A STUDY OF THE METHOD OF REPRODUCTION OCCURRING IN THE
REDIA OF PROTEROMETA MACROSTOMA HORSFALL (1933)

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INTRODUCTION

There have been various attempts to explain the production of progeny by such larval generations as the sporocysts and rediae of digenetic trematodes. Whether these generations are sexual or asexual has never been demonstrated to the complete satisfaction of everyone. If they are sexual individuals (either monoeicous or dioecious types), one would expect to find gonads in them, or at least germ cells which display meiosis, or possibly polar body formation.

Investigations of the germ cell cycle in the digenetic trematodes, especially the more recent studies of Brooks (1930) and Woodhead (1931), have resulted in interpretations so opposite in nature that further study of this long disputed question has become increasingly desirable.

Cable (1934) pointed out that a comprehensive study of the germ cell cycle of the trematodes involves some of the most difficult, as well as fundamental, problems of parasitology. It is probable that germinal development has changed with the evolution of the Digenea, resulting in modifications occurring in their life histories. It is conceivable that the interrelationships of various families of digenetic trematodes are not sufficiently close to expect all members of this group to be characterized by the same
type of germ cell cycle. In a group of animals as large and as old as the Trematoda, it is conceivable that evolutionary changes have accounted for considerable diversity of reproduction, and, therefore, it is possible that sporocysts and rediae of some groups may reproduce by sexual means while in other groups these larval stages may reproduce by asexual methods. It is possible that in the life histories of the Monogenea there are stages of modes of development, such as polyembryony, internal budding, or parthenogenesis, that foreshadow or correspond to the sporocysts and rediae of the Digenea. If such stages do not occur in the Monogenea, one might be justified in assuming that endoparasitism, alone, is responsible for the larval stages of the Digenea.

The method by which sporocysts and rediae are produced in the life cycle of digenetic trematodes is a controversial issue. In attempting to explain the method of reproduction, Brooks (1930), Chen (1937), and Rees (1940), favored the theory of germinal lineage with polyembryony. However, Woodhead (1931, 1936, 1951, 1955) rejected this hypothesis, proposing a theory of polymorphism, in which he advanced the idea that the sporocysts and rediae of digenetic trematodes reproduce sexually.

Divergent views in the interpretation of the method by which sporocysts and rediae reproduce prompted the present investigator to make a study of these stages of the digenetic trematode Proterometra macrostoma. This species is a member of the trematode family Azygiidae. Its life history is known. The snail, Coniobasis livescens, which harbors the sporocysts and rediae stages, is found
in plentiful numbers in the Olentangy River, Columbus, Ohio. This factor, together with the fact that the redia and cercaria are large, make this trematode suitable for study.

According to Dickerman (1934), various fish belonging to the family Centrachidae serve as definitive hosts for the adult stage of Proterometra macrostoma. These include the Black crappie, Pomoxis nigro-maculatus; White crappie, P. annularis; Green sunfish, Lepomis cyanellus; Orange-spotted sunfish, L. humilis; Pumpkinseed, L. gibbosus; Northern blue gill, L. macrochirus macrochirus; Warmouth bass, Chaenobryttus coronarius; Northern rockbass, Ambloplites rupestris; Largemouth black bass, Micropterus salmoides; Spotted black bass, Micropterus punctulatus; and the Smallmouth black bass, M. dolomieu dolomieu.

The tissue site of the adult trematode in infected fish is the esophagus, where it lays unsegmented eggs that are carried along the digestive tract of the fish. Such eggs are voided in the fecal material of the fish. These unsegmented eggs undergo no further development while in the water.

The furtherance of the cycle is dependent upon the ingestion of the unsegmented eggs by coprozoic snails such as Coniobasis livescens, G. opaca, and Pleurocera acuta. The eggs complete their incubation within the intestine of the snail where the miracidia hatch and penetrate the intestinal wall, migrating into the coelom. Proterometra macrostoma, therefore, does not have a free-swimming miracidium. The miracidium changes into a mother sporocyst in the coelom of the snail. Since it is difficult to separate these two
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stages, they are sometimes referred to collectively as the
miracidium-mother sporocyst. The mother sporocyst in the digestive
gland of the snail produces a progeny of rediae. There is only one
generation of redia in the life cycle. The method by which this
progeny arise within the mother sporocyst is controversial, inves-
tigators disagreeing relative to the interpretation of this process.
In turn, each redia produces a number of cercariae within its body.
Again, the manner by which cercariae arise within a redia is ques-
tionable. This is the controversial point which this present
study is concerned.

The fully developed cercariae leave the body of the redia by
way of a rudimentary birth pore, and, after a short period of moving
about within the snail tissue; emerge into the surrounding water,
where they exist now as free-swimming cercariae. The cercaria of
Proterometra macrostoma is distinguished as a typical representative
of the Mirabilis group of Cystocercus Cercariae (Sewell, 1922), in
that the body of the cercaria is retracted into a large chamber
within the tail. The tail extends out into a flat stalklike struc-
ture terminating into two broad flaplike plates. While the cercaria
is still within the snail, the cercarial body is not retracted into
the tail chamber, this apparently occurring after the liberation of
the cercaria from the snail. The factors governing this behavior
are not clearly understood. Perhaps, the pH and oxygen tension
changes, which the organism experiences in emerging from the internal
environment of the snail body into the new outside environment, may
have some effect upon this behavior.
The cercaria of Proterometra macrostoma is precociously developed, possessing functional gonads. Eggs with shells are already present in the ovarian end of the uterus in some specimens. In this respect, the cercaria of this trematode is comparable to a young adult trematode with a tail.

The completion of the life cycle is dependent upon the cercaria being ingested by the fish host. When this occurs, the body of the cercaria is freed from its tail chamber, after which it migrates anteriorly from the stomach of the fish into the esophagus. In this tissue site it remains, having completed its life cycle. It may be considered as an adult trematode when it lays eggs in the esophagus of the fish.

Since it is a question of whether meiosis or any structures which suggest sexuality, such as gonads, are observable in the larval generations of digenetic trematodes, any observation of these stages occurring in the sporocysts or rediae of P. macrostoma is to be considered evidence that these generations possess the potential for sexual reproduction. A study of the behavior of the chromosomes of cells suspected as being germinal in nature should help one in determining whether sporocysts and rediae reproduce sexually. For example, the question would be resolved if one were to observe meiosis in a redia.

It should be noted here that the number of systematic attempts to trace the germ cells throughout the life cycles of Digenea has been very few. Only two attempts have been carried through to completion. Chen (1937) described the complete germ cell cycle in Paragonimus
Kellicott and Rees (1940) worked out the complete germ cell cycle in *Parorchis acanthus*.

There are two methods of research which might be employed in determining the method by which sporocysts and rediae reproduce. There is the method of morphology, which has been employed by various investigators in searching for gonads, meiotic divisions, or some sexual phenomena. This method has been employed by such investigators as Grobben (1882), Thomas (1883), Wegener (1886), Dolfus (1919), Brooks (1930), Woodhead (1931), Chen (1937), and Rees (1940).

On the other hand, histochemical methods supplemented by cytophotometry might be employed in determining the reproductive methods of the various life cycle stages of digenetic trematodes. Such techniques employ chemical reagents for the localization of various components of the cell, such as lipids, carbohydrates, and nucleoproteins, after which cytophotometric analysis determines the quantity of these. In this fashion, the cytophotometrist has been able to determine the variations of amounts of desoxyribonucleic acid (DNA) in the chromosomes of many plants and animal tissues.

The present investigator became interested in variations of DNA in the chromosomes of those cells of the sporocysts and rediae which participate in reproduction, because a determination of the amount of DNA suggests the type of activity of the cell.

While the latter method of research has been employed in the analytical-cytological studies of various plant and animal tissues, it has never been used, until this present study, in the analysis of those cells of the sporocysts and rediae which participate in
reproduction. This method was used in an attempt to demonstrate
(a) whether maturation phenomena occur in the redia of Proterometra
macrostoma; (b) the origin and distribution of the cells that give
rise to the cercaria of this species; and (c) the method of multipli-
cation of the cells which give rise to the redia.
HISTORICAL SURVEY

Theories of Reproduction by Sporocysts and Rediae

The first experimentally traced life history of a digenetic trematode was that of Fasciola hepatica. This was completed by Thomas (1883). The opinions of the early investigators prior to this time regarding the life histories of digenetic trematodes were based upon homologies of the larval and adult stages.

Metagenesis.- The theory of metagenesis, the alternation of a sexual with an asexual stage or stages, was applied to the trematodes by Steenstrup (1842), who thought their development was comparable to the reproductive cycle of the coelenterates and tunicates. This view was supported by Houlié (1865), Pagenstecher (1867), and Behringer (1884). Balfour (1885) maintained, on theoretical grounds, that the asexual generation of the trematode arose from endogenous cell formation or internal budding in the sporocyst. Wagener (1886) described the differentiation of germ cells from somatic cells in the body walls of sporocysts.

Heterogenesis.- The theory of heterogenesis, or the alternation of a bisexual generation with a parthenogenetic generation, as applied to trematodes, was initiated by Grobben (1882), who declared that germ cells within the sporocysts and rediae were true parthenogenetic
eggs, which gave off polar bodies and then developed.

Reus (1903) found a type of cell in the body wall of *Histomonum duplicatum*, which he thought gave rise to the germ cell. Haswell (1903) believed that the embryos in the sporocysts of *Echinostomum* developed from an ovary that projected into the body cavity at the posterior end of the sporocysts. Tennent (1904) reported two methods by which he thought germ cells were produced in the branching sporocysts of *Ducephalus haimeanus*: (1) by scattered production of germ cells into the sporocysts body cavity from the body wall of the sporocyst, and (2) by localized production of germ cells within a blunt process at one end of the body.

**Paedogenesis.**—Paedogenesis, a term indicating a type of life history in which reproduction is carried on by the larval stages and not by the adult, as in the fly, *Chironomus*, was introduced by von Baer (1866) in an attempt to explain the reproductive cycle of trematodes. In this theory, von Baer conceived of sporocysts and rediae as larval stages of the trematodes. Cary (1909) reported that the body wall of young sporocysts of *Diplodiscus temperatus*, gave rise to what he interpreted as egg cells, while in older sporocysts such cells arose from localized ovaries. He conceived of sporocysts as parthenogenetic individuals. Cary claimed to have found maturation division in the eggs which he found in the sporocysts of *Diplodiscus temperatus*, and therefore he supported Claus' (1889) doctrine of heterogony with paedogenesis. Ssinitzin (1911) supported this view on theoretical grounds. Faust (1917) also supported this view, reporting the
formation of polar bodies in the redia of a holostome *Cercaria flabelliformis*.

**Extended metamorphosis.** Balfour (1885) and, later Looss (1892) believed that the reproductive cycle of the digenetic trematodes was a matter of extended metamorphosis. Leuckart contended that if a series of different development phases could contract into a single continuous process, then, conversely, it was possible that this could spread itself out into a number of phases. He laid the foundation for the idea of germinal lineage through the life history of digenetic trematodes.

Leuckart believed that the germ cells of sporocysts and rediae could be traced directly back to the eggs of a previous adult, not having become differentiated in the formation of the sporocysts. In this manner, he believed that the germ cells remain similar to their antecedent cells, and that these germ cells had come over into the body cavity of the sporocysts. Thomas (1883), Schwarze (1886), and Coe (1886) subscribed to this theory.

**Germinal lineage.** Dolfus (1919) revived Leuckart's hypothesis of germinal lineage. This was supported by the studies of Brooks (1930), Chen (1937), and Rees (1940). Dolfus believed that certain cells, which he called germ cells, of the sporocysts and rediae could be traced directly back to the fertilized egg. Since these cells were never localized in germinal epithelia, he believed that they never became true germ cells which give rise to ova and spermatozoa; however, they remained separate from the somatic cells during the development
of the sporocysts and rediae. The sporocysts and rediae were referred to as reproductive sacs. The multiplication of the cells of the germinal line, according to Brooks, was accounted for as polyembryony of the original zygote (fertilized egg), extended throughout the whole development of the "germinal sacs" (rediae and sporocysts) while they were in the molluscan hosts. Brooks believed that numerous somatic cells, which were split off from the cells of the germinal line during this process, formed primary and secondary germinal sacs, sporocysts and rediae respectively. These germinal sacs contained both multiplying germinal line cells and embryos. He believed that such germinal line cells coming over into the cercaria from the radial generation, gave rise to gonads which produced sperm and ova. This hypothesis contended further that mature spermatozoa and ova were formed by the cercaria and, later, by the adult trematode, these being the only cells in all the stages of the life cycle of the digenetic trematodes which underwent meiosis, and therefore these cells were the only ones that had a reduced number of chromosomes.

Chen (1937) gave a complete account of the germ cell cycle of Paragonimus kelliotti, in which she believed that germinal lineage was found without interruption through all the larval stages, sporocyst and two generations of redia.

Polyembryony.—Brooks (1930) made a cytological study of various sporocysts and rediae which produced cercariae. He found what he thought were germ cells, calling them antecedent cells, scattered in
the body cavity of young sporocysts and rediae. Neither polar bodies, nor maturation phenomena were found in connection with these antecedent cells. Germinal multiplication was accounted for by successive divisions of antecedent cells, which form loosely organized cell masses in the body cavities of both sporocysts and rediae. Secondary components of the germ masses, later, underwent regular cleavage forming cercarial embryos. Evidence was presented to show that the life histories of the digenetic trematodes should not be interpreted as metagenesis, heterogony, paedogenesis, or metamorphosis extending over several generations; however, Brooks offered a substitute hypothesis to account for reproduction by sporocysts and rediae. He postulated that the germ cells passed through these larval stages, and therefore he interpreted the life cycle as one in which germinal lineage prevailed. In his hypothesis, polyembryony featured as a mode of multiplication. Brooks maintained that many individuals resulted from a single fertilized egg. He defined polyembryony as any case in which multiple embryos resulted from a single fertilized egg.

Brooks compared certain features of the life cycle of trematodes to polyembryony as it is described by Patterson (1921) in the insect Paracopidosomopsis. Patterson believed this polyembryony was an inherent tendency of the egg, being related to its simplicity. On the other hand, the work of various experimental cytologists suggests that polyembryony is initiated by various environmental factors of the egg. Stockard (1921) induced polyembryony in fish by arresting the development of various embryonic stages at low temperatures. He
found the lack of oxygen supply, also, produced the same results, and Marshall (1922) suggested that this was responsible for polyembryony in mammals.

Cable (1934) opposed the views of Brooks, contending that germinal lineage supplemented by intercalated polyembryony was insufficient to explain the germ cell cycle of trematodes in general. He suggested that the changes brought about in the trematodes had not appeared suddenly but gradually, and are the result of the parasitic habit of trematodes. He believed that the primitive trematodes had become sexually mature in the molluscan host, in which they completed their life cycle at a time before the vertebrates had appeared. According to Cable's view, parasitism in the subsequent evolution of the Digenea included either the postponement or loss of sexual reproduction by stages in the molluscan host.

The work of Rees (1940) gave support to the theory of germinal lineage with polyembryony. In her investigation of the germ cell cycle of Parorchis acanthus, the cycle was carried from the fertilized egg through the redia stage and up to the adult form. The germ cell cycle of Parorchis acanthus resembled in many respects that of Paragonimus kelicotti described by Chen (1937), who believed that the germ cells developed directly into embryos without the formation of germ masses or maturation phenomena. However, they differed in that sporocysts are not represented in the life cycle of Parorchis acanthus, a single redia being formed from a cell that developed directly from a propagatory cell in each miracidium. It also differed from Paragonimus kelicotti in that the redia, when first formed within the
miracidium, contains a number of germ cells, which, by division, develop into germ balls, which in turn develop into second generation rediae.

**Polymorphism.** Woodhead (1931) rejected the theory of germinal lineage with polyembryony, reporting that he had found nuclei with chromatin arranged in crescents in the redia of Gasterostomes. He interpreted this as indicating a stage in spermatogenesis, and therefore that the rediae were sexual individuals. He studied the mother sporocysts, the redia, and finally, the adult stage of *Bucephalus elegans*, finding what he interpreted to be stages of spermatogenesis in each of these generations, and he concluded that each was a sexual generation. Woodhead also reported a Laurer's canal in the redia, describing it as a long tube containing sperm cells, and he postulated that it was used in fertilization. He also found what he interpreted as an ovary in both the mother sporocyst and the redia, and therefore he concluded that each generation began with a fertilized egg. Woodhead further contended that reduction division and fertilization occurred in each generation, there being no parthenogenesis or metagenesis in the life cycle of *Bucephalus elegans*. Woodhead interpreted reproduction as being sexual in all stages of the life cycle of this species. However, he conceived each stage as being an adult, and therefore the species as being polymorphic.

In his discussion of *Bucephalus elegans*, he stated that

. . . we have three "adults" I believe certain. The three adults are mature and not larval. All three have a well defined anterior sucker with mucin and penetration glands. The reproductive and nutritive systems in each are well
developed. I see no "larval" structure in either the first, second or third adults and therefore no "paedogenesis." The gasterostome is therefore polymorphic having three generations, each generation having homologous stages; 1st the fertilized egg, 2nd the free-swimming larvae (miracidium, pro-redia and cercariae), 3rd the adult with a pupal (cyst) stage retained or evolved only in the last generation.

Later, Woodhead (1936) reported that Leucochloridium, Spp. have a polymorphic life cycle similar to Bucephalus; however, the former trematodes are not generally regarded as being closely related to the Bucephalidae.

Woodhead (1954) pointed out that bisexual reproduction is the method followed by the mother sporocyst in producing the first generation of rediae in the trematode Paragonimus kellicotti. This contradicted the investigations of Chen (1937). Woodhead further contended that the mature mother sporocyst of P. kellicotti is hermaphroditic, possessing both ovaries and testes, capable of fertilization, and therefore it is a true generation.

Histochemical and Cytophotometric Backgrounds
Since this investigation will embrace a histochemical and cytophotometric study of the cells of the rediae and cercariae of Proterometra macrostoma, it seems appropriate to mention some of the histochemical and cytophotometric backgrounds which lead up to this study.

Cassperson (1936) used a photographic method in conjunction with a crude monochromatic illuminator and a monochromatic quartz microscope to obtain data for absorption curves of chromatin of cell nuclei.
He demonstrated that the marked ultraviolet absorption of these nuclear masses, known since the first application of the quartz microscope in 1902, was probably due to the nucleic acid content within the cell nucleus. Cassperson and his students developed the ultraviolet technique to a high degree of perfection, and in 1917, made quantitative measurements in ultraviolet light of cell parts in microscopic sections.

Darlington and La Cour (1940) reported that there were variations in the quantity of DNA in the chromosomes of the nucleus during mitosis. They believed the changes in the content of DNA paralleled the relative distinctness of chromosomes in cytological preparations, increasing in prophase and gradually decreasing in telophase to a low amount in interphase.

Stowell (1942, 1945) and Stowell and Cooper (1945) used a modified Feulgen reaction with a system of photometric recordings to estimate the amount of DNA in a variety of normal and neoplastic tissues. They measured the mean amount of DNA per cell, thus correlating quantitative results with morphological data. Their results indicated that the mean amount of DNA per cell was similar for most tissues in the animals they measured, except for some carcinomas in which it was significantly increased.

Brachet (1944) pointed out that chromosomes vary in their behavior and appearance according to the nucleic acid supply available for the "synthesis" of the chromosomes during the prophase of mitosis. He found that the supply of nucleic acid is reduced during the segmentation of eggs, and consequently the chromosomes appeared smaller.
Stowell (1956) reported that the "Feulgen nucleal reaction" is commonly used as a histochemical test for DNA. Darlington (1947) pointed out the vital importance of the Feulgen nucleal reaction in making chemical processes in the nuclei structurally visible.

Pollister and Ris (1947), working with both plant and animal tissue, made nucleoprotein determinations in cytological preparations, using one wave length of a single beam of monochromatic light of the visible spectrum. Ris (1947), following the method of Pollister in investigating the composition of chromosomes during mitosis and meiosis, concluded that the DNA of chromosomes increased twofold during the mitotic prophase and also in the first meiotic prophase; however, he found no such increase taking place in the prophase of the second meiotic division.

Investigators disagreed as to the variations of the amount of DNA in chromosomes, and Vendrelly and Vendrelly (1948) suggested that the amount of DNA per nucleus is probably constant in all nuclei of any species at all times. More recent data, obtained by similar methods, by Mirsky and Ris (1949), indicate that DNA is approximately constant within the nuclei of one species at all times, and also of each type of tissue regardless of its activity.

However, all quantitative studies on DNA have not shown this constancy in amount of DNA per nucleus. Evidence that DNA varies widely from tissue to tissue in *Tradescantia*, has recently been brought forward by Schrader and Leuchtenberger (1949).

Swift (1950) measured the DNA in the nuclei of ten different formalin-fixed tissues from young and adult mice, employing the
conventional single beam of monochromatic light. He found that in this animal the nuclei, regardless of tissue type, showed approximately the same amount of DNA, except for the nuclei in liver, pancreas, thymus, blood lymphocytes, and Sertoli cells. On the basis of the amount of DNA in cell nuclei, he classified as Class I the somatic cells of this animal, as having the most common amount of DNA, with a range expressed in arbitrary units as 3.00-3.44; Class II, those which contained twice the amount, having a range of 6.00-6.40; and Class III, those which contained four times the common amount of DNA in the cell nuclei, having a range of 12.4-13.2. He classified the sex cells of the mouse as Class I premeiotic spermatogonia, with a range of 3.20-3.28; Class II premeiotic spermatogonia, with a range of 5.91-5.98; primary spermatocytes with a range of 5.81-6.28; secondary spermatocytes with a range of 3.21-3.35; and spermatids with a range of 1.42-1.68. Occasionally, rare intermediate values were found, presumably associated with mitoses. In the liver of a ten day old mouse, Class I nuclei occurred in the greatest numbers, while in the adult mouse liver, Class II nuclei were more abundant.

Mouse spermatid nuclei contained half the amount of DNA that was found in Class I somatic nuclei, and primary spermatocytes contained four times the amount of DNA as did the spermatids. Each primary spermatocyte had the amount of DNA, which would later be divided into four parts, distributed equally to the spermatids, with each spermatid getting one-fourth the amount of DNA.

Swift also reported that the amount of DNA found within the
nucleus of a cell was correlated with the stage of mitosis of the cell. He found that interphase nuclei showed a large number of values intermediate between Class I and Class II amounts of DNA. All early mitotic prophase nuclei that were measured were Class II nuclei, whereas all mitotic interphases were of Class I. It thus appears, in the tissues studied, that DNA in cell division is "synthesized" in the interphase nucleus before the visible onset of the prophase. During the visible stages, such as prophase, metaphase, and anaphase, no DNA is synthesized, and the amount is cut in half at anaphase.

Patau (1952) devised a method whereby quantitative determinations could be made of DNA in irregular-shaped nuclei. He used two different wave lengths of a single beam of monochromatic light of the visible spectrum. Patau's method reduced the mathematical computations necessary when using the conventional method of the single beam of monochromatic light of one wave length. It further compensated for (1) the distributional errors; (2) the stray light error (including the effects of diffraction); and (3) the wavelength error; all of these errors can always be kept much below 3 percent of the true dye content of the cells. Since in Patau's method, cell nuclei are measured in replication, and at two different wave lengths, this greatly reduces the total number of cell nuclei of any given type that would have to be measured in making determinations of DNA in cell nuclei. Patau's method is adapted also for study of irregular-shaped nuclei, such as would be encountered in photometric studies of the nuclei of the cells of sporocysts and rediae of digenetic
trematodes. Moreover, the method is adapted to sections as well as smears, provided complete nuclei are selected and the nuclei are slightly separated from one another. It is preferable that a space of at least one-half the nuclear diameter be between the nuclei that are being measured, because this would prevent the overlapping of nuclei, and therefore prevent erroneous results caused by such overlapping. The method can be applied to any shape of cell, sufficiently stained, since the shape of the object as well as the distribution of the dye molecules within it have only a small effect on the result.
MATERIALS AND METHODS

The material used in this study was obtained from two types of hosts. Mature specimen of Proterometra macrostoma were obtained from the esophagus of the definitive host, the green sunfish Lepomis cyanellus. The larval stages were taken from the digestive gland and body cavity of the intermediate host, the snail Coniobasis livescens.

In order to obtain adult forms of Proterometra macrostoma, it was necessary to experimentally infect the green sunfish. Fifteen specimen of this species of fish were obtained from the Fish and Wildlife Service, Department of Interior, U.S. Government. These fish were maintained in separate aquaria into which cercariae of the trematode were introduced. The fish ingested the cercariae.

Approximately 1500 specimen of the snail, Coniobasis livescens, were collected, at various times, and maintained in shallow wide-mouth bottles. After three days, they were observed for evidence of emerging cercariae. Infected snails were crushed in Ringer's saline for cold-blooded animals, and the rediae and young cercariae were teased out of the crushed tissue and fixed.

The preparation of cytological material for photometrical analysis has been found by many investigators to have rather different requirements than those developed primarily for cellular morphology. For morphological cytology, fixation is employed to
preserve cells in as near a natural condition as possible, and to facilitate the application of stains or metallic impregnations which will give maximum contrast between the component parts of cells.

This is accomplished by selecting methods of staining in which considerable physical adsorption either predominates (Heidenhain's iron-alum haematoxylin or crystal violet staining), or else is almost the whole process (silver or osmium impregnation). These techniques are notoriously erratic, and demand much experience and subjective judgement on the part of the cytologist.

The reaction of Feulgen and Rosserbeck (1924) has been generally used as a specific test for DNA in the cell nucleus. The mild acid hydrolysis that precedes the staining of the cell nucleus, (1) removes the purine bases completely; (2) removes the histones and nucleic acids progressively; and (3) releases the aldehyde groups from the desoxyribose sugar of DNA. Following hydrolysis, the tissues, or sections, are washed in water, after which they are transferred to a solution of Schiff's reagent (leucofuchsian), which reacts with the exposed aldehyde groups to produce a purple dye in the DNA portion of the nucleus alone. This procedure makes the presence of DNA visible in the cell nucleus, and has become an important method which is used in the analysis of cells for their constituent materials.

In cytophotometry, on the other hand, it has been found necessary to select methods which can be rigidly standardized, and which will give reproducible results as measured with a photometer. There must be prescribed conditions of concentrations of the hydrolyzing agent. The pH of the hydrolyzing agent must be kept
constant and its temperature must be regulated according to the fixing reagent that was used in fixing the specimen. Furthermore, the time for the hydrolysis must be regulated, according to the fixing reagent used. The stain must be known to react with some particular chemical component of the cell, which in this case is DNA. The fixing fluid must produce no color in the tissue; the tissues must be left in such condition that chemical staining or tests can be carried out; the tissue must be in such condition that chemical extractions or digestions can be carried out; and the refractive index among the cellular elements must be minimized. These results are generally accomplished with considerable sacrifice of cellular morphology, particularly in the techniques most widely used for nucleoproteins.

The Feulgen reaction, as it is applied to cytophotometry, generally fulfills the aforementioned conditions. It is held to be specific for desoxyribonucleic acid because (a) only nuclear chromatin of the cell gives a positive reaction; (b) after chemical extraction of nucleic acids from a section, the nuclear chromatin gives a negative Feulgen reaction; (c) the specific removal of DNA by desoxyribonuclease digestion makes the section Feulgen-negative; and (d) cytophotometric measurements of the Feulgen reaction of haploid spermatozoa and diploid somatic tissues, show the same quantitative relationships as does the chemical analysis of the masses of isolated cells and nuclei.

It becomes necessary for both the cytologist and cytophotometrist to examine fixed and stained preparations microscopically.
A prolonged search must be made to locate the best oriented illustrative cells in order that valid inferences may be drawn. Generally, the cytologist must select stages of cells that indicate a sort of average for the whole group of cells of that type. However, variability from cell to cell will likewise characterize the data obtained by cytophotometry, which in this case, is objective. A group of cells, therefore, can be treated statistically. This will rarely demand measurements of more than 100 cells of any one type, since measurements by replication supply data from 200 readings.

Cytological Methods

Fixation.—Material for the cytological study of whole mounts of rodia, cercaria, and adults of Proterometra macrostoma was fixed in Lavidowsky’s AFA. Material for sectioning was fixed in Bouin’s, Carnoy’s, Sanfelice, and Flemming’s fixing reagents. Flemming’s and Sanfelice fixing reagents were found to be most satisfactory. Smear preparation were fixed in aceto-cresin.

Dehydration and Sectioning.—The dioxan-xylol method was employed for dehydrating and clearing material preparatory for cytological study. In preparations for sectioning, the material was embedded in paraffin impregnated with bees wax. Sections were cut at 4 - 10 microns thick. Six microns proved most satisfactory in this study. Whole mounts were dehydrated in alcohol, and then cleared in clove oil.
Staining.- In preparation for staining, the sections were deparaffinized, then passed through the alcohols into water. They were stained in Heidenhain's iron-alum haematoxylin, dehydrated, cleared, and finally mounted under a cover glass in Permount. Some sections were mounted in green Euparal. Smear preparations were fixed and stained simultaneously in aceto-orcein, and mounted in Permount. Whole mounts were stained in Semichon's paracarmine, and mounted in Permount.

Microscopic examination of cytological materials was accomplished with the aid of 10X, 15X, and 97X apochromatic objectives, and 8X, 10X, and 15X periplan oculars. Line drawings were made by microprojection using a Bausch and Lomb Tri-Simplex microprojector. A Leitz Panphot was employed to make the microphotographs.

Histochemical Methods

Fixation.- Materials for histochemical study were fixed in a solution of alcohol-mercuric chloride. They were then transferred to 95 percent alcohol and held for 24 hours. This procedure prevented the possibility of a plasmal reaction occurring later, which would introduce another variable when photometric measurements were conducted. The sections were placed into a 70 percent iodized alcohol solution to remove the mercuric chloride from the tissues.

Dehydration and sectioning.- The material was dehydrated by the alcohol method, cleared in chloroform, and infiltrated with 56°C paraffin, impregnated with bees wax. Sections were cut at a thickness
of 10, 12, and 14 microns.

Staining. - The sections were deparaffinized, passed down the alcohols into water, and then stained, using the Feulgen technique (de Tomasi, 1936, modification). Eventually, the sections were dehydrated, by the alcohol method, cleared in xylene, and mounted under a cover glass in Permount.

Method for making Cytophotometric Measurements

In making measurements of the DNA content of a nucleus, it becomes necessary to use cytophotometric equipment. In using this apparatus, the absorption by the Feulgen-stained nuclei of a single beam of monochromatic light of the visible spectrum is determined by a photometric apparatus similar in basic principle to that described by Pollister and Ris (1947) (Fig. 1). Goldberg (unpublished) has made several modifications of Pollister's apparatus. In the apparatus used in the present study, the light source was produced by a tungsten lamp, G. E. 1193, which provided a beam of white light that passed through a monochromator. A single beam of monochromatic light was provided in this fashion, and the wave length of the monochromatic light beam was shifted from one end of the visible spectrum to the other by manipulating dials. The monochromatic light beam was directed to the mirror of the microscope, which reflected the beam up through the object on the microscope stage. An extension tube containing a field diaphragm was connected to the draw tube of the microscope.
The field diaphragm eliminated all the image except the area of the nucleus which was to be measured. A side viewing tube was connected to the extension tube, thus providing a means of viewing the object on the microscope stage while measurements of DNA in the nucleus were being made. The phototube was connected, in light-tight fashion, to the extension tube, and a micro-ammeter was connected to the phototube. The transmission of light through the object on the microscope stage was read from the dials on the micro-ammeter. The whole system was powered by a series of storage batteries which
supplied direct electric current. In Goldberg's modification a potentiometer was incorporated into this apparatus in order to control any fluctuations in the operation of the phototube or light source.

The flint glass prism within the monochromator was placed upon a pivot turning point, and by turning this prism various wavelengths of monochromatic light of the visible spectrum was provided. This adapted the monochromator for use in photometric determinations of DNA in irregular-shaped nuclei, according to the methods of Patau (1952), which employs two different wave lengths of monochromatic light. The apparatus also measured the amount of light absorption of Feulgen-stained nuclei, and therefore the dye content of the nuclei was computed in relative arbitrary units. As a result, inferences were drawn relative to chromosome variations, indicated by the dye content of the nuclei.

When making computations of the dye content of a Feulgen-stained nucleus, it is necessary that the aperture of the phototube be located in the plane of the microscope image of the Feulgen-stained nucleus which is to be measured, since a determination of the amount of DNA suggests the type of activity of the cell. The field area (in the plane of the object) corresponds to the aperture, and the entire object (stained nucleus) is contained within the aperture area. The field area is illuminated by a beam of monochromatic light with normal incidence and uniform intensity. The phototube has a constant sensitivity all over the field area, and its response to the light entering the phototube is registered in galvanometer units.
In using the apparatus, the light flux, or illumination, is defined as \( I_1 \), when it is passing through a stained nucleus in the field area \( B \). The illumination is defined as \( I_0 \) when it is passing through a blank field. The transmission of light is greater through a blank slide than the transmission of light through a stained nucleus, since the Feulgen dye in the nucleus absorbs the light, therefore, \( I_0 > I_1 \). The ability of a dye to absorb light of a given wavelength is referred to as the extinction factor \( E \) of that dye. \( E \), therefore varies directly with the number of absorbing molecules and is expressed as \( E = \frac{1}{(\log T)} \).

The mathematical formulae Patau derived for use in his method are indicated in the following paragraphs. The transmission of light \( T \) through the field area \( B \) containing an object (stained nucleus) is expressed as \( T = \frac{I_1}{I_0} \). The loss of light \( L \) due to its passage through media of various refractive indices is expressed as \( L = \frac{I_1 - I_0}{I_0} = 1 - T \). The amount of Feulgen dye \( T \) in the object is expressed as \( T = KHC \), in which \( C \) is a correction factor and \( K \) is a constant.

Actual measurements of DNA in a cell nucleus cannot be made until the two wave lengths that will be used in the determinations have been selected. A test object (stained nucleus similar to the nuclei to be measured) must be used in determining the two wave lengths that will be used in making the measurements of DNA in the stained nuclei. For accurate results, the two wave lengths, expressed as wave length_1 (\( \lambda_1 \))
and wavelength \( \lambda_2 \) must be selected so that the test object displays an extinction (E) at \( \lambda_1 \) = \( 2E \) at \( \lambda_2 \). This is essential in Patau's method, since as he contends, the selection of the proper extinction values of the two wavelengths will materially reduce the errors generally encountered in photometric determinations. Patau also suggests that the wavelengths selected must be sufficiently removed from the peak (E) for the material that is being measured so that valid results may be obtained.

Patau's method requires four photometric readings at each wavelength, two with and two without the object in the field area. These readings will indicate the transmission of light through the whole field (B), in which the transmission at wavelength \( \lambda_1 \) (\( T_1 \)) is expressed as \( T_1 = \frac{I_{11}}{I_{10}} \), and the transmission at wavelength \( \lambda_2 \) (\( T_2 \)) is expressed as \( T_2 = \frac{I_{21}}{I_{20}} \). The loss of light at wavelength \( \lambda_1 \) is expressed as \( L_1 = 1 - T_1 \); and the loss of light at wavelength \( \lambda_2 \) is expressed as \( L_2 = 1 - T_2 \). The extinction coefficient of the dye in the object is expressed as \( k_1 \).

When computing the average dye content of a Feulgen-stained nucleus, there are two important factors. These are \( Q \), which is the ratio between \( L_2 \) and \( L_1 \), and \( C \), which is the correction factor that Patau claims reduces the errors and variables that might arise in photometric measurements. The computation of the Feulgen dye content \( V \) can be shown to be:
\[ \gamma = KB \frac{1}{C} \]

K = \frac{1}{k_1 \log 10} (this constant can be ignored in final computations)

Q = the ratio between \( L_2 \) and \( L_1 \)

C = \frac{1}{2 - Q} \log \frac{1}{Q - 1}

The actual computation of the correction factor (C) is made unnecessary by using Table 1, derived by Patau (1952), which gives (C) as a function of (Q). Linear interpolations are possible unless (Q) approaches 1. For example, if (Q) is found to equal 1.345 in a set of computations, Table 1 will only give the values of \( Q = 1.34 \) and \( Q = 1.35 \). However, by interpolation, the value of \( Q = 1.345 \) in terms of (C) is found to be 1.625.
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The Redia

The redia of *Proterometra macrostoma* parasitizes the digestive gland (liver) of the snail, *Goniobasis livescens*. Rediae were recovered from snails by crushing the shell, and then dissecting the liver with needle-point dissectors. The living, full developed redia is an elongated, spindle shaped, lemon-yellow body, which may attain a length of more than 8-mm. It shows an average length of 2.5-mm., and an average width of 0.7-mm., after it has been killed and fixed in various fluids.

The fully developed redia is a thin walled sac, which contains (1) cell masses in the body cavity identified as germ balls; (2) developing cercarial embryos; and (3) young cercariae (Figs. 3 & 10). The oldest cercaria appear to be located in the anterior portion of the body cavity. Since it is progenic, the oldest cercaria is actually a young adult, having a full complement of genital organs, and often a small number of eggs with shells. It differs from the adult fluke, as in most other trematodes, in the possession of a tail appendage. In those that have emerged from the snail host, the body of the cercaria is retracted into the anterior chamber of the tail, and therefore it has been referred to as a Cystocercous cercaria.

The walls of the redia consist of a relatively thin cuticle,
a thin middle layer composed of circular and longitudinal muscles, and an inner parenchymatous tissue layer. The young redia possesses a pharynx and a rhabdocoeil gut, but when this stage increases in size, becoming fully developed, the intestine ruptures and the pharynx, surrounded by a few circular and longitudinal muscles, becomes the birth pore. This birth pore, located in the anterior wall of the redia, provides a means of escape to the cercaria when it leaves the body of the redia.

Laurer's canal and gonads, which were reported by Woodhead (1931) in the redia of certain species of trematodes, were never observed in any of the rediae of *P. macrostoma*. Such structures, suggesting a sexual form, were not demonstrable by morphological examinations of the redia stage of *Proterometra*.

Morphological Observations of the Redia

**Germinal line cells of the redia.**—Certain cells were observed within the parenchymatous tissue of the body wall at the posterior end of the young redia (Figs. 4 & 11). The cytoplasm of these cells has an irregular shape, and it extends into a stalk or pedicle, which appears to attach each cell to the wall of the redia. A single centrally located nucleolus, and lightly-stained chromatin granules may be observed in the nuclei of these cells. The size of the nuclei ranges from 10 - 12 μ. These cells were generally observed in the interphase stage of mitosis as was evident by the presence of the scattered chromatin granules and centrally located nucleolus in the nucleus. At this time they contained darkly-stained bodies that
appear to be either confluent with the nuclear membrane or closely adjacent to it. It is possible that such bodies might be the "ectosomes" that Nachtwey (1925) believed to be identical with the "membrankörper" in the trematode Asplanchna priodonta. Chen (1937) reported that similar structures were present in the first cleavage of the fertilized egg of Paragonimus kellicotti. The exact function of these bodies is unknown; however, according to Chen, when the propagatory cells begin to divide, the "ectosomes" disappear.

This type of cell is referred to as a germinal line cell in this study. These are the same type of cells that Brooks identified as the "antecedent cell" in his investigation; however, Brooks reported that they were found scattered within the body cavity of the rediae and sporocysts that he studied. Neither Chen, Cable, nor Rees, were able to confirm this point in their investigations. The present investigator could find no evidence to indicate that the germinal line cells were scattered in the body cavity of the redia of Proterometra.

Failure to obtain the miracidium-mother sporocyst generation of Proterometra in this study, prevented the present investigator from tracing the germinal line cells back into this generation; however, the general characteristic of these cells, such as their adherance to the redial walls, and their subsequent development into germ balls within the body cavity of the redia, suggest that they are germinal line cells rather than gonads.

Some investigators have contended that such germinal line cells adhering to the walls of the redia are true ova, in which maturation
figures and polar bodies have been observed. The present investigator observed germinal line cells within the posterior walls of a number of young rediae of *P. macrostoma*. An intensive examination of the germinal line cells for any evidence of germinal epithelium, which might suggest gonadal tissues, did not reveal any evidence that indicated that the germinal line cells were surrounded or inclosed in germinal epithelium. Neither were maturation figures, meiosis, nor polar bodies observed in these cells.

Germ balls of the redia. - It was found that germ balls developed from the cleavage of germinal line cells that had been found within the parenchymatous tissues of the wall of the redia. This was made evident by the fact that various gradations and various sizes were found between the germinal line cells at one extreme to completely developed cercariae at the other. The germ balls were observed either attached to the body wall or within the body cavity of the redia (Figs. 12 & 13). Apparently the developing cell mass eventually detaches itself from the radial wall, after which multiplication of the cells within the cavity of the redia continues. Finally, the mass of cells transform into a cercarial embryo. Cleavage of the germinal line cells appears to be unequal, resulting in cellular differentiation (Fig. 14). Certain of the cells of the germ ball retain the general characteristics of the original germinal line cell. These cells possess a large nucleus, with a darkly-stained, centrally located nucleolus, and they have chromatin granules scattered within the nucleus. Such cells are comparable to
the germinal line cells; however, another type of cell, the somatic cell, was also observed in the germ ball.

Investigators have disagreed as to whether the germinal line cells are larger than the somatic cells. Brooks (1930) observed unequal cleavage of the antecedent cells in his investigations of the sporocysts of *Cercaria laruei*. The larger cell, the macromere, Brooks contended, accounted for the germinal line. The smaller cell, the micromere, he thought, gave rise to the somatic cells. Chen (1937) reported unequal cleavage in the first cleavage division of the fertilized egg of *Paragonimus kellicotti*, however, the smaller cell, with its centrally-located nucleolus and "ectosomes", was called the propagatory cell, and represented the germinal line while the larger cell, the ectodermal cell, gave rise to somatic cells. Rees (1940) also reported unequal cleavage, but she referred to the larger cell as the propagatory cell, and the smaller one as the somatic cell.

In this study, it was found that the smaller cell could be distinguished from the germinal line cell in that the former are smaller in size, possess coarse darkly-stained chromatin, and lack a nucleolus. It was found that multiplication of the somatic cells proceeded more rapidly than that of the germinal line cells, resulting in the formation of a mass of cells that varied in size, largely within the limits of 6 - 10 μ. By examining microscopically a number of sections which show germ balls, it was found that a germ ball continues to increase in size. The factors which govern the size of it are not clearly understood. The cytoplasmic boundaries
of the individual cells of the germ ball are not distinct; however, the nuclei of the cells are quite distinct (Fig. 12).

In one series of sections, a cell mass was observed within the parenchymatous wall tissue of a redia. Some of the cells of the mass had a centrally located nucleolus with lightly-stained chromatin granules in the nuclei, while the other cells of the mass were smaller in size and had coarsely-stained chromatin and no nucleolus in their nuclei. This indicated that the mass was composed of germinal line and somatic cells and that it was a germ ball. This same phenomena was observed in another series of sections stained by the Feulgen technique. The germ ball bulged out into the body cavity of the redia (Figs. 13 & 14). This is probably the structure that many investigators in the past have called an ovary. Microscopic examination of the cell mass failed to reveal any maturation figures, although some of the dividing cells were observed in various mitotic phases. Examination of the nuclei of the cells failed to reveal any with the haploid number of chromosomes, and, also, no germinal epithelium was observed in the cell mass indicating that such a mass was a developing germ ball and not a gonad.

In another germ ball consisting of about thirty cells, it was observed that one of the lightly-stained cells had assumed a flattened ellipsoidal shape, being located on the periphery of the cell mass (Fig. 15). Chen (1937) reported such a flattened peripheral cell which appeared in the germ ball of Paragonimus kellicotti, and she claimed that it marked the anterior end of the future embryo.

Germ balls may be distinguished from cercarial embryos by
(1) the presence of the large germinal line cells and smaller somatic cells, both of which are characteristic of germ balls; (2) the differences in staining between the germinal line cells and the somatic cells, germinal line cells containing a darkly-staining nucleolus while somatic cells exhibit a coarsely stained chromatin material but do not have a nucleolus; (3) a thin investing membrane which occurs around the germ ball; and (4) the absence in even the larger germ balls of any arrangement of cells that would indicate any tissue or organ formation.

When a germ ball was examined microscopically, neither maturation figures nor polar bodies were observed, indicating that it was not a sexual organism. Germ balls which develop in the posterior portion of the radial body cavity apparently are transformed directly into cercarial embryos, after detachment from the body wall. Such germ balls did not dissociate into secondary masses as was reported by Brooks for the sporocyst of *Cercaria laruei*. Also, the multiplying cells within the germ balls indicate that they are composed of multiplying germinal line and somatic cells.

*Cercarial embryos.*—An examination of sections of the cercarial embryos showed that cellular differentiation within the embryo had occurred. The shape of the cercarial embryo was ovoid, and a firm investing membrane could be seen surrounding it. In some of the sections of the cercarial embryos dividing somatic cells were observed. Mitotic figures were observed in the nuclei of some of these cells and in a few of these nuclei, the polar view of the
metaphase stage could be distinguished, and, also, the diploid chromosome number (18) for this species could be counted. Anderson (1935) established the chromosome number for this species of trematode.

As might be expected, germ balls did not form from the germinal line cells which were carried over into the cercarial embryos. A mass of small darkly-stained cells appeared in the posterior end of the cercarial embryo. These cells were smaller than either the germinal line cells or somatic cells that had been observed previously and the nuclei were stained very intensely. These cells were interpreted to be the genital primordia of the developing cercaria. Further examination of various serial sections of a number of cercariae showed that these cells give rise to the reproductive organs of the cercaria, and therefore the adult generation of Proterometra. This point was substantiated by later studies of sections stained by the Feulgen technique. Chen (1937) reported that these darkly-stained cells in the cercarial embryos of Paragonimus kellicotti were derived from the multiplication of germinal line cells that came over into the cercarial embryo from the redia.

In the microscopic examination of several sections of cercariae, a structure was observed which was interpreted to be the excretory bladder primordium. This structure appears posterior to the mass of cells representing the genital primordium. The excretory bladder cells possess clear nuclei, some of the cells appearing to elongate, forming a row of cells end to end, on either side of a lumen (Fig. 17). A comparison of various sets of serial sections of cercariae showed
that the genital primordial mass divides, with one of the masses appearing anterior while the other mass appears posterior to the primordium of the excretory bladder. Later studies of sections of older cercariae indicate that the female reproductive organ appears in the region anterior to the excretory bladder primordium, and that the male reproductive organs appear posterior to the primordium. This leads one to infer that the reproductive organs developed from the original genital primordium.

Cable (1931) identified similar cells in the region anterior to what he believed to be the primordia of the excretory bladder in *Cryptocotyle lingua*. He thought that these cells developed into germ cells; however, he was unable to trace them back into the germ cell stage in the redia. Cercarial embryos, in various stages of development, were observed by the present investigator in the body cavities of the rediae (Fig. 10). Embryos that were more advanced in their development showed distinct organs, tissues, and both ventral and oral suckers. These cercarial embryos possessed tails. The cercarial body was not retracted into the tail chamber of the cercaria.

**The Cercaria**

The first morphological evidence of sexuality in the life cycle of *Proterometra macrostoma* was observed in the cercaria. Spermatogenesis and spermiogenesis were observed in longitudinal sections of both young and fully developed cercariae that were still within the redia (Fig. 18). Ova were observed in sections cut through the ovary (Fig. 19).
Young cercariae continue to grow and move about within the body cavity of the redia; however, because of pressure, the birth pore of the redia eventually ruptures, allowing the cercariae to escape into the tissues of the snail. Finally, after a brief period within the snail tissue, during which time other cercariae are being released from the redia, the wall between the body cavity and the mantle chamber of the snail ruptures. This permits passage of the cercariae to the outside through the mantle cavity.

Cercariae that had emerged from the snail always had their bodies retracted into the tail chamber. The cercaria is able to exist as a free-swimming organism for several days. Fully developed gonads, testes and ovary, were observed in longitudinal sections of the cercaria. The ventral and oral suckers as well as other organs of an adult Proterometra were observed in the cercaria, which apparently only differs from the adult fluke in that the cercaria possesses a tail (Fig. 20).

The Adult Stage of Proterometra macrostoma

Adult stages of Proterometra were obtained by experimentally infecting the green sunfish, Lepomis cyanellus, one of the definitive hosts for Proterometra. Food was withheld from ten green sunfish for two days. On the third day, nine fish were each fed approximately twenty-five cercariae of Proterometra macrostoma. The tenth fish, the control, was fed a normal diet of daphnia and bloodworms. The control and the nine fish that had been experimentally fed were sacrificed and examined for infection with the adult stage five weeks
after the initial feeding with the cercariae. When fed to the fish, the body of the cercaria was retracted into the tail chamber (Fig. 1). The results are indicated in Table 2.

Table 2

<table>
<thead>
<tr>
<th>Tank</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>IX</th>
<th>Con</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cercariae placed in each tank</td>
<td>24</td>
<td>26</td>
<td>25</td>
<td>28</td>
<td>25</td>
<td>27</td>
<td>24</td>
<td>22</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>No. of adult trematodes obtained</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

The morphology of the adult forms was studied from the specimens obtained in the experimental feeding of the fish. It is evident that the number of adult flukes recovered from the fish host was far below the number of cercariae introduced to the tank.

Cytological Observations of the Redia and Cercaria

Cytological preparations of the rediae and also of both young and emerged cercariae stained in Heidenhain's iron-alum haemotoxylin were studied because the observations from this source were to be correlated later with cytophotometric determinations of the DNA in the nuclei of the cells of rediae and cercariae. Since some investigators have called the germinal line cells of the redia true ova, particular attention was given to these cells. No ovary, testes, germinal epithelium, maturation figures, or meiosis were observed in the cytological sections of the rediae, therefore, it was concluded
that the rediae lack sexuality and that they reproduce asexually; however, in the young and older emerged cercariae, evidences of sexuality were quite apparent. Spermatogenesis and spermiogenesis was followed in serial sections of the cercariae. The long slender black threadlike lines in some of the sections are spermatozoa (Fig. 18). In serial sections cut through the ovary, ova were observed (Fig. 19). In the various sections of cercariae in which the gonads were completely developed, stages of maturation and meiosis were observed. Attention was paid to the size and number of the chromosomes, since this cytological information was also to be correlated with later histochemical observations.

**Histochemical and Cytophotometric Observations**

It is known that histochemical staining by the Feulgen technique localizes certain cellular constituents in preparation for cytophotometric measurements. The cytoplasm of the cells of the redia and cercaria that have been stained in this fashion appears indistinct, since it is Feulgen-negative and does not stain by this technique. On the other hand, the chromatin portions of the nucleus stain vividly, being Feulgen-positive (Fig. 23).

Cells of various rediae, stained in this manner, were examined in a search for nuclei that might be undergoing maturation division. A search was also made for polar bodies. No evidence of either of these phenomena was observed. In one series of sections, a developing germ ball showing distinctly the unequal cleavage of cells was observed still within the walls of the redia (Fig. 14). Neither maturation nor
polar body formation was observed in the cells of this germ ball. In serial sections of Feulgen-stained rediae in which the germ balls were usually observed within the body cavity of the redia, there was no evidence of any structures that would suggest sexuality. These results are in agreement with those secured when the cytological preparations were examined.

Again, the first evidence of bisexuality was observed in longitudinal sections of young cercariae still within the radial body cavity. Spermatogenesis could be followed clearly in serial sections of the testes of Feulgen-stained young and emerged cercariae (Fig. 21). Spermiogenesis, however, could not be followed so well in the Feulgen-stained sections of the cercariae as in the cytological preparations. Spermatozoa could not be detected in these sections, in spite of the fact that spermatogonia and spermatocytes were clearly visible. Sets of serial sections from cercariae that had been Feulgen-stained were examined carefully in an attempt to follow oogenesis; however, it was not followed so well as spermatogenesis in the sections. This was to be expected since in the development of the gonads of the trematode, the testes develop before the ovary.

Non-hydrolyzed sections stained in Schiff's reagent (leucofuchsin) served as control sections, these sections gave a Feulgen-negative reaction. The distinctness of the chromatin material was completely lost in such sections, since hydrolysis is required to release the aldehydes of the deoxyribose sugar of DNA, and it is these aldehydes that stain purple (Fig. 22). On the other hand, hydrolyzed sections of cercariae gave excellent Feulgen-positive results (Fig. 23).
Sections stained by the Feulgen technique gave further support to observations made earlier in this study that the reidia exhibit no evidence of sexuality. Neither Laurer's canal nor any structures that would suggest bisexuality were observed in the reidia stained by this technique. Furthermore, no evidence of meiosis was observed in the cells of any of the reidia. Once more, evidence of sexuality appeared first in the developing cercarial embryos, in which fully developed gonads were observed. Spermatogenesis was observed occurring in both young and emerged cercariae that were stained with this technique.

Cytophotometric measurements.- Quantitative determinations of deoxycytomidinucleic acid in the nuclei of cells of both reidia and cercariae were made after the cells had been stained by the Feulgen technique. The quantitative determination of DNA in the nucleus of a cell is based upon the amount of absorption of a single beam of monochromatic light of the visible spectrum by the dye content of the Feulgen-stained nucleus. By means of this technique, the range of DNA in the nuclei of cells of a tissue can be computed, thus enabling the investigator to draw certain inferences concerning the type of tissue he is investigating.

The monochromator that was used in this study was calibrated in terms of millimicrons; therefore, these units will be used in referring to the various wave lengths that were used. Wave length$_1$ ($\lambda_1$), at the 475 m $\mu$ band of the visible spectrum, and wave length$_2$ ($\lambda_2$), at 525 m $\mu$ band, were the wave lengths selected for use in this study. Wave length$_1$ has an extinction factor (E) of 0.194 while wave length$_2$
has an extinction factor of 0.388. These extinction values satisfy
the requirements of Patau, who devised the method of making
determinations of DNA by employing two different wave lengths of
monochromatic light of the visible spectrum.

A primary spermatagonium in the longitudinal section of the
testis of an emerged cercaria was selected for the preliminary
measurement of the amount of DNA in its nucleus. The apparatus was
adjusted so that the photometer gave a reading of 100. When the
apparatus is set at this point, deviations from this value can be
read easier and with more accuracy. A blank slide without an object
in the field was measured for the transmission of light. At the
475 m u band of light, the reading was 92.3, which indicated that the
monochromatic light beam had passed through the blank slide with a
minimal amount of light absorption, although, as has been seen, a
small amount of light was lost due to (1) the refractive index of the
mounting medium; (2) the thickness of the slide and cover glass; and
(3) the size of the field area, which in this case was 1.1 u in diameter.

When the object (a stained nucleus of a primary spermatagonium)
was placed in the light field, transmission through the object was
recorded at 61.0. It is known that light transmission does not vary
directly, but, rather, that it decreases logarithmically with a
linear increase in absorbing molecules. The above reading indicated,
therefore, that some of the light was absorbed by the dye particles
that were in the stained nucleus.

The field area was next reduced to 8 u in diameter and measure-
ments of light absorption were made at the wave length band 475 m u.
A transmission of 91.4 was recorded for the blank slide while a reading of 69.8 was recorded for transmission through the stained nucleus of the primary spermatogonium.

The wavelength band was shifted to 525 mµ, and measurements were made at the field areas 1µ and 8 µ, respectively. At the field area of 1µ, transmission through the blank was recorded at 92.5, while transmission decreased to 69.5 when the light beam passed through the stained nucleus of the primary spermatogonium. In the field area of 8 µ, transmission was recorded at 92.6, while transmission through the stained object was recorded at 27.2.

The mean amount of Feulgen dye in the nucleus of a single primary spermatogonium, computed in average arbitrary units at the field area of 1µ, was 7.05. At the field area of 8 µ, the mean average of Feulgen dye in the nucleus was computed to be 6.58. The data compiled in these measurements are recorded in Table 3.
Table 3. Two determinations (with replications) of the amount ($\gamma$) of Feulgen-dye (in arbitrary units of a section of the nucleus of a primary spermatogonium (fixed in mercuric chloride and alcohol). Data obtained at two different field areas, 8 u and 14 u.

<table>
<thead>
<tr>
<th>$\gamma_1$ = 475 mu</th>
<th>$\gamma_2$ = 525 mu</th>
<th>(T_1)</th>
<th>(I_{11}/I_{10})</th>
<th>(L_1)</th>
<th>(T_2)</th>
<th>(I_{21}/I_{20})</th>
<th>(L_2)</th>
<th>(Q)</th>
<th>(C)</th>
<th>(T=HL_1C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reading of galvanometer</td>
<td>with nucleus</td>
<td>without nucleus</td>
<td>(l = T)</td>
<td>Reading of galvanometer</td>
<td>with nucleus</td>
<td>without nucleus</td>
<td>(l = T)</td>
<td>from Table 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>61.0</td>
<td>92.3</td>
<td>60.8</td>
<td>91.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>61.2</td>
<td>91.0</td>
<td>61.1</td>
<td>90.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>122.2</td>
<td>183.3</td>
<td>0.670</td>
<td>0.330</td>
<td>98.7</td>
<td>183.1</td>
<td>0.538</td>
<td>0.462</td>
<td>1.400</td>
<td>1.527</td>
<td>6.65</td>
</tr>
<tr>
<td>60.8</td>
<td>91.3</td>
<td>61.1</td>
<td>90.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>121.7</td>
<td>182.2</td>
<td>0.669</td>
<td>0.331</td>
<td>99.7</td>
<td>185.5</td>
<td>0.534</td>
<td>0.436</td>
<td>1.345</td>
<td>1.625</td>
<td>7.53</td>
</tr>
<tr>
<td>49.9</td>
<td>91.4</td>
<td>48.8</td>
<td>92.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>98.6</td>
<td>184.0</td>
<td>0.531</td>
<td>0.469</td>
<td>54.7</td>
<td>185.3</td>
<td>0.295</td>
<td>0.705</td>
<td>1.501</td>
<td>1.385</td>
<td>6.49</td>
</tr>
<tr>
<td>48.9</td>
<td>92.5</td>
<td>49.0</td>
<td>92.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>97.9</td>
<td>185.1</td>
<td>0.527</td>
<td>0.473</td>
<td>55.2</td>
<td>185.6</td>
<td>0.299</td>
<td>0.701</td>
<td>1.480</td>
<td>1.412</td>
<td>6.67</td>
</tr>
</tbody>
</table>

mean | 7.05 | 6.58
The results of the photometric measurements shown in Table 3 indicated that a certain amount of the monochromatic light had been absorbed as it passed through the stained nucleus of a primary spermatogonium. It has been pointed out previously in this study that the aldehydes of the desoxyribose sugar of DNA, which are released during the hydrolysis of the sections, will take up the stain, and, also, that these aldehydes represent the chromatin portions of the cell nucleus. This factor accounts for the differences in the transmission that appeared when measurements through a blank slide were compared with measurements through a stained object such as the nucleus of a primary spermatogonium. It further indicates that the size of the field has a bearing upon the transmission of light through a stained nucleus. In this case, as the field area was decreased, transmission readings decreased, due to the fact that light absorbing dye molecules were decreased with the reduction of the field area. The histochemical treatment of the cell nucleus localized the DNA in the nucleus, after which cytophotometric readings provided a means of measuring the mean average of the dye content of the nucleus, these results being expressed in average arbitrary units.

Seventy-nine nuclei of Feulgen-stained cells, representing 15 primary spermatocytes, 21 secondary spermatocytes, 18 germinal line cells, and 25 somatic cells, all selected from sections of different rediae and cercariae, were measured following the procedure outlined in Table 3. All of these nuclei were measured in replication. The data represents a summary of the determinations of the average amount
of Feulgen dye (in arbitrary units) and the range of the dye content of the stained nuclei of various cells in mitosis and meiosis in sections of the redia and cercaria of *Protermetra macrostoma* (Table 4).
Summary of measurements, at different field area, of the amount ($T$) of Feulgen-dye (in arbitrary units) of various cells in sections of rediae and cercaria in mitosis and meiosis. Fixed in mercuric chloride-alcohol.

<table>
<thead>
<tr>
<th>Material</th>
<th>Mean amount of dye content in the cell nuclei</th>
<th>Range of dye content in cell nuclei</th>
<th>Number of nuclei measured</th>
<th>Field area (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Spermatogonia from testis of cercaria (prophase)</td>
<td>6.68</td>
<td>6.58-7.53</td>
<td>15</td>
<td>11 u</td>
</tr>
<tr>
<td>Secondary spermatocytes from testes of cercaria (prophase)</td>
<td>3.11</td>
<td>2.96-3.75</td>
<td>21</td>
<td>8 u</td>
</tr>
<tr>
<td>Germinal line cells from walls of rediae (interphase)</td>
<td>3.48</td>
<td>3.29-3.75</td>
<td>18</td>
<td>16 u</td>
</tr>
<tr>
<td>Somatic cell from rediae (prophase)</td>
<td>6.30</td>
<td>6.21-7.33</td>
<td>15</td>
<td>16 u</td>
</tr>
<tr>
<td>Somatic cells from rediae (interphase)</td>
<td>3.22</td>
<td>3.01-3.46</td>
<td>10</td>
<td>16 u</td>
</tr>
</tbody>
</table>
The results of the measurements of the nuclei of the seventy-nine cells from different redia and cercaria of Proterometra, summarized in Table I, show a remarkable unity in the computed amounts of the regenerated fuchsin per nucleus. Previous investigators working on photometric determinations in plant and animal cells have found a similar constancy in the amount of DNA in the cells they have investigated. However, it appears that the mitotic or meiotic phase of the nucleus will determine, to a large extent, the amount of DNA that is found in it. The prophase stage of both somatic cells and primary spermagonia of the redia and cercaria of Proterometra are in close agreement relative to the mean averages of the dye content of these Feulgen-stained nuclei. The range of 6.21-7.33 of the dye content in the somatic cells in prophase is in agreement with the results of Swift (1950), who placed somatic cells of the mouse in Classes I, II, and III, based upon the amount of DNA in their cell nuclei. According to Swift's classification, the somatic cells in prophase of the redia of Proterometra would be placed in Class II. As might be expected, the somatic interphase cells show a range of 3.01-3.46. This result agrees with the findings of various cytologists who have claimed that DNA reaches its lowest level in the interphase stage of a mitotic nucleus. Following Swift's classification, the somatic interphase nucleus would be placed in Class I.

The germinal line cells, which are neither somatic, nor, in the strictest sense, true germ cells, are usually found in the interphase of mitosis. The range of the mean average of the dye content of
these cells is 3.29-3.375 which closely approximates the range of
the somatic cells in mitotic interphase.

The range of the premeiotic spermatogonia of the cercaria is
6.58-7.53, and therefore these cells must be considered as being
in the prophase stage. Swift classified such cells as Class II
premeiotic sex cells. The secondary spermatocytes of the cercaria
of Proterometra show a range of 2.97-3.27, indicating a decrease in
the amount of DNA in these cells, which is to be expected since these
cells have undergone meiosis. Swift classified such cells as
secondary spermatocytes, with a range of 3.21-3.35.

When Swift measured the spermatids of the mouse, he secured a
range of 1.42-1.68. However, spermatids were not observed in the
Foulgen-stained material in this study, and therefore Swift's
findings in the spermatids of the mouse could not be substantiated
by examination of the Foulgen-stained sex cells of the cercaria of
Proterometra.

In applying cytophotometric techniques to the study of those
cells of Proterometra, which are suspected of being sexual, the
morphology of the cell must be studied carefully, and the meiotic
or mitotic phase designated. By correlating the range of the dye
content of the stained nucleus with the morphological data, it can
be determined whether or not the cell is sexual in nature. For
example, a nucleus that gives a reading in the range 6.21-7.53
indicates this nucleus is in prophase. Morphological examination
would determine whether it is somatic or sexual. On the other
hand, a nucleus that gives a reading below 3.00 would be
considered as a nucleus undergoing meiosis, since the reading indicates a decrease in the DNA of the nucleus. A morphological examination should then be made of the nucleus to see if the structure would support this inference. If it did, such cells would be considered as sex cells, since one finds meiosis only in the sex cells.

It is interesting that morphological observations were confirmed by the application of cytophotometric measurements of DNA in the cell nuclei of the radia and cercaria of Protocometra. Variations in the chromatin material of the nuclei were made visible, and the increase or decrease of DNA was recorded and computed by the use of histochemical methods supplemented by cytophotometry. These methods supply both a qualitative and quantitative measurement of the DNA in the nuclei of cells. Since maturation and mitosis can be detected by these methods, they can be employed in determining whether the larval stages of digenetic trematodes contain sexual structures such as gonads.

The results of the morphological and cytophotometric study of the germinal line cells of the radia of Protocometra are an example of how this method can be applied to a study of the germ cell cycle of any other species of digenetic trematode. The germinal line cells of the radia of Protocometra macrostoma have been suspected as being ovaries by some investigators. Morphological examination of these cells revealed that their nuclei contained a large nucleus, with a centrally located nucleolus, and some of these had "ectosomes". Therefore, neither germinal epithelium nor maturation figures were
observed in these cells. Moreover, the chromatin material was scattered in the nucleus of these cells. All of this morphological evidence pointed to them as being in the interphase of mitosis; therefore, they could not be classified as sexual in nature. Further, when such germinal line cells were followed through a number of sets of serial sections, it was observed that they developed into germ balls within the redial body cavity, these germ balls were observed to later develop into cercarial embryos.

Finally, cytophotometric measurements of the amount of DNA in the nuclei of these germinal line cells indicated a range of 3.29-3.75 for the dye content of the stained cells, which indicated that the cells were in the interphase stage. This information correlated with the morphological evidence indicates that these cells are not sexual, but that they are cells of the germinal line. The cytophotometric measurements of the germinal line cell confirm the findings that this type of cell is in the interphase of mitosis. The cytophotometric findings corroborate the morphological findings that germinal line cells are not sexual. The present investigator believes that the cytophotometric method can be applied to a similar study of other species of digenetic trematodes.
DISCUSSION

Germinal line cells were found within the parenchymatous tissue of the body wall of the reidia of *Proterometra macrostoma*. This type of cell possesses a large nucleus that contains lightly-stained chromatin granules with a centrally located nucleolus. Darkly-stained "ectosomes" were observed in the cytoplasm closely adjacent to the nuclear membrane. These characteristics are similar to those that Chen (1937) found in the propagatory cell derived in the first cleavage of the fertilized egg of *Paragonimus kellicotti*; Rees (1940) observed similar characteristics in the propagatory cell of *Parorchis acanthus*. The characteristics, mentioned above, establish the fact that the germinal line cells of the reidia are probable descendants of the propagatory cell (primordial germ cell) that was derived in the first cleavage division of the fertilized egg of *Proterometra macrostoma*. However, since the sporocyst generation of this species was not obtained in this study, it was impossible to trace the germinal line cells back through the sporocyst generation into the fertilized egg.

The subsequent development of the germinal line cells into germ balls, which were found in the body cavity of the reidia, and the metamorphosis of the germ balls into cercarial embryos, indicates
that the germinal line cells represent germinal lineage with polyembryony.

The germ balls of the redia that developed from germinal line cells have been called ovaries by some investigators. Neither germinal epithelium, polar bodies, maturation figures, nor meiosis were observed in connection with the germ balls of the redia, and therefore this stage does not represent a sexual generation.

Unequal cleavage was observed in the germ balls, with the germinal line cells maintaining their characteristics. A smaller type of cell with coarsely-stained chromatin and no nucleolus was observed in the germ balls. These cells were interpreted to be somatic cells. The unequal cleavage of the germinal line cells takes place rapidly, and the cell mass is completely lacking in organization and at no time is there any suggestion of the formation of the primordia of organs within the germ balls.

The continued mitoses of germinal line cells in the formation of germ balls within the body cavity of the redia apparently exerts some influence upon the somatic processes of this stage. In the older redia there is no pharynx, gut, or gonads, the redia appearing as a mere sac inclosing the developing germ balls. The redia lack any sexuality whatsoever. The germ balls within the cavity of the redia are transformed into cercarial embryos. Since germinal line cells were originally in the germ ball before its transformation into a cercarial embryos, it follows that some germinal line cells are consequently in the cercarial embryos. This is further evidence of germinal lineage. It is interesting to note, however,
that the germinal line cells in the cercarial embryos do not give
rise to germ balls as they did in the previous redial generation.
It is believed that the germinal line cells have passed through
the miracidium-mother sporocyst and rediae into the cercariae.
Probably, due to the physiological age of the germinal line cells
as they came over into the cercariae, the tendency to multiply and
form germ balls within the cercariae has been restrained. The
germinal line cell now in the cercariae undergo their normal
development to produce sex cells and gonads in cercarial embryos.
This is, therefore, the first evidence of sexuality in any of the
larval stages of Proterometra macrostoma.

Many theories have been advanced to account for reproduction
in the sporocysts and rediae of digenetic trematodes. The absence
of any internal budding in the redia of Proterometra macrostoma
suggests that metagenesis cannot be used as an explanation of
reproduction in this species. Moreover, since neither ovaries nor
polar bodies were observed in the redia, the occurrence of partheno-
genesis and likewise heterogony can be excluded. The absence of
any mature sexual organs in the redia eliminates the possible appli-
cation of paedogenesis or polymorphism to reproduction in this stage.
Finally, germinal lineage with polyembryony, as expressed by the
observed actions of the germinal line cells and germ balls, can
account for reproduction in the redia of Proterometra macrostoma.
The relationship of the germinal line cells in the life cycle of
Proterometra macrostoma may be shown graphically by the following
figure, modified from Brooks.
Fertilized egg

Primordial germ cells that pass through the miracidium into the mother sporocyst

Cells of the germinal line continued in the redia

Cells of the germinal line found in the cercaria which becomes the genital cells of the adult

Figure 2. Suggested pattern of germinal lineage in Proterometra macrostoma (after Brooks). On the left, the cells of the germinal line. On the right, tissues that disappear without descendants.
It was impossible to trace the germinal line cells from the miracidium-mother sporocyst into the redia generation, since mother sporocysts were not obtained in this study, and therefore this portion of the figure was drawn in dotted lines.

There was no morphological evidence of sexuality in the redia of Proterometra macrostoma, indicating that this stage reproduced asexually. The cercaria, on the other hand, exhibited sexuality. They possessed fully developed gonads, which contained sex cells undergoing meiosis in the formation of mature ova and spermatozoa. The cercaria of Proterometra possessed all the structures of an adult trematode.

Histochemical methods afforded an excellent technique for use in interpretations of the germ cell cycle of digenetic trematodes. By a technique of hydrolysis, the chromatin material was localized, after which variations of the chromosomes were followed in Feulgen-stained sections. Microscopic examination of Feulgen-stained rediae gave support to observations that the redia of this trematode is asexual, and that germinal lineage with polyembryony accounted for reproduction in the redia.

One important point which stands out in the cytophotometric study of redial and cercarial cells of Proterometra is that the quantitative results secured by cytophotometric measurements complement the data secured from the morphological examination of the cells. Morphological examination of the cells gives a clue as to the status of the cell, and cytophotometric measurements of the DNA in the nuclei established the category of the cell.
The controversy of the method of larval reproduction in digenetic trematodes revolves around the question of the presence or absence of maturation figures or polar bodies in the larval stages of the life cycle. Cytophotometric measurements confirm the absence of these structures and suggest that sexuality does not exist in the larval forms of Proterometra macrostoma. The results secured in the cytophotometric study of various cells of the redia of Proterometra indicate that this technique is useful in studying the germ cell cycle, especially when this method is correlated with morphological data secured by examination of the cells.

Quantitative measurements of DNA in the cells of the redia have shown that meiotic cells have less DNA in their nuclei than premeiotic sex cells. These results were evident in the study of the primary spermatogonia when compared to the secondary spermatocyte. There was a decrease in the DNA content of the nucleus in the spermatocyte of the cercaria. This is further evidence that cytophotometry complemented by morphological examination can be used as a method in the study of the larval stages of trematodes in determining their sexuality.

Patau's method, which utilizes two different wavelengths of monochromatic light, is well adapted to the type of cell analysis necessary in studies of the germ cell cycle of digenetic trematodes. The present investigator realizes that the cytophotometric phase of this investigation is a pilot study to determine its potential for use in the analysis of cells of the larval stages of digenetic
trematodes. The results of the studies by other investigators of smears and sections of various animal and plant tissue, together with the results of this study on the cells of rediae and cercariae, emphasize the utility of this method for studying the germ cell cycle of digenetic trematodes.

SUMMARY

1. The literature on the germ cell cycle of digenetic trematodes was reviewed.

2. Germinal lineage with polyembryony can be applied to the germ cell cycle of Prouterometra macrostoma, as has been shown by the study of the redia and cercaria of this species.

3. The redia contain germinal line cells within the parenchymatous tissue of the posterior wall of the body cavity.

4. The germinal line cells of the redia develop into germ balls within the parenchymatous tissues of the radial body wall, after which they become detached and continue further development within the cavity of the redia.

5. The germ balls of the redia develop directly into cercarial embryos; there is no dissociation of the germ balls into secondary germ masses.

6. Neither maturation figures nor polar bodies were observed in the cells of the redia.

7. The redia of Prouterometra macrostoma shows no sexuality, and exhibits no evidence of sexual maturity.

8. The cercaria of Prouterometra is bisexual, spermatogenesis and oogenesis are observable in the testes and ovary, respectively, of the cercaria.

9. Histochemical techniques in conjunction with cytophotometric determinations of DNA in the nuclei of the cells of the redia and cercaria of Prouterometra macrostoma have shown that both methods can be applied to studies of the germ cell cycle of digenetic trematodes.
10. The method of Patau, which utilizes two different wavelengths of monochromatic light of the visible spectrum, has been adapted to studies of the irregular-shaped cells encountered in investigations of the germ cell cycle of digenetic trematodes.
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BIBLIOGRAPHY (continued)


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EXPLANATION OF PLATES

All line drawings were made with the aid of a Tri-Simplex microprojector. Microphotographs were made with a Leitz Panphot.

Abbreviations used

- acetabulum
- body cavity
- cercaria
- cirrus sac
- egg
- ectosome
- excretory bladder primordium
- female genital primordium
- germinal line cell
- genital primordium
- mammilation
- male genital primordium

- mitotic cell
- ovary
- oral sucker
- opening of tail cavity
- ovary
- ovum
- excretory bladder primordium
- flattened peripheral cell
- stalked cell
- somatic cell
- spermatozoa
- tail
- testis
- vitellaria

Fig. 1. Mature cercaria showing distome retracted into tail chamber.
Fig. 2. Diagram of adult showing principal structures.
Fig. 3. Longitudinal section of a redia showing young cercaria and embryos in body cavity.
Fig. 4. Section of redial wall showing germinal line cells in parenchymatous tissue.
EXPLANATION OF PLATES (continued)

Fig. 5. Longitudinal section of developing cercarial embryo showing differentiation of cells.

Fig. 6. Longitudinal section of young cercaria showing principal organs and structures.

Fig. 7a. Germinal line cells showing centrally located nucleolus.

Fig. 7b. Germinal line cells showing centrally located nucleolus.

Fig. 8. Mitotic division of germinal line cell, forming a somatic cell and a germinal line cell.

Fig. 9. Somatic cell of a cercaria.

Fig. 10. Longitudinal section of a redia with developing cercaria and several embryos in various stages of development. X60.

Fig. 11. Section of redial body wall showing germinalline cells with centrally located nucleolus. Ectosomes show faintly in some of the cells. X1000.

Fig. 12. A germ ball of a redia showing germinal line cells and somatic cells. Two dark spots at upper right are artifacts. X1000.

Fig. 13. Germ ball developing in parenchymatous wall of a redia. Germinal line cells can be seen, some cells show mitotic figures. X1275.

Fig. 14. Germ ball developing in the wall of a redia, stained by Feulgen technique. Unequal cleavage of cells can be seen. X1000.

Fig. 15. Germ ball transforming into a cercarial embryo, flattened peripheral cell is seen at lower right side of germ ball. X1275.

Fig. 16. Isolated cells of a germ ball prepared by the smear technique. Mitotic figures can be seen indistinctly. X2000.

Fig. 17. Longitudinal section of developing cercaria showing genital primordia and primordium of excretory bladder. X720.

Fig. 18. Section of a young cercaria, developing within the redial body cavity, cut through the region of the testis. Spermatogenesis and spermiogenesis can be seen. The slender black threadlike structures are spermatozoa. X700.

Fig. 19. Section of young cercaria, developing within the redial body cavity, cut through the region of an ovary. Oogenesis can be seen. X1000.

Fig. 20. Longitudinal section of a free-swimming cercaria with distome retracted into tail chamber. X60.

Fig. 21. Section of testis of cercaria stained by Feulgen technique. Spermatogenesis can be seen. X810.
EXPLANATION OF PLATES (continued)

Fig. 22. Longitudinal section of a free-swimming cercaria, showing distome body retracted in tail chamber. This section was not exposed to acid hydrolysis, but was later stained with leucofuchsin. Note the indistinctness of cellular structure. X60.

Fig. 23. Longitudinal section of free-swimming cercaria, showing distome body retracted in tail chamber. This section was exposed to acid hydrolysis, then stained with leucofuchsin. Note how distinctly the chromatin material of the cells stands out against the background of the colorless cytoplasm of the cells. X70.
PLATE I

Fig. 1

Fig. 2

Fig. 3

Fig. 4
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PLATE V

Fig. 12

scm cl.

gc cl.
Fig. 13
PLATE VIII

Fig. 14.
Fig. 15
PLATE X

Fig. 17
PLATE XIII

Fig. 20
PLATE XVI

Fig. 23
AUTOBIOGRAPHY

I, Claude Randolph Taylor, was born in New York, N.Y., October 1, 1907. I received my grammar and secondary school education in the public schools of New York City, and my undergraduate training at Tufts College, Medford, Massachusetts, which granted me the Bachelor of Science degree in 1927. I received the Master of Science degree in 1932 from The Ohio State University. I taught in several Negro colleges of the South for a number of years, returning to The Ohio State University in 1953 to resume my graduate studies. While in residence there, I was assistant to Professors J.A. Miller and J.W. Price during the years 1953-1956. I am presently employed as Assistant Professor in the Department of Microbiology, College of Medicine, Howard University, Washington, D.C.