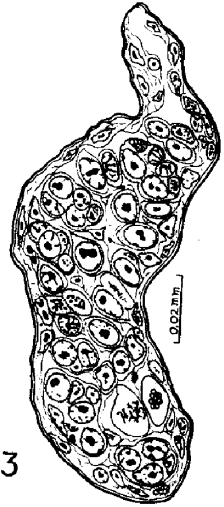
FIG. 11. A section of a germinal mass from a young daughter sporocyst of *Diplostomum flexicaudum* (Van der Woude, Cort and Ameel, 1953, Fig. 3).



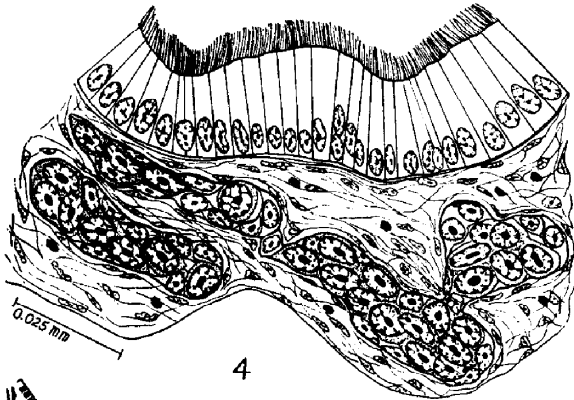
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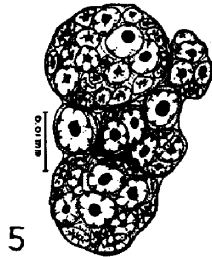
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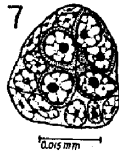
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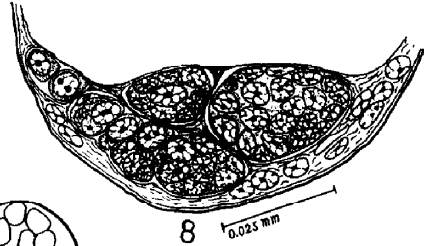
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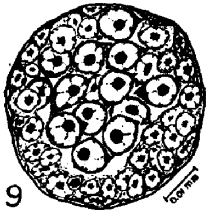
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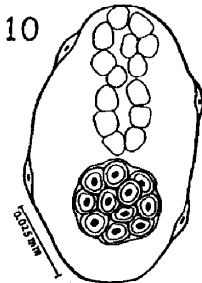
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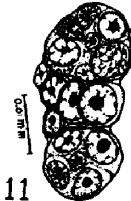
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PLATE II

FIG. 12. Immature daughter redia of *Allassostomum parvum* (Cort, Ameel and Van der Woude, 1948, Fig. 4).

FIG. 13. Posterior end of a large mature redia of *Psilostomum ondatrae* (Cort, Ameel and Van der Woude, 1948, Fig. 18).

FIG. 14. Daughter redial embryo of an unidentified echinostome from *Physa* sp. (Cort, Ameel and Van der Woude, 1948, Fig. 35).

FIG. 15. Posterior end of a mature mother redia of an unidentified echinostome from *Physa* sp. (Cort, Ameel and Van der Woude, 1948, Fig. 33).

FIG. 16. A very immature mother redial embryo of an unidentified echinostome species from *Helisoma campanulatum* (Cort, Ameel and Van der Woude, 1949, Fig. 1).

FIG. 17. Daughter redial embryo of *Psilostomum ondatrae* (Cort, Ameel and Van der Woude, 1949, Fig. 11).

FIG. 18. Very immature mother redia of *Euryhelmis monorchis* (Ameel, Cort and Van der Woude, 1950, Fig. 1).

FIG. 19. Almost mature mother redia of *Euryhelmis monorchis* (Ameel, Cort and Van der Woude, 1950, Fig. 5).

FIG. 20. Very young mother redial embryo of *Paragonimus kellicotti* in the "germ ball" stage (Ameel, Cort and Van der Woude, 1951, Fig. 17).

FIGS. 21 and 22. Mother redial embryos of *Paragonimus kellicotti* (Ameel, Cort and Van der Woude, 1951, Figs. 18 and 22).

FIG. 23. Posterior end of almost mature daughter redia of *Paragonimus kellicotti* (Ameel, Cort and Van der Woude, 1951, Fig. 34).

FIG. 24. Very young mother sporocyst of *Halipegus eccentricus* (Ameel, Cort and Van der Woude, 1949, Fig. 3).

FIG. 25. Posterior end of immature mother sporocyst of *Halipegus eccentricus* (Ameel, Cort and Van der Woude, 1949, Fig. 7).

FIG. 26. Very young redial embryo of *Halipegus eccentricus* (Ameel, Cort and Van der Woude, 1949, Fig. 10).

FIG. 27. Redial embryo of *Halipegus eccentricus* (Ameel, Cort and Van der Woude, 1949, Fig. 12).

FIG. 28. Young redia of *Halipegus eccentricus* (Ameel, Cort and Van der Woude, 1949, Fig. 16).

FIG. 29. Posterior end of mature daughter redia of *Triganodistomum mutabile* (Cort, Ameel and Van der Woude, 1950a, Fig. 7).

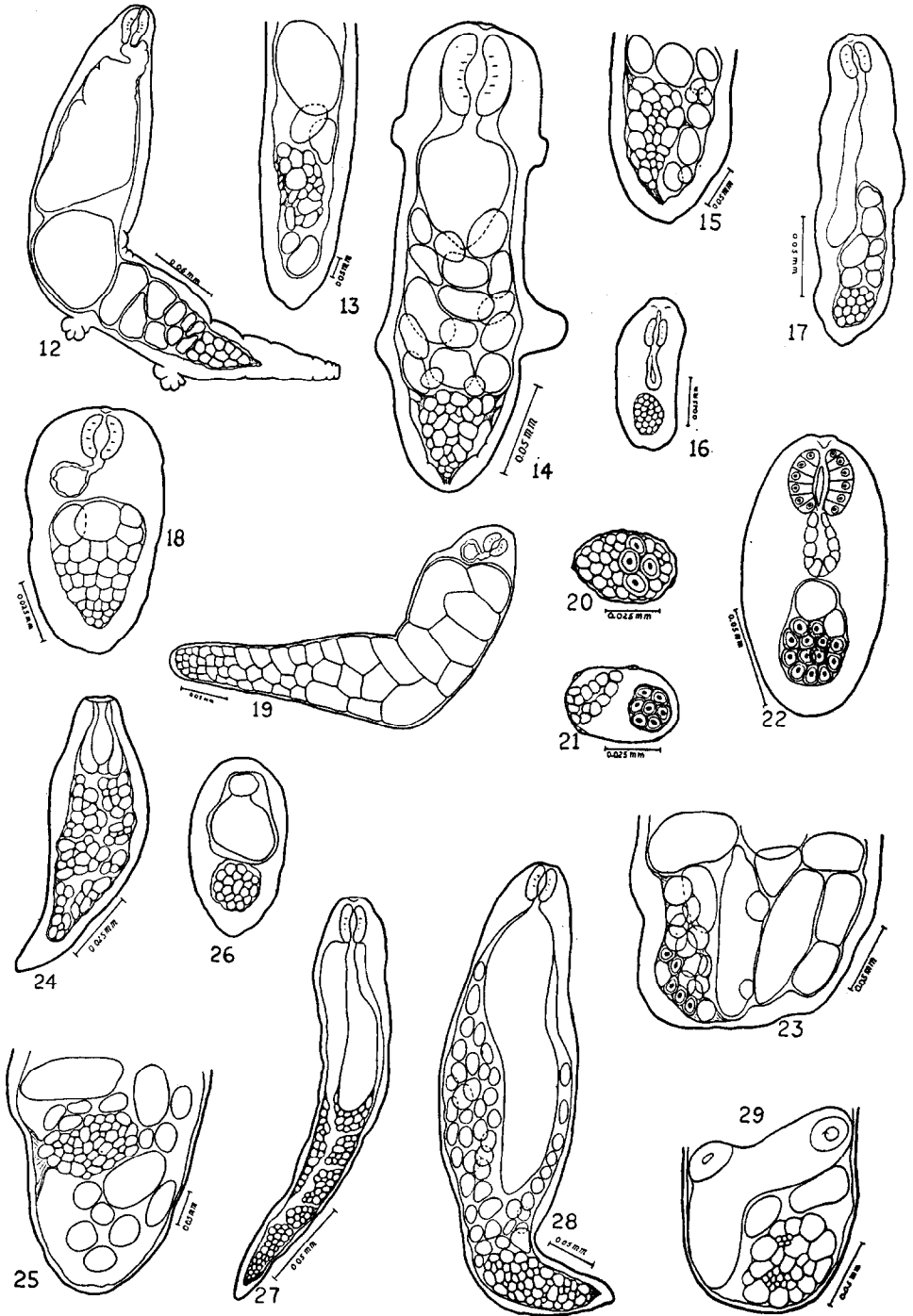


PLATE II
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PLATE III

FIG. 30. Very young mother sporocyst of *Lechriorchis primus* attached to the outside of the intestinal wall of the snail intermediate host (Cort, Ameel and Van der Woude, 1952, Fig. 1).

FIG. 31. Young branching mother sporocyst of *Lechriorchis primus* (Cort, Ameel and Van der Woude, 1952, Fig. 7).

FIG. 32. Very immature mother sporocyst of *Plagiorchis proximus* attached to the intestinal wall of the snail intermediate host (Cort and Ameel, 1944, Fig. 4).

FIG. 33. Very immature mother sporocyst of *Plagiorchis muris* (Cort and Ameel, 1944, Fig. 1).

FIG. 34. Three very immature daughter sporocyst embryos of *Lechriorchis primus* (Cort, Ameel and Van der Woude, 1952, Fig. 12).

FIG. 35. Daughter sporocyst embryo of *Plagiorchis muris* about ready to escape from the mother sporocyst (Cort and Olivier, 1943, Fig. 8).

FIG. 36. Immature daughter sporocyst of *Macroderoides typicus* (Cort and Ameel, 1944, Fig. 10).

FIG. 37. Anterior end of almost mature mother redia of *Clinostomum marginatum* (Cort, Ameel and Van der Woude, 1950b, Fig. 1).

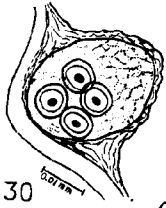
FIG. 38. Immature daughter redia of *Clinostomum marginatum* (Cort, Ameel and Van der Woude, 1950, Fig. 13).

FIG. 39. Germinal mass from the mother sporocyst of *Cercaria modicella* (Van der Woude, Cort and Ameel, 1953, Fig. 1).

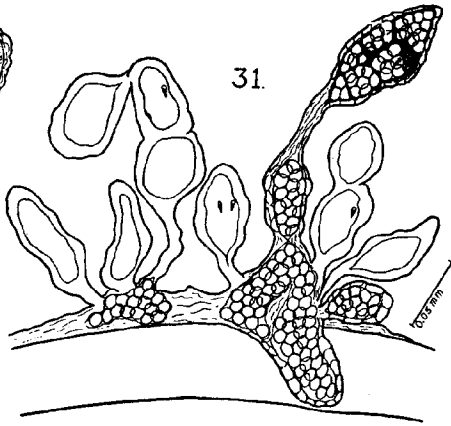
FIG. 40. Very young stage of a mother sporocyst of *Diplostomum flexicaudum* (Cort, Ameel and Van der Woude, 1951, Fig. 3).

FIG. 41. Young embryo daughter sporocyst of *C. modicella* (Van der Woude, Cort and Ameel, 1953, Fig. 10).

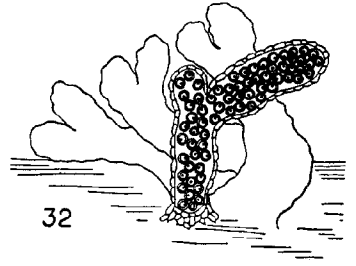
FIG. 42. Young daughter sporocyst of *C. modicella* (Van der Woude, Cort and Ameel, 1953, Fig. 16).



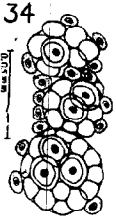
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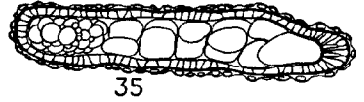
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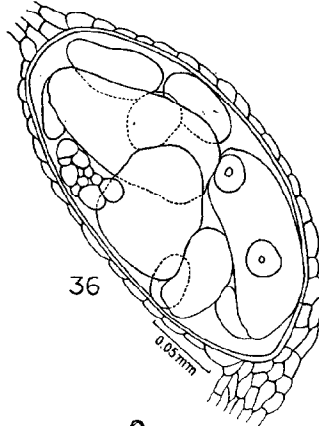
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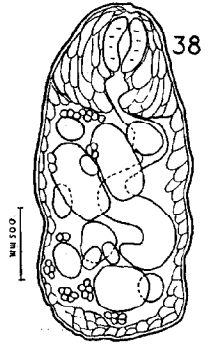
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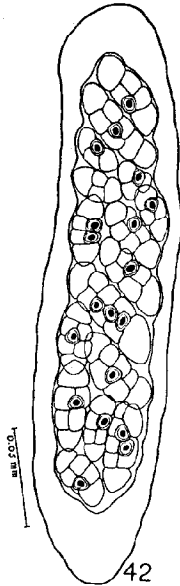
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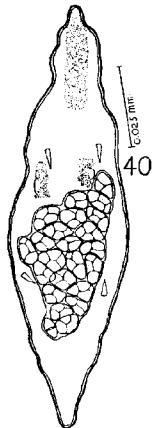
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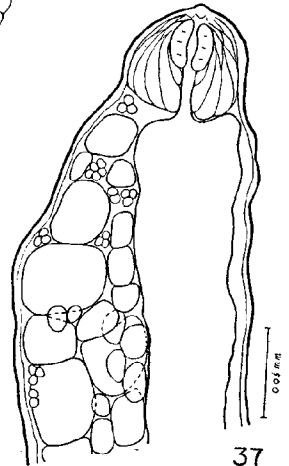
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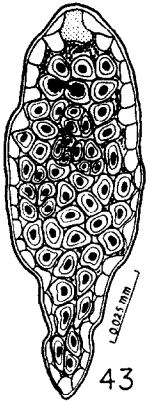
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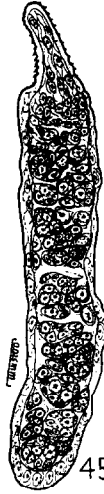
37

PLATE IV

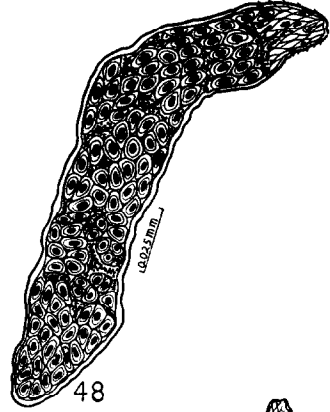
- FIG. 43. Very young mother sporocyst of a spirorchiid.
- FIG. 44. Section through a part of a mother sporocyst of a spirorchiid.
- FIG. 45. Longitudinal section of young free daughter sporocyst of a spirorchiid.
- FIG. 45. Longitudinal section of young free daughter sporocyst of a spirorchiid.
- FIG. 46. Section of daughter sporocyst embryo of *Schistosomatium douthitti* (Cort, Ameel and Van der Woude, 1953, Fig. 10).
- FIG. 47. Three day old mother sporocyst of *Trichobilharzia physellae*, 0.35 mm long.
- FIG. 48. Daughter sporocyst embryo of *Trichobilharzia physellae*, 0.21 by 0.03 mm.
- FIG. 49. Immature daughter sporocyst of *Trichobilharzia physellae*, 0.38 by 0.03 mm.



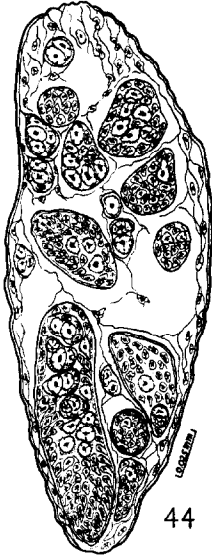
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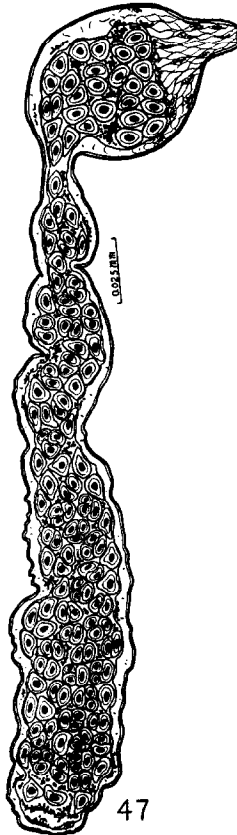
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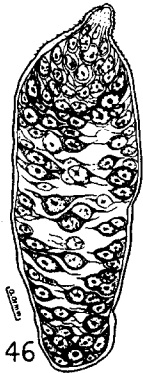
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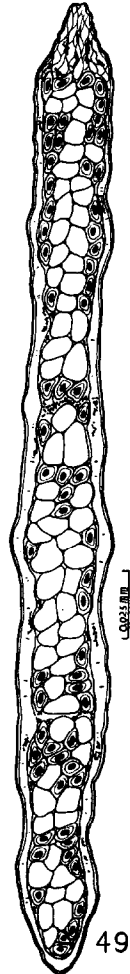
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