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Studies on the Morphology and Growh Rate of the Cestode Hymenolepis Microstoma After Gamma Irradiation of the Cysticercoids and Eggs

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To the Graduate Council:

I am submitting herewith a dissertation written by Huldrich H. Kuhlman entitled "Studies on the Morphology and Growh Rate of the Cestode Hymenolepis Microstoma After Gamma Irradiation of the Cysticercoids and Eggs." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in .

Dr. Arthur W. Jones, Major Professor

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To the Graduate Council:

I am submitting herewith a thesis written by Huldrich H. Kuhlman entitled "Studies on the Morphology and Growth Rate of the Cestode Hymenolepis Microstoma After Gamma Irradiation of the Cysticercoids and Eggs." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Zoology.

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STUDIES ON THE MORPHOLOGY AND GROWTH RATE OF THE CESTODE *HYMENOLEPIS MICROSTOMA* AFTER GAMMA IRRADIATION OF THE CYSTICERCOIDS AND EGGS

A Dissertation

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

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by

Huldrich H. Kuhlman

August 1961

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This dissertation is dedicated to my wife for her understanding and unselfish cooperation.

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I. INTRODUCTION

This study was undertaken in an attempt to determine some of the morphological effects of gamma-irradiation on the cestode, Hymenolepis microstoma (Dujardin, 1845). The cysticercoids of the cestode in the intermediate host, Tribolium confusum, were irradiated with gamma rays. Mice were experimentally infected with irradiated cysticercoids by way of a stomach tube. After the cestodes had developed to maturity in the definitive hosts, the hosts were sacrificed and the cestodes were removed by autopsy and prepared for morphological study.

The adult cestode seems excellent material for radiation study both in cytology and morphology. According to Wardle and McLeod (1952) the general features of a cestode show that several regions should prove sensitive to irradiation. The short neck region is a place of rapidly dividing cells where new proglottids are constantly being formed; growth and maturation of the young proglottids involve further cell division. L. B. Russell (1954) pointed out that embryonic tissues are especially sensitive to low doses of irradiation since, in them, growth is rapid. One might expect that irradiation damage to cestode cells would not differ greatly from that in higher forms. Rather high doses of gamma irradiation were used since it was pointed out by Bloom and Bloom (1954) that going down the scale of animal (and plant) complexities the forms become increasingly resistant. Nevertheless, the kinds of cellular changes seen in vertebrates also occur in lower forms, although the amount of irradiation necessary to elicit these changes in the latter is usually much greater. Each proglottid of the cestode may be considered

as an individual unit and since each cestode is composed of a large number of proglottids, much information may be gathered from a single cestode.

A survey of the literature reveals that work on irradiation of the helminths is rather limited and scattered. Perhaps the most extensive work has been done on Trichinella spiralis. Tyzzer and Heneiji (1916) used radium irradiation on the surface of the abdomen of rats infected with fully developed T. spiralis. They found that surface radiation did not injure the Trichinella. The worms appeared well developed and persisted longer than worms not irradiated in control animals. However, similar treatment from the second day after the ingestion of cysts apparently resulted in a retardation of development, 30 per cent of the females being immature. Levin and Evans (1940) subjected T. spiralis larvae digested out of rat muscle to various intensities of X-irradiation. Larvae exposed to 20,000 r of irradiation developed, but the embryos retained in the females were retarded and injured. It was evident that irradiation had selective action on the germ cells, and that it affected them in such a way as to interfere with subsequent development. Evans, Levin and Sulkin (1941) demonstrated that a dosage of 5,000 r inhibited the development of T. spiralis larvae. Levin and Evans (1942) demonstrated that T. spiralis larvae treated "in vitro" with 3,500 r of X-irradiation would grow to maturity in the intestine of rats but would produce no offspring. Apparently the developing embryos were damaged beyond the point of recovery.

Alicata and Burr (1949) subjected trichinous rat meat to gamma rays at the rate of 2,000 r per twenty-four hours at 4° C. There were increased and variable deleterious effects on the reproductive cells of the adult female worms with increased dosage. Meat irradiated for six days resulted in the sterility of about 60 to 100 per cent of the females. Irradiation produced no lethal effect on the larvae nor did it affect their power to develop to the adult stage. These results show that gamma irradiation at the dose intensity and temperature applied is not lethal to Trichinella larvae encysted in meat, but under proper dosage renders the female worms sterile and unable to complete their life cycle. Alicata (1951) exposed infected rat meat to various doses of X-irradiation. Larvae exposed to 15,000 r to 20,000 r reached maturity in the intestine of the host. No larvae reached maturity following irradiation at 30,000 r. Some larvae receiving 100,000 r to 600,000 r were found alive in the intestinal tract of the host twenty-four hours after infection. The power of any trichina larvae to become established and develop in the intestine of the host was completely destroyed by exposure to 700,000 r of irradiation. Some of the morphological changes evident were: shrinkage and degeneration of the ovary, inability of egg cells to undergo complete cleavage, production of cuticular thickenings in the body wall, and stunted growth. Gould, Van Dyke and Gomber (1953) exposed Trichinella larvae to X-irradiation ranging from 3,500 r to 750,000 r. It was found that their results agree with those of previous workers when the irradiated larvae were injected into a suitable host.

Cook (1939) demonstrated that Ascaris eggs irradiated with X-rays would not embryonate if allowed to develop at 25° C. soon after fertilization, but if these eggs were kept inactive for several weeks by refrigeration before being allowed to develop they would embryonate. Bachofer and Phal (1955) irradiated eggs of Ascaris lumbricoides suum with X-rays which produced a cleavage delay and interference with normal embryogenesis. When the irradiated eggs were refrigerated at 0° C. to 5° C. for periods up to thirty-five weeks and subsequently incubated at 30° C. in order to initiate cell division, the cleavage delay remained unchanged. Survival (completion of embryogenesis) shows a continuous decrease over a period of thirty-five weeks of post irradiation exposure at 0° C. to 5° C. on subsequent incubation at 30° C. This decrease in viability becomes evident after the first month of storage at low temperatures.

Babero (1952) showed that eggs of Ascaridia galli (Schrank, 1788) which had received doses of X-rays ranging from 20,000 r to 40,000 r were retarded in development and showed abnormal cleavage. Morphological abnormalities were more varied and pronounced in eggs exposed to 20,000 r to 40,000 r than in those exposed to 5,000 r to 10,000 r of irradiation. No size difference was apparent in larvae developed from irradiated eggs and from non-irradiated eggs.

Attempts by Katz (1956) to induce successive free-living generations of Strongyloides papillosus by irradiation of the sperm and/or ova were not successful. The major part of the work was done by the use of cobalt^{60}, although X-rays were also used. Extensive data were accumulated

concerning immediate effects of irradiation. As the dosage increased there was a decrease in the percentage of hatching and survival of larvae. Cleavage rate and development of embryos were delayed. Spermatozoa were still active in the uteri five hours after exposure to 20,000 r. Killing effects of 100 per cent occurred in gravid females subjected to 20,000 r and in bisexuals subjected to 40,000 r. The lethal effects increased with increasing dosage.

Ciordia (1957) studied the effects of X-rays on Trichostrongylus axei. Larvae were exposed to X-irradiations ranging from 1,000 r to 20,000 r. Larvae exposed to 5,000 r showed an increase in infectivity rate. A dose of 10,000 r had no obvious effects on the larvae and those receiving 20,000 r failed to reach maturity.

From the literature review it was found that the effect of irradiation on cestodes has been greatly neglected. Palais (1933) studied the morphological effects on Hymenolepis diminuta developed from eggs which had been subjected to X-ray and ultra violet rays. There were no visible changes in the cestodes which could be attributed to effects of irradiation. Schiller (1957, 1959) used X-irradiation as a mechanism for facilitating the study of morphological variation in Hymenolepis nana (Von Siebold, 1852). Eggs and cysticercoids which had been subjected to X-irradiation ranging from 5,000 r to 40,000 r were injected into experimental mice respectively. It was found that, within the errors of the experiment, the number of mutations is directly proportional to the dose given. These findings agree with the statements of Lea (1955), Dobzhansky (1955) and Hollaender (1955) that ionizing irradiations are known to

increase greatly the rate at which known anomalies are expressed in a number of organisms. The cestodes probably do not differ from other organisms in this respect. Kisner (1957) subjected white rats infected with H. diminuta to whole body gamma irradiation ranging from 120 r to 720 r. This was done in an attempt to determine the morphological effects of gamma irradiation on the adult rat cestode. At the level used there was no significant difference between irradiated and non-irradiated specimens.

Dvorak (1959) studied the cestode Baerietta sp. from salamanders. Naturally infected salamanders were irradiated with cobalt^{60}, the doses ranging from 800 r to 10,000 r. Worms from infected irradiated and non-irradiated hosts were prepared for cytological study. The results were essentially negative; however, more experimental work should probably be done before reaching a definite conclusion.

Child (1900, 1903), Kerr (1949) and Voge (1952) have made reference to many abnormalities found in non-irradiated cestodes. Many of these factors will be treated later in this study.

The present study is concerned primarily with morphological effects of gamma irradiation on the adult cestode H. microstoma. Effects of irradiation on growth rate were also studied. High doses of irradiation (2,000 r to 50,000 r) were used since it was known that the intermediate host could withstand relatively high doses of gamma rays (Cork, 1957) and that notable damage to parasitic worms is produced only after exposure to relatively high doses of irradiation. All mice infected with cysticercoids which had received 30,000 r or more were negative for cestodes

upon autopsy. The results of this study were derived from adult cestodes developed from eggs and cysticercoids receiving gamma irradiation ranging from 2,000 r to 20,000 r. In this study the following points were to be studied morphologically and analyzed where reasonably possible.

1. Normal growth rate and development of H. microstoma.
2. Effects on growth rate of H. microstoma after gamma irradiation of the cysticercoids.
3. Morphological abnormalities occurring in adult non-irradiated cestodes.
4. Qualitative and quantitative effects on the morphology of H. microstoma after gamma irradiation of the cysticercoids.
5. Qualitative and quantitative effects on the morphology of H. microstoma after gamma irradiation of the eggs.
6. Effects on later generations of H. microstoma after gamma irradiation of the cysticercoids.
7. Effects on later generations of H. microstoma after gamma irradiation of the eggs.

II. MATERIALS AND METHODS

The cestode used in the present study was recovered from Mus musculus (L.) near Chattanooga, Tennessee, in September, 1959. Stock cultures of Tribolium confusum (Duval) larvae established and maintained in the laboratory were used as the intermediate host. Schiller's (1959) method of infecting Tribolium larvae was modified slightly as follows: Discs of filter paper were placed in the bottom of Petri dishes and slightly moistened with 0.85 per cent saline. The gravid portion of the cestodes was spread over the moist filter paper. T. confusum larvae were placed into the dish containing the cestodes for a period of twelve to fifteen hours of feeding time. After feeding, the Tribolium larvae were removed from the Petri dish and placed in a jar containing "enriched" flour. The development of the cysticercoids of H. microstoma in Tribolium larvae is undoubtedly very similar to that described by Schiller (1959) for H. nana. The cysticercoids completed their development in the larvae of T. confusum and survived metamorphosis of this insect. The adult T. confusum beetles remained infected during the remainder of their life. Tribolium larvae were used as the intermediate host in preference to adult beetles because they are more easily infected since they are more voracious eaters and they were easier to handle. Mature cysticercoids were recovered from the hemocoele of the insect larvae after seventeen days by dissection of the larvae in 0.85 per cent saline with the aid of a dissecting microscope. A polyethylene tube 0.030 inches inside diameter attached to a tuberculin syringe was used to transfer the cysticercoids to experimental mice. The mice were slightly etherized and the desired

number of cysticercoids was given each mouse via stomach tube. The mice used in this study were albino "Rockland all purpose" mice secured from Rockland Farms, New City, New York. All mice used in this study were maintained on Purina laboratory chow for rats, mice and hamsters.

The irradiating was done by personnel of the University of Tennessee Atomic Energy Commission Agricultural Research Program, Oak Ridge, Tennessee. The source for irradiation during this study was cobalt^{60}. In irradiating cysticercoids, T. confusum larvae containing mature cestode cysticercoids were placed in small plastic vials 20 to 25 mm. in diameter with a little flour added. These vials were placed in a larger irradiating capsule at Oak Ridge so that the material to be irradiated could be placed very close to the source. In all material irradiated the dosage was between 560 and 566 roentgens per minute until the desired dosage was attained. A similar vial of material which served as control material was prepared but not irradiated. On the day following irradiation experimental mice were separated into groups of five and each mouse was given the desired number of cysticercoids. One group of five mice was infected with non-irradiated cysticercoids to serve as a control group.

In irradiating cestode eggs, gravid proglottids were placed in a small plastic vial with just enough water to cover them and were treated the same as cysticercoids. After irradiation the proglottids were spread on moist filter paper in a Petri dish as described above and Tribolium larvae which had been starved for twelve to fifteen hours were permitted to feed on these proglottids in the manner previously described. A similar vial which served as a control for this part of the study was

prepared but not irradiated. Seventeen days were allowed for the cysticercoids to reach maturity in the Tribolium larvae. The same procedure was followed to infect mice with cestode cysticercoids from irradiated eggs as was used to infect mice with irradiated cysticercoids.

After infection the mice were kept in wooden cages and maintained on Purina laboratory chow for the desired period of time. For one part of this study the experimental mice were sacrificed on the fourteenth day; for the rest of the study they were sacrificed on the twenty-first day after infection. Much difficulty was encountered in removing intact cestodes from the definitive host, due to the cestode's attaching itself in the small bile passages of the host. Therefore the liver and greater part of the small intestine of the definitive host were removed at autopsy and refrigerated in 0.85 per cent saline from one to two hours at approximately 4° C. This chilling effect seemed to relax the cestodes so they could be removed from the bile passages of the definitive host with less difficulty. Upon being freed from the bile passages and removed from the duodenum the cestodes were placed in a Petri dish containing 0.85 per cent saline and again refrigerated at 4° C. for about two hours to completely relax them before fixing. For the sake of record keeping each mouse was given a number in the order in which it was sacrificed and the total number of cestodes recovered was recorded in a table.

For morphological studies the cestodes were fixed in hot (70° C.) Alcohol-Formalin-Acetic (A. F. A.) fixative (50:10:2:40 of 95 per cent ethyl alcohol, commercial formalin, glacial acetic acid, distilled water). Using hot A. F. A. prevents shrinkage and contraction of the cestode.

The cestodes were stored in the fixative for eighteen to twenty-four hours after which they were transferred to 70 per cent ethyl alcohol for permanent storage or until needed for further study. For cytological study some cestodes were fixed immediately after removal in Carnoy's fixative (6:3:1 absolute ethyl alcohol, chloroform, glacial acetic acid). The cestodes were carefully placed on the upper inside wall of a small glass vial and the fixative was gently added without coming in contact with the cestodes. The thumb was placed over the mouth of the vial and the cestodes were shaken vigorously in the fixative for about thirty seconds, and then stored in the fixative for eighteen to twenty-four hours when they were transferred to 95 per cent ethyl alcohol until used or in which they could be kept indefinitely.

To determine the size of the cestodes a twelve inch celluloid ruler with a millimeter scale was used. A small camel's hair brush was used to moisten the ruler and to extend the cestode out on the ruler. Measurements taken by this method are not entirely accurate because of the irregularities of the cestodes, but the probable error is not believed to be significant for the purpose of this study.

Stained whole mounts of the specimens were prepared for study by employing Semichon's acetic carmine (Mackie, Hunter and Worth, 1945). The specimens were transferred from 70 per cent alcohol to Semichon's and allowed to overstain. This usually took from forty-five minutes to one hour, depending on the size of the specimen. They were then transferred to acid alcohol (1 per cent HCl in 70 per cent alcohol) used for destaining. After proper destaining they were passed through a series

of alcohols (70, 80, 95 and 100 per cent respectively) for dehydration. They were kept ten to fifteen minutes in each of the alcohol series. From 100 per cent alcohol they were transferred to carbolxylene (70:30 xylene, phenol) which was used as a clearing agent and which kept the specimens soft and pliable (Gray, 1952). At this point the specimens were flattened between two standard microscope slides and cleared further in xylene for about ten minutes. The xylene also hardened the material. All cestodes prepared for whole mount study were mounted in permount. Three cestodes were prepared for analysis from each mouse sacrificed except in one or two cases where the infection was less than this number. As each cestode was prepared and mounted, the slide was labelled to correspond with the experiment number, control or irradiation dose used, generation of cestodes, mouse number and cestode number (signified by a letter a, b, or c).

The microscope used in studying the morphological characteristics was a Spencer Binocular scope with 10 x wide angle ocular, N. A. 1.25 condenser and 5, 10, 43 and 97 x objectives. All morphological work shown in the data was done with the 10 x objective. The source of light was a 100 watt Burton microscope lamp with a blue filter. For photomicroscopy an Olympus PM-6 photo micrographic camera was used with an Olympus UCE-B IV microscope.

Analysis of variance for samples within samples (Model II, single classification; see Snedecor, 1956) was used to determine if the cestodes varied significantly from radiation dosage to radiation dosage, from mouse to mouse of the same dosage, or from cestode to cestode of the same mouse.

The estimate of the percentage of binomial to total variation in the number of abnormalities of the cestodes was found to be less than 30 per cent. Therefore equal weights were employed for the analyses on which tests of significance were based (Cochran, 1943).

III. DESCRIPTION AND LIFE HISTORY OF HYMENOLEPIS MICROSTOMA

In September, 1959, Minnick and Filler (1960) discovered the cestode H. microstoma (Dujardin, 1845) in Mus musculus (L.) near Chattanooga, Tennessee. A literature review indicates that this is a rather rare cestode, having been reported only once before in North America (Harkema, 1946). According to Joyeux and Kobozieff (1927), H. microstoma was first discovered in France by Dujardin in 1845; his description is rather short, but nevertheless sufficient for identification of the cestode. Several specimens are preserved in the Zoological Museum in Berlin. Joyeux and Kobozieff (1928) recognized it in a colony of white and black mice and apparently were the first to use it in experimental research. These authors have reported its distribution and occurrence as follows:

Locality	Author	Form
Rennes (France)	Dujardin	adult
Lille (France)	Moniez	larva
Froyes (France)	Joyeux	adult and larva
Potsdam (Germany)	Stein	larva
Berlin	Janicki	adult
Catane (Sicily)	Grassi and Rovelli	larva
Beni-Ounif (N. Africa)	Joyeux	adult

In addition to the above mentioned localities the cestode has been found by Meggitt and Subramanian (1927) in Rangoon, by Bacigalupo (1928a) in Argentina, and by Baylis (1934) in Tanganyika. Harkema (1946) reported it from North America.

H. microstoma (Dujardin) has been found over an extensive geographical range and can live in a relatively cold climate, a subtropical climate or the dry hot climate of the Sahara region. Moreover, its cysticercoids can undoubtedly develop within more species of insects than have been listed by Joyeux and Kobozieff (1927, 1928). The infection of the intermediate as well as the definitive host presents no difficulties. It would therefore seem that the percentage of parasitized animals in nature would be great and the cestode would thus be common. However, since this is not the case the distribution and apparent rareness of H. microstoma in nature appears unexplainable. With the ease of propagation and the high infectivity maintained in the laboratory, it would seem that the cestode would be found frequently; however, it is very rare. The life cycle of H. microstoma has been fairly well described by Joyeux and Kobozieff (1928); moreover, it has been reaffirmed and the growth pattern in the definitive host has been established (Dvorak, Jones and Kuhlman, 1960).

HABITAT

H. microstoma is unique in its location in the definitive host. The cestode inhabits the bile duct and duodenum. In all infected mice examined, all but two cestodes were found attached in the bile passages of the host. The most common place of attachment was the first 4 or 5 mm. of the bile duct; however, a number were found to have attached themselves in the smaller lateral ducts within the lobes of the liver and in the cystic duct leading to the gall bladder. From the point of

attachment the cestodes may extend in two different directions, either further up into the bile passageways or gall bladder and looping back over themselves and extending toward the duodenum or they may extend down the bile duct directly into the duodenum. In two instances the posterior proglottids of the cestode, and in one instance a complete cestode, occurred in the gall bladder of the definitive host. In infected mice it was noted that the bile duct became greatly distended because of the infection; in many cases its diameter was found to be as large as, or larger than the duodenum, especially in heavy infections. Upon autopsy, in the latter case, the bile duct was found to be packed with cestodes to the point of appearing to be completely obstructed. Generally the gall bladder was slightly enlarged, the bile content being about the same as in non-infected mice; however, in a few cases there was a creamy substance in the bile. In most cases the liver presented no visible manifestations due to the infection, but occasionally, especially in infections of twenty-one days or more, small white spots were found on the surface of the liver. Whether these were due to the cestode infection or not has not been determined in our laboratory. Kobozieff (1933) referred to these spots as a cancerous condition and suggested the infection as a cause. However, of 293 non-cancerous "Waltzing" mice used in his experiments a larger proportion of tumors developed in 146 which were not infected than in 143 mice artificially infected with H. microstoma. Dobrovolskaia-Zavadskaia and Kobozieff (1927) in describing the liver condition of infected mice pointed out that sometimes the liver presents no visible manifestations, while at

other times it is increased somewhat in volume and becomes yellowish in color. In some situations they found on its periphery small white spots, drawn out or confluent. In one mouse the inferior surface of the left lobe contained white sinuses which were caused by cestodes enclosed under the capsule of the organ. Microscopic examination revealed the presence of parasites in the substance of the liver itself. In two cases H. microstoma in the bile passageways and Cysticercus fasciolaris (Taenia crassicollis) in the liver tissue were coexistent. Another phenomenon which was characterized by the appearance of white spots on the surface of the liver was necrobiosis of hepatic parenchyma. In the places where this process started, pale nuclei were found in the hepatic cells and sometimes, in addition, small vacuoles.

DESCRIPTION OF ADULT WORM

The adult cestode may reach a length of 280 mm. as was found to be the case in one single infection; with heavy infections the cestodes are usually much smaller, ranging from 115 mm. to 175 mm. The scolex measures 190 μ to 217 μ in width. It is equipped with four suckers and a single row of rostellar hooks numbering from twenty-three to twenty-eight. The rostellar hooks appear to be very similar to those of H. nana but with a shorter handle. The neck is about 600 μ to 900 μ in length. The individual segments are much wider than long. Gravid proglottids, for example, vary from 800 μ to 1000 μ in width and from 250 μ to 350 μ in length.

The eggs have a very characteristic appearance and one who is not familiar with them can easily mistake them for H. nana eggs, to which they are very similar in every respect except size. H. microstoma eggs range from 75 μ to 90 μ in length as compared with 40 μ to 50 μ for those of H. nana. The eggs of cestodes develop within themselves small spherical embryos characterized by the presence of three pairs of claw-like hooks. The fully developed embryo, which grows no more until ingested by a suitable host, is known as the oncosphere. It is surrounded by a lemon-shaped membrane (embryophore) which has a small knob on each end from which arise three to six polar filaments. The embryophore is surrounded by the egg shell proper which is oval in shape and practically colorless.

The testes normally number three per segment, one poral and two antiporal. The antiporal testes may lie either in a straight line with the poral one, or they may lie one slightly in front of the other depending on the state of contraction of the cestodes. The testes are slightly oval in shape and measure from 70 μ to 130 μ along the long axis.

All of the above measurements agree quite well with those of Joyeux and Kobozieff (1928) with the exception of the size of the proglottids which they describe as considerably smaller and the testes size which they gave as 120 μ to 170 μ which is somewhat larger than the testes of our specimens. It is the opinion of the writer that the cestode described by Joyeux and Kobozieff and the one used in the present work are two varieties of the same species.

LIFE CYCLE

The life cycle of H. microstoma has been worked out experimentally using T. confusum larvae as the intermediate host. The Tribolium larvae were experimentally infected as explained elsewhere in this paper. The cestode egg hatches and the onchosphore penetrates the intestinal wall of the larva and can be recovered from the hemocoele fifteen to twenty hours after feeding. In the hemocoele the cysticercoid reaches maturity in eight to seventeen days depending on the temperature, after which no essential growth occurs until ingested by the definitive host. The cysticercoids survive ecdyses and metamorphosis of the Tribolium larvae and the adult insect remains infected for the remainder of its life. The cysticercoids of H. microstoma resemble those of H. nana quite closely. Their length ranges from 125 μ to 190 μ with a caudal appendage, or cercomer, 165 μ to 1500 μ in length. Some abnormally long cercomers have occasionally been found on cysticercoids recovered in single infections of old Tribolium cultures. The cysticercoids of this cestode have a pear-shaped appearance with the embryonic hooks located in the caudal portion. The anterior portion contains the rudiments of the four future suckers and the rostellum with the fully developed rostellar hooks of the scolex. These two portions are joined by a narrow stalk and in the definitive host the caudal portion is lost and from the anterior portion the strobila will develop. The definitive host becomes infected in nature by ingesting cysticercoids. Infection was produced experimentally by dissecting mature cysticercoids from the hemocoele of insect larvae in 0.85 per cent saline under a dissecting microscope and injecting them into the

definitive host by way of a stomach tube. Forty-eight hours after introducing the cysticercoids into a mouse, young worms were found in the upper region of the small intestine of the mouse. After ninety-six hours young cestodes appeared in the bile duct of the mouse. Having reached this location the cestodes grew and developed rapidly and began producing eggs in about twelve to fourteen days. Experimentally infected mice usually pass eggs in the feces fourteen days after they have been infected. When experimental mice containing mature cestodes were sacrificed, small chains of gravid proglottids were found in the lower part of the small intestine, indicating that H. microstoma sheds mature proglottids which are expelled in the feces. In nature the intermediate host becomes infected by feeding on egg infected mouse droppings and the mouse becomes infected by ingesting infected beetles which serve as an intermediate host.

Autoreinfection does not occur in H. microstoma. On two occasions a single mouse was heavily infected with H. microstoma eggs with a stomach tube. Each mouse was sacrificed eighteen days after infection and there were no signs of the mouse's harboring cestodes. There has been no evidence of accidental autoreinfection in our laboratory. During the course of this work all cestodes recovered from experimentally infected mice were approximately of the same size and stage of development in each mouse with the exception of one heavily infected mouse in which severe crowding effects were evident.

One mouse was experimentally infected with one hundred cysticercoids to demonstrate crowding effects. This mouse was sacrificed fourteen

days after infection. The condition of the bile duct and duodenum showed no greater enlargement than in infections of five to ten cestodes. The cestodes were recovered and were found to be much smaller, measuring from one-tenth to one-fourth the size of those recovered from mice harboring five to ten cestodes. Ninety-three cestodes whose length varied from 10 mm. to 30 mm. were recovered from this heavily infected mouse.

It was experimentally demonstrated that H. microstoma and H. nana can exist in the same host simultaneously. Two mice were infected with ten cysticercoids of H. nana and ten cysticercoids of H. microstoma each in an attempt to determine whether one host would harbor both cestodes. Both mice were sacrificed fifteen days after infection with the following results: One mouse was negative for both cestodes, the second mouse harbored ten H. microstoma and nine H. nana. In each case the cestodes were found in their natural habitats within the host. H. microstoma was found in the bile passages and anterior region of the small intestine and H. nana in the posterior region of the small intestine. With the growth region of the two species of cestodes being separated by almost the full length of the small intestine (varying from 35 cm. to 45 cm. in length) it appears that one can develop quite independently of the other.

During the present study H. microstoma has been established and maintained in two strains of laboratory mice. One strain is the albino "Rockland all purpose" strain secured from Rockland farms, the other is a brown strain secured from the Bacteriology Department of the University of Tennessee.

Unsuccessful attempts have been made to establish an infection in white rats, Rattus norvegicus. A total of ten white rats were experimentally infected in pairs on five different occasions. The members of the first pair were given ten cysticercoids each, the second twenty-five cysticercoids each, and the other three pairs one hundred cysticercoids each, by way of a stomach tube. The rats were sacrificed thirty-six, twenty-four, twenty, fourteen and twenty-two days respectively after infection. All rats were negative; the liver, gall bladder, bile duct and intestine were normal and showed no signs of having been infected. Bacigalupo (1928b) tried unsuccessfully to infect two white rats with eggs by making them swallow gravid proglottids. From these observations it is probably safe to conclude that albino rats do not play the part of a definitive host for H. microstoma by either direct or indirect infection.

During this study two golden hamsters, Mesocricetus auratus, were experimentally infected with H. microstoma. Each animal was given twenty-five cysticercoids by means of a stomach tube. The animals were maintained on the same diet as were the mice. Fourteen days after infection one hamster was sacrificed and found to harbor seven cestodes. The cestodes were in the same region as those in infected mice but were about one-third the length of those found in similarly infected mice. At the time the hamster was sacrificed it appeared to be in a poor state of health. Autopsy showed that it was badly jaundiced and that the first portion of the intestine was filled with a yellowish fluid and seemed to be inflamed. The other hamster died sixteen days after infection; it also was found to be infected with H. microstoma and also appeared

to have suffered from jaundice. It seems that although the golden hamster, M. auratus, may harbor an infection of H. microstoma, the cestode has an adverse physiological effect upon hamsters which is unlike its effect upon mice.

DURATION OF INFECTION IN DEFINITIVE HOST

During the course of this work an experiment was carried out to establish the time when the cestode reaches maturity and the duration of time the definitive host may remain infected. Twelve mice were infected with one cysticercoid, each with a stomach tube. The mice were kept in separate cages and the feces of each mouse were examined daily for cestode eggs beginning on the tenth day after infection. All twelve mice began passing cestode eggs on the fourteenth day. Six of these mice were later placed in one cage and were sacrificed at thirty day intervals in an attempt to establish the longevity of the cestode in the host. The two mice sacrificed last were both negative. The last positive mouse of this group was sacrificed five and one-half months after the initial infection. From this observation it can be concluded that a single H. microstoma infection may last from five and one-half to six and one-half months in Mus musculus.

The early development of the cestodes in Mus musculus has been experimentally established during this study. Twelve mice were infected with cysticercoids of H. microstoma; each mouse was given one hundred cysticercoids by means of a stomach tube. The mice were sacrificed in pairs at intervals of twenty-four, forty-eight, seventy-two, ninety-six,

one hundred and twenty, and one hundred and forty-four hours respectively. No young worms were recovered from the two mice sacrificed twenty-four hours after infection; in the forty-eight and seventy-two hour period young worms were recovered mostly from the area of the duodenum below the orifice of the bile duct. In the two mice which were sacrificed ninety-six hours after infection a number of small cestodes had found their way into the bile duct; however, most of the worms recovered were in the region three to six centimeters below the orifice of the bile duct. This seems to indicate that the worms move down past the opening of the bile duct and migrate back up into the bile duct where they establish themselves. It was noticed that the young worms recovered from the bile duct were three to four times as long as those which had not yet reached the bile duct. This would lead one to believe that upon reaching the bile duct there is a very rapid increase in size.

INTERMEDIATE HOST

The intermediate host used throughout the course of this study was the larval stage of T. confusum (Duval). This beetle has never been reported as an intermediate host for H. microstoma but is being used very successfully by numerous workers studying H. nana and H. diminuta at the present time. At the time of the finding of this cestode (Minnick and Filler, 1960) a colony of T. confusum was being maintained in the laboratory which prompted its use as an intermediate host and has proved very successful. T. confusum were infected in the larval stage by spreading gravid proglottids on moist filter paper in a Petri dish

and letting the larvae feed on them overnight. The Tribolium larvae were maintained on "enriched" flour. The cysticercoids are able to withstand ecdyses and metamorphosis of Tribolium. Other beetles which might act as an intermediate host for this cestode were tested. Fifty larvae of Oryzaephilus surinamensis (L.), (saw-tooth grain beetle) were exposed to feed on gravid proglottids in the same manner as described above for T. confusum. After feeding, these larvae were placed in a jar containing grits and granules of laboratory chow. Seventeen days after exposing them to gravid proglottids they were dissected in 0.85 per cent saline under a dissecting microscope and found to be 94 per cent infected. However, since Tribolium larvae are much larger than those of Oryzaephilus and show an equally high infectivity rate they were used in preference to Oryzaephilus larvae. Thirty-five larvae and thirty-five adult Tenebrio molitor (Fab.) were fed on gravid proglottids as described above. They were maintained on oatmeal and shredded paper for a period of twenty-one days. Neither the larvae nor adult beetles were found to be infected with H. microstoma cysticercoids when examined. In the literature, it appears that there are a number of insects which reputedly serve as intermediate hosts for H. microstoma. Contrary to our findings, Joyeux and Kobozieff (1927) reported Tenebrio molitor (Fab.) as an intermediate host and also that they had been able to observe experimentally the development of the cysticercoid in the beetle Geotropus sylvaticus (Panzer). Again, in 1928, they reported that the cysticercoids develop in Tenebrio beetles. However, they were unable to establish them in Tenebrio larvae. They also reported them in Ceratophyllus fasciatus, the rat flea. Bacigalupo

(1928b) stated that the larval stage of H. microstoma had been found in Argentina in the larva of Tenebrio molitor (Fab.) by Swafford. Bacigalupo (1928b) also reported that he had tried to infect forty-one Tenebrio molitor larvae, ten of which he had examined and found to be negative; the other thirty-one had not been examined at the time of his report.

A survey of the literature as well as the present study seem to indicate that the role of an intermediate host can be satisfied by a number of the smaller beetles and the cysticercoid can be easily maintained in them. The problem of the definitive host seems more complicated. Even though the cestode is very easily maintained in Mus musculus and apparently does not seriously harm the mouse, it was found to have rather serious effects on the golden hamster. We have been able to establish H. microstoma in only two definitive hosts in the laboratory. At the present time a project is under way in an attempt to establish H. microstoma in a number of small rodents to see whether they will serve as definitive hosts.

IV. STUDIES ON GROWTH RATE OF *HYMENOLEPIS MICROSTOMA* AFTER GAMMA IRRADIATION OF CYSTICERCOIDS AND EGGS

The following studies of the effect of gamma irradiation on *H. microstoma* were based on specimens resulting from three experiments. Experiment I was a pilot experiment conducted in an attempt to establish a dosage range best suited for subsequent studies; it consisted of two parts. In part one of this experiment three groups of mature cysticercoids in infected *T. confusum* larvae were irradiated at 2,000 r, 10,000 r and 50,000 r respectively. A fourth group of non-irradiated larvae was used for control material. Twenty mice were separated into groups of five. Five non-irradiated cysticercoids were injected into each mouse of one group which served as a control. Each of the remaining three groups was infected with cysticercoids which had been exposed to a particular dose of gamma irradiation. The mice were sacrificed on the fourteenth day after infection and the cestodes were recovered and prepared for morphological study. Part two of this experiment was conducted in the same manner as part one, differing only in the dosage of irradiation which ranged in the following dosages: 15,000 r, 20,000 r, 25,000 r, 30,000 r and 40,000 r. The data for experiment I are shown in Tables I and II respectively. Autopsy showed all mice infected with cysticercoids receiving more than 20,000 r of irradiation were negative for cestodes; therefore, irradiation doses above 20,000 r are not shown in the tables. In experiments II and III irradiation doses of 10,000 r and 15,000 r were used.

In experiment II mature cysticercoids in infected T. confusum larvae were subjected to gamma irradiation doses of 10,000 r and 15,000 r. From these irradiated cysticercoids the P_1, F_1 and F_2 generations of adult cestodes were produced for this part of the study. In experiment III H. microstoma eggs were subjected to gamma irradiation doses of 10,000 r and 15,000 r. From these irradiated eggs the P_1, F_1 and F_2 generations of adult cestodes used for experiment III were produced. A separate control group was used for each experiment. However, in experiment II a supplementary experiment was conducted separately for each part using a dose of 20,000 r and a separate control group.

The purpose of experiment II was to compare growth rate and morphological effects in the parent (P_1) and the F_1 and F_2 generations of cestodes developed from irradiated cysticercoids. In part one of experiment II infected T. confusum larvae containing mature cysticercoids were subjected to gamma irradiation of 10,000 r and 15,000 r respectively. Fifteen mice were separated into three equal groups and infected with five cysticercoids each. The first group of mice received cysticercoids irradiated at 10,000 r and the second group received cysticercoids irradiated at 15,000 r. The third group which served as a control group was infected with non-irradiated cysticercoids. All mice used in experiment II were sacrificed on the twenty-first day after infection. The cestodes recovered from mice of part one are the P_1 adult cestodes. They were fixed in A. F. A. as previously described and representative specimens were prepared for morphological study. Free gravid proglottids recovered from the intestine of infected mice were used to infect Tribolium larvae

for the next generation of cestodes. In order to secure enough material for study and also to infect larvae for succeeding generations of cestodes, individual mice were infected with ten cysticercoids in parts two and three of experiment II and also in experiment III.

In part two of experiment II groups of Tribolium larvae were fed on gravid proglottids obtained from the respective groups of mice of part one. This was done to obtain cysticercoids for the F_1 generation of cestodes. Mice separated into groups of five were infected with cysticercoids from the respective groups of Tribolium larvae. Each mouse received ten cysticercoids. The mice were sacrificed twenty-one days after infection. The cestodes recovered were fixed in A. F. A. and representative specimens were prepared for morphological study. Gravid proglottids recovered from the intestine of infected mice were used to infect Tribolium larvae for the next part of this experiment.

In part three of experiment II Tribolium larvae were fed on gravid proglottids obtained from cestodes of the F_1 generation obtained from each respective group of mice. After the cysticercoids had matured in Tribolium larvae they were removed from their host by dissection. Mice separated into groups of five were infected with cysticercoids from the respective groups of larvae. Each mouse was given ten cysticercoids. The mice were sacrificed twenty-one days after infection and the cestodes recovered were fixed and prepared for morphological study. The cestodes recovered from this part of the experiment are the adult F_2 generation of cestodes. The data on growth rate for parts one, two and three of experiment II are shown in Tables III, IV and V. The data for the

supplementary experiments to experiment II are shown in Tables VI, VII and VIII.

In experiment III, as has already been mentioned, H. microstoma "eggs" instead of cysticercoids were subjected to gamma irradiation. The purpose of experiment III was to compare the growth rates and morphological effects in the parent (P_1), F_1 and F_2 generations of H. microstoma reared from eggs which had been subjected to 10,000 r and 15,000 r of gamma irradiation respectively for the initial infection.

Experiment III consisted of three parts. In part one Tribolium larvae were separated in groups and were fed on eggs which had been subjected to gamma irradiation of 10,000 r and 15,000 r respectively. One group of larvae which served as a control was fed on non-irradiated eggs. Mice separated in groups of five were infected with mature cysticercoids developed from irradiated eggs of the respective doses. One group of mice was infected with cysticercoids from non-irradiated eggs. This group of mice served as a control group. Each mouse was given ten cysticercoids. The mice were sacrificed twenty-one days after infection. The cestodes recovered from this infection are the P_1 generation for experiment III. They were fixed and prepared for morphological study in the same manner as the cestodes in experiment II. For part two gravid proglottids were recovered from the intestine of infected mice and were placed in respective Petri dishes and prepared for Tribolium larvae infection. Mature cysticercoids were recovered from infected Tribolium larvae seventeen days after infection. Mice separated in respective groups of five were infected with mature cysticercoids. Each mouse was

given ten cysticercoids. The mice were sacrificed twenty-one days after infection and the cestodes recovered from these groups of mice were fixed and prepared for morphological study in the usual manner. The cestodes recovered from this part of experiment III represent the F_1 generation of cestodes. For part three of experiment III gravid proglottids were recovered from the intestine of mice harboring F_1 cestodes. The gravid proglottids were placed in respective Petri dishes prepared for Tribolium larvae infection. Mature cysticercoids recovered from these Tribolium larvae were injected into respective mice in groups of five. Each mouse received the usual number of ten cysticercoids. The mice were sacrificed, as usual, twenty-one days after infection. The cestodes recovered from this infection were fixed and prepared for morphological study. The cestodes recovered from this infection represent the F_2 generation of this experiment. The data on growth rate for parts one, two and three of experiment III are shown in Tables IX, X and XI.

Upon statistical analysis, some experimental results were significant, others were not.

For statistical analysis, Tables I and II were combined into one table as indicated in Table XII. It seemed permissible to do this since these two tables are the result of growth rate for experiment I which was done in two parts as already explained. From Table XII one can readily see that there is a decrease in cestode length as the dosage of irradiation increases. It would, therefore, seem safe to conclude that the mean length of the cestodes decreased as the irradiation dose increased. An analysis of variance test confirmed that the mean length of

cestodes receiving different dosages of irradiation varied significantly at the 1 per cent level. This, however, was not found to be the case in experiments II and III.

The statistical results are not quite so consistent in experiments II and III. In experiments II and III the mice were sacrificed twenty-one days after infection and some of the mature proglottids of the cestodes had already become detached from the cestodes and eliminated by the mouse. The comparative average length of cestodes developed from cysticercoids which had been subjected to doses of 10,000 r and 15,000 r respectively, is shown in Table XIII. An analysis of variance test was run on experiment II with the following results. The P_1 generation showed no significant change in cestode length as the irradiation dose increased. In the F_1 generation a change significant to the 1 per cent level was observed. Again in the F_2 generation there was no significant change. It should be noted that in the P_1 generation each mouse received five cysticercoids, whereas in the F_1 and F_2 generations each mouse received ten cysticercoids. This increase in infection was necessary to secure material for the subsequent generation. It is altogether possible that because of a lighter infection in the P_1 generation the cestodes might have matured more rapidly and thus have shed mature proglottids sooner than in the F_1 generation, thereby increasing the variability in length of the worms recovered, a situation which could in part account for our non-significant results.

In the F_1 generation where each mouse received ten cysticercoids the cestodes could have possibly developed a bit slower and not have

reached maturity quite so early as the P_1 cestodes did and thus a more accurate measurement of each cestode might have been attained. In observing Table XIII for the F_2 generation of this experiment, it is noted that one of the control mice died. One of the mice infected with cysticercoids irradiated at 15,000 r also died and one was negative. These factors could easily account for a discrepancy in the F_2 generation.

In experiment III no significant results were obtained on growth rate where eggs of H. microstoma were irradiated at 10,000 r and 15,000 r respectively. In the P_1 generation only enough mice were infected to get material for the F_1 generation. An analysis of variance test which was run on the F_1 generation proved that the variation in growth rate was not significant. In the F_2 generation three of the control mice and two of the mice infected with cysticercoids exposed to 10,000 r of irradiation died. Because of the death of the mice there was insufficient information; consequently no statistical analysis was done on this part of experiment III. The cause of the death of the mice was not determined. It was noted, however, that the mice which died during this part of the experiment seemed to have developed diarrhoeic stools a few days prior to death. This phenomenon was also noticed in mice and rats kept in the embryology and physiology laboratories at the University of Tennessee.

V. MORPHOLOGICAL STUDIES OF HYMENOLEPIS MICROSTOMA AFTER GAMMA IRRADIATION OF CYSTICERCOIDS AND EGGS

In this part of the study a series of investigations was undertaken in an attempt to show something of the nature, extent and directional trends of morphological variations in the cestode H. microstoma due to gamma irradiation. There is no record in the literature that any morphological work of any sort has been carried on with this particular species of cestode. However, it is known that a certain amount of morphological variation is normal for the genus Hymenolepis. In their work with H. diminuta Palais (1933), Voge (1952) and Kisner (1957) found that there were a number of morphological variations normal for this species. In working with H. nana Baylis (1924) reported that this cestode also showed a number of variations. A number of other species of the genus Hymenolepis (Voge, 1952) normally show morphological variations which are normal to the genus. From these reports in the literature, it was concluded that H. microstoma would not deviate from the pattern of morphological variations normal to cestodes. Controls for the experiments were examined to determine the extent of variation for this species. Some of the known variations could not be observed from a study of the gravid region of the cestode. These include such factors as the number and position of the testes and cirrus pouch. Since morphological variation represents an abnormal growth of cells, it was concluded that an injurious agent such as irradiation might change the frequency of such occurrences; therefore, any abnormality consistently appearing more frequently in the irradiated cestodes than in the controls would serve as

an indication that an irradiation-induced change has occurred. According to the literature on radiation biology (Lea, 1947; Dobzhansky, 1955; Hollaender, 1955), ionizing radiations are known to increase the rate at which usual variant characters are expressed in a number of different organisms. X-rays and ultra violet radiation (Dobzhansky, 1957) induce mutations in organisms ranging from plant viruses and bacteria to higher plants, and from protozoans to mammals. Studies with H. nana (Schiller, 1957) indicated that cestodes probably do not differ from other organisms in this respect.

A sample data sheet of the variations observed is shown in Table XIV. It can be seen from Table XIV that the variations studied were grouped under four main categories, namely, abnormal testes development, abnormal cirrus pouch, sterility, and abnormal segmentation. In experiment I two cestodes from each infected mouse were prepared for morphological study and the data recorded on a data sheet. In experiments II and III three cestodes were prepared for morphological study in every case where the infection consisted of three or more cestodes per mouse. From these data sheets tables were prepared for all three experiments for an analysis of variance test.

The results for experiment I are shown in Tables XV, XVI, XVII and XVIII. In studying Table XV one can readily see that there is a gradual increase in the incidence of abnormal testes development as the irradiation dose increases, except at the 20,000 r dose level. At this dose level there was a decrease in incidence in the development of abnormal testes. However, there was also a sudden decrease in cestode

length which may help to account for this decrease. An analysis of variance test shows that these results are significant at the 5 per cent level.

Table XVI shows the incidence of the appearance of abnormal cirrus pouches in the control worms is greater than that of those developed from cysticercoids which received 2,000 r of irradiation. Cestodes developed from cysticercoids which received 10,000 r, 15,000 r and 20,000 r of irradiation showed a marked increase in the incidence of abnormal cirrus pouches. An analysis of variance test applied to the data on Table XVI shows that the results are statistically significant.

Table XVII shows that the incidence of sterility increased as the irradiation dose increased except at the 20,000 r level. At the 20,000 r level there was a decrease in sterility; an analysis of variance test shows that these figures are significant at the 1 per cent level. Table XVIII shows that the rate of abnormal segmentation increased as the irradiation dose increased. An analysis of variance test indicated that the figures are significant at the 5 per cent level.

In experiment II which involved the inheritance of radiation effects, three cestodes from each mouse were prepared for morphological study. The data for each cestode examined were recorded on a data sheet, from which Tables XIX, XX and XXI were prepared for analysis. The only significant results obtained in experiment II were in abnormal testes development in the F_2 generation. An analysis of variance test showed that abnormal testes development in the F_2 generation was significant at the 1 per cent level.

Experiment III was to study morphological effects of H. microstoma after gamma irradiation of the eggs. The cestodes recovered from the infected mice were prepared for morphological study in the same manner as for experiments I and II. The data for each cestode examined were recorded on a data sheet as described for experiments I and II. From the data sheets Tables XXII, XXIII and XXIV were prepared for statistical analysis. In the parent generation only two mice were infected. This was done because the mouse colony was running low and only enough material was desired to get an F_1 generation of cestodes developed from irradiated eggs. An analysis of variance test was run on the data obtained from the F_1 generation of cestodes. The only data in which the results were significant in the F_1 generation were those concerned with abnormal testes development. These data were significant at both the 5 and 1 per cent levels. The data from the F_2 generation of cestodes developed from gamma irradiated eggs were tabulated but not statistically analyzed. One of the F_2 generation control mice was negative, and two died before the cestodes matured. Two of the mice infected with 10,000 r material also died. There was, therefore, not enough material available to do an analysis of variance test. From the tabulated data, however, one can see that the results of the F_2 generation would probably not prove significant.

TYPES OF VARIATIONS

All types of variations recorded in the data sheets and tables are illustrated in Plates I to V. All variations recorded and shown

were found to be present in normal controls as well as in irradiated cestodes. There are variations of each particular abnormality shown. It is not possible to give a single description which would fit each abnormality. Each abnormal proglottid was recorded under that type which it most closely resembled. The following are some observations of the types of proglottids and their variations. Voge (1952) found that these abnormalities occur in a number of cestodes of the genus Hymenolepis.

Normal proglottids are usually much wider than long. Immature proglottids (Fig. 1, Plate I) appear to be about eight times as wide as long. Gravid proglottids (Fig. 1, Plate IV) appear to be about three times as wide as long. In greatly expanded cestodes the gravid proglottids may be nearly as long as wide or becoming nearly square in form. In the immature proglottids the reproductive organs are distinct and quite easily recognized in well stained specimens. In gravid proglottids the reproductive organs are in a state of disintegration. The uterus is packed with mature embryos and occupies all available space in the proglottid. As the proglottids mature and become packed with embryos the terminal portion of the cestode breaks free and the gravid proglottids are expelled by the host.

Testes abnormalities are variations from the normal position of the testes in this species which is two testes on one side and one testis on the other side. The side with one testis normally has the cirrus pouch located on that side. One of the most common occurrences of testes abnormality was that of testes reversal (Fig. 2, Plate I and Fig. 1,

Plate II). The question came up during the course of this study as to whether this was reversal of testes or inversion of the whole proglottid. As the proglottids were examined for testes position, it was noted in a few cases where the cirrus pouch as well as the testes were visible, that the cirrus pouch was also reversed. This would indicate an inversion of the whole proglottid. However, in most cases where the testes were reversed the cirrus pouch was in its normal position. A number of proglottids showed one extra testis (Figs. 1, 3 and 4, Plate I) located in every case on the side with two testes. In a number of cases it was found that all three testes were on one side (Fig. 2, Plate I). In all cases where this type of abnormality was present the three testes were located on the side opposite the cirrus pouch. Other abnormalities of testes arrangement were two testes on one side (Fig. 3, Plate I) and one testis per side (Fig. 2, Plate I). Whenever one testis was missing leaving two testes, the latter were opposite the side on which the cirrus pouch was located. In one instance a double set of testes was observed (Fig. 2, Plate II). There is a question as to whether this is really a double set of testes in one proglottid, or whether two proglottids are closely fused leaving no crosswall between them. The latter could very likely be the case since the proglottid which contains the double set of testes is slightly longer than the adjoining proglottids.

Cirrus pouch abnormalities such as the appearance of a double cirrus pouch (Figs. 3 and 4, Plate II) were not very common; however, they did occur frequently enough to warrant study. In many cases there were two distinct cirrus pouches in the proglottid with the adjoining

proglottids having a single cirrus pouch. In a number of cases, however, the cirrus pouch branched over from an adjoining proglottid into a common opening. In one case, not pictured, one proglottid was found to contain two distinct cirrus pouches within the proglottid with the one from the proglottid next to it joining these to form a common opening for the three. There were also noted a number of cases in which the cirrus pouch was reversed and in one outstanding case about half of the proglottids were inverted, thus making it appear as if the cirrus pouch were reversed. Cirrus pouch reversal was also noted by Voge (1952) in H. diminuta. The proglottids with these abnormalities appeared to be normal in other respects.

Sterility was observed quite frequently in both controls and irradiated cestodes. Proglottids exhibiting sterility were either completely sterile (Figs. 2 and 4, Plate III), or partly sterile (Figs. 3 and 4, Plate II, and Figs. 2 and 3, Plate III). It was observed that sterility ranged from a single proglottid to an entire group of proglottids. Some of the proglottids were completely empty with no indication that reproductive organs had ever developed. In others, the cirrus pouch (Fig. 3, Plate II) was visible along with some other structures which appeared quite disintegrated. In most cases where the proglottid was termed as partly sterile (Fig. 3, Plate II) one side of the proglottid was completely empty with no sign of reproductive organs having developed. In the other side it was observed that there had developed a cluster of embryos. In many cases where sterile proglottids were observed it was noted that the adjacent proglottids contained fewer

embryos than the more distant proglottids (Fig. 4, Plate II). This seemed to be true more in the irradiated cestodes than in the controls. Complete and partial sterility was noted in incompletely developed proglottids (Fig. 4, Plate III) as well as in fully developed proglottids.

Proglottids with few and large embryos occurred in a number of cases. Certain gravid proglottids contained fewer and larger embryos (Fig. 2, Plate IV) than in normal segments. Proglottids with few and large embryos were noted by Voge (1952) in Hymenolepis citelli and by Jones (1951) in Hymenolepis pitymi. Jones suggested that this size difference might be due to cross fertilization with other species of cestodes.

Reversal of proglottids (Fig. 3, Plate V) is a type of abnormality quite rare in this material, appearing only fifty-two times in the total number of proglottids observed in both control and irradiated cestodes. Kerr (1949) observed one strobila which exhibited a complete double reversal of twelve proglottids. At the site of the primary reversal there was a ring of tissue giving the general impression of a greatly compressed proglottid. Where the proglottid again reversed to its normal position the proglottid was bulbous and gave the appearance of having been injured. The internal organs of the reversed proglottids observed in the present study seemed quite disorganized. The cross walls of the proglottids involved were very irregular. In the reversed proglottids it is not possible to determine the exact position of the internal organs due to their irregularity. Neither is it possible to determine from the affected area alone which is the scolex end of the cestode, as can be seen in Fig. 3. In the specimens examined the reversed regions involved only three

or four proglottids. In no instance was there more than one case of proglottid reversal per cestode.

Incomplete separation of proglottids (Fig. 3, Plate I and Fig. 1, Plate V) was quite a common occurrence. There was no evidence that incomplete separation of proglottids was more common on one side than on the other. The degree of non-separation varied greatly. In some cases only two or three proglottids failed to separate, and in some cases as many as fifty proglottids. In practically all cases, where this abnormality was observed, the internal organs appeared normal. Voge (1952) and Kisner (1957) observed the same type of abnormal development in H. diminuta.

Incomplete and irregular development of proglottids was observed frequently in all material examined. In many cases an incompletely developed proglottid was wedged in between a gravid and a sterile proglottid (Fig. 4, Plate III). Kisner (1957) found this type of abnormality in H. diminuta. In other cases the abnormal proglottid was wedged between two gravid proglottids (Figs. 3 and 4, Plate IV). Many of the incompletely developed proglottids were gravid and many were either sterile or partly sterile. Voge (1952) observed these abnormalities in H. citelli and H. diminuta. Perhaps the most unusual abnormality observed was irregular development (Fig. 4, Plate V). This abnormal structure was observed in only four specimens. In each case the scolex and neck region of the cestode were normal, but the rest of the strobila was so distorted that it could not be analyzed. It can easily be seen from Fig. 4 that where this abnormality was present one could not make a

study of the individual proglottids nor of the organs within the proglottid. This extreme type of abnormality has not been reported previously.

Failure of the lateral wall to segment on one side was observed in a number of cases. The lateral walls of two or three proglottids failed to show a separation on one side. However, one case was observed where the lateral walls on one side failed (Fig. 2, Plate V) to separate in more than thirty proglottids. A very definite line of fusion was observed, with the external segmentation furrow completely missing. Nearly one-fourth of the width of the proglottids had also failed to separate so they appeared as a solid mass. The opposite sides of the proglottids seemed normal in every respect.

An irregular lateral segmentation wall was another variation of the lateral wall of the proglottids (Fig. 1, Plate IV). The lateral segmentation furrow was very irregular. In some cases it appeared as if two proglottids were fused, and in some cases it appeared as if three or four proglottids were fused into one proglottid. In many cases it was difficult to correlate the internal structure with the external form. This form of irregularity was found in control as well as in irradiated cestodes.

All of the forms of abnormalities listed here were separated into groups on the basis of their probable relationships. The data obtained from the prepared cestodes were recorded and prepared for analysis. The results of the analysis tests are recorded earlier in this section.

VI. DISCUSSION

All cestodes recovered from infected mice for this study were carefully measured and the lengths recorded for comparative studies. Each type of abnormality observed was recorded without regard to the possibility that some kinds would probably not be changed by radiation. It was assumed that the analysis of variance test would indicate whether the cestode length and which abnormalities had been affected by radiation. The analysis showed that in experiment I where the infected mice were sacrificed and the cestodes recovered before they shed mature proglottids, the difference in mean cestode length between the irradiated and non-irradiated cestodes was significant to the 1 per cent level. It is felt that the non-significant results in mean cestode length obtained in experiments II and III are due in part to the fact that many of the cestodes had already shed some terminal proglottids at the time they were recovered from the mice. It was impossible to include the shed proglottids in the data.

With the limited amount of material available for study it was difficult to determine the exact cause of the difference between the P_1 irradiated and the F_1 and F_2 worms. The significant results obtained in the P_1 generation are probably due to effects of irradiation, while the non-significant results in the F_1 and F_2 generations are probably due to inheritance, since they were not directly irradiated. If one were to find quantitative effects in the F_1 and F_2 generations, the results would probably show up in the F_1 and F_2 population as increased variability because of irradiation-caused variation in the cells of the germ

line from which these generations are derived. Dvorak's (1961) incomplete results on H. diminuta which has been given cumulative doses of gamma radiation up to 60,000 r show an increased variability in strains descended from irradiated ancestors. However, in his work each succeeding generation of cysticercoids received an additional 15,000 r of gamma irradiation whereas in the above study only the parent generation of cysticercoids was irradiated. The analysis of the morphological abnormalities in general showed that in some cases the difference between irradiated and non-irradiated forms was significant to the 5 per cent level. In some cases the difference was significant to the 1 per cent level. In most cases, however, there was no significant difference between irradiated and non-irradiated forms.

Even though some of the morphological differences between irradiated and control forms are not statistically significant, there does, however, exist the problem of normal variations. Morphological abnormalities in cestodes are by no means rare, as Clapham (1939), in making a thorough study of the subject, found. Voge (1952) made a study on abnormalities found in H. citelli, H. diminuta and H. horrida. She found that abnormalities occur frequently in these three cestodes. Kisner (1957) studied a number of abnormalities in H. diminuta which had also been described by Voge (1952). Child (1900, 1903) made an extensive study of the abnormalities of Moniezia expansa and found them to be quite numerous. Schiller (1957, 1959) observed a number of morphological abnormalities in H. nana. Many of the abnormalities mentioned and described in the above mentioned studies were also observed in the present study on H. microstoma. It is

interesting to note that even though there is an extensive amount of literature on the morphological abnormalities of cestodes, few attempts have been made to offer an explanation of the possible cause or origin of these abnormalities.

Perhaps one of the earliest attempts to offer an explanation of cestode abnormalities was that of Child (1900, 1903) in his study of the abnormalities of M. expansa in which he concluded that the abnormalities in the adult cestodes resulted from irregularities in the embryological development rather than modification of structures after they had formed. Barker (1916) concluded that abnormalities are possibly due to partial or incomplete separation of the blastomeres of the cestode embryo. Jones (1946) described a degenerate cestode sucker and indicated that it could possibly be due to failure of development of structures rather than to an embryonic disturbance. The opinions expressed as to possible causes of cestode abnormalities seem to agree that the latter are due to some disturbance in the growth region of the developing cestode.

In attempting to understand the basis for the effects of radiation on cestodes it seems logical to give a brief summary of the basic ideas relative to the effects of radiation on other animals, since practically all of the available literature on the effects of radiation on animals refers to the latter. A review of the literature indicates that the nucleus of the cell is more sensitive to irradiation than the cytoplasm (Perthes, 1903; Mitchell, 1943; Puck, 1960). Since the action of irradiation is primarily exerted on the nucleus of the cell one would expect chromosomal damage resulting in mutations and inhibition of

mitosis. Muller (1927) discovered that the number of mutations in Drosophila can be increased by the use of X-rays. A number of reports in the literature (Lea, 1947; Dobzhansky, 1955; Hollaender, 1955; Schiller, 1959) have indicated that the number of mutations produced by radiation is directly proportional to the amount of irradiation to which the individual is exposed. The findings of several investigators (Carlson, 1938; Henshaw and Cohen, 1940; Lea, 1955; Errera, 1960) support the view that irradiation may alter the chromosomes at any stage in the mitotic cycle.

Among the earliest known effects of radiations on living organisms were their capacities to produce changes in cell morphology (Carlson, 1954) which were frequently followed by death of the cell. There seems to be a direct relationship between cell damage and the anomalies resulting after exposure to radiation. It is also reported in the literature (Carlson, 1954) that the biological effects of radiation do not become observable immediately on the cessation of treatment, but that they may become detectable from within a few minutes to years after the treatment.

From the reports on radiation damage referred to in this study one can conclude that the primary site of damage is in the genetic apparatus, and that the damage consists of the destruction of the capacity to reproduce in some cells and the induction of mutations in other cells. Damage to the chromosomal and mitotic figures would bring about similar results.

Insofar as the writer is aware, the literature contains only three reports (Palais, 1933; Kisner, 1957; Schiller, 1959) in which an attempt

has been made to determine the effects of radiation on cestodes.

Palais (1933) compared the frequencies of abnormalities in X-irradiated and non-irradiated proglottids in H. diminuta and concluded that radiation had no effect in increasing the frequency of abnormal proglottids. Unfortunately it has not been possible to compare the amounts of radiation used in the present study with those of Palais because the information on dosage in her report is meaningless without a knowledge of the original source of energy.

Kisner (1957) subjected all possible stages of embryonic development of the proglottids of H. diminuta to gamma irradiation. His report indicated that irradiated cestodes were shorter than the controls; but the morphological effects upon the irradiated worms compared with controls were non-significant. The negative results obtained by him were undoubtedly due to the low doses of radiation. The dosage he employed ranged from 120 r to 750 r. If the dose is too low, the change may not be detectable on the basis of morphological criteria. Therefore, insufficient radiation may account for the failure of any morphological differences to be observed between the irradiated and control cestodes in his study.

Schiller (1959) reported that the frequency with which certain variants occur in H. nana can be increased by exposing either eggs or cysticercoids to X-irradiation and that the rate of increase is proportional to the amount of radiation employed. Radiation doses ranging from 5,000 r to 30,000 r were used in his study. He is of the opinion that the effects may be attributed to some disturbance of the embryonic

organization. The embryonic development of cestodes differs from that of other animals in that a mature cestode is not a single individual, but a group of individuals, one behind the other, forming a chain of proglottids. Each proglottid is a reproductive individual within itself. The embryonic growth region of the cestode is in the short neck region just behind the scolex. From this embryonic region new proglottids are formed, pushing back the segments previously formed and producing a chain of proglottids called a strobila. If the cause of abnormalities in normally developing cestodes is due to some disturbance in the growth region, it would seem logical to assume that abnormalities influenced by radiation would also be due to some disturbance in the growth region. If this assumption is correct, then it would perhaps be safe to conclude that proglottids which lack a definite form before radiation would have a greater number of abnormalities than proglottids which are formed before radiation. In Kisner's (1957) study no mention was made that gravid or mature proglottids showed a greater number of abnormalities than were found in the young regions.

In the present study on H. microstoma cysticercoids were exposed to radiation doses ranging from 2,000 r to 50,000 r. Total lethal effects were produced with doses greater than 30,000 r. Cestode eggs used in the present study were exposed to radiation doses of 10,000 r and 15,000 r. The results of the present study are comparable with those of Schiller (1959) even though they are not as consistent as his. The difference in mean length of irradiated worms compared with controls is possibly due to a quantitative effect upon cell population, where the

growth region was affected by radiation resulting in the failure of some cells to develop, thus accounting for the shorter length of cestodes in irradiated material. It seems possible that certain of the abnormalities which have been observed in H. microstoma, particularly those which are characterized by the absence of one or more of the reproductive organs and consequent sterility, might also be attributed to a change in the growth apparatus and that radiation only serves to increase the frequency of these disturbances.

Further work on the embryology, genetics and cytology of cestodes is highly recommended. Although some cytological studies have been reported in a few species (Child, 1911, in Moniezia; Harman, 1913, in Taenia; Young, 1919, in Taeniidae; Jones, 1945, in Hymenolepididae; Jones and Ciordia, 1956, in Taenia; and Jones and Wyant, 1957, in Taenia), comparatively little is known about the heredity of these organisms. With the present state of knowledge in regard to cestode cytology, genetics and embryology, it is not possible to know whether such events as chromosome or gene mutations occur and how important a contribution they make to the variations observed. These studies would certainly be a tremendous undertaking but, nevertheless, one to stimulate the scientist.

VII. SUMMARY

T. confusum larvae containing mature cysticercoids of *H. microstoma* were irradiated with gamma rays with doses ranging from 2,000 r to 50,000 r. *H. microstoma* eggs were irradiated with doses of 10,000 r and 15,000 r. All materials were in plastic vials which were placed inside the radiation capsule during irradiation. The dose rate ranged from 560 r to 566 r per minute. Twenty-four hours after irradiating the cysticercoids, mice were infected with a given number of cysticercoids. *T. confusum* larvae were infected with irradiated eggs and seventeen days later the cysticercoids developing from these eggs were removed from the larvae and injected into mice.

Upon recovery of the cestodes from the mice a number of abnormalities involving proglottid form, sterility, testes, cirrus pouch reversal, and segmentation were noted in both control and irradiated forms. All abnormalities were recorded on data sheets. An analysis of variance test was applied to the frequencies of the abnormalities. This test indicated that, where the worms were recovered before they had shed terminal proglottids, there was a significant difference to the 1 per cent or 5 per cent levels in the occurrence of abnormalities in irradiated cestodes as compared with controls. The variations in mean cestode length were significant to the 1 per cent level. The irradiated forms showed a significant decrease in length. In most respects where the cestodes had shed terminal proglottids before they were recovered from the mice, there was not a significant difference in the occurrence of abnormalities or mean cestode length in irradiated cestodes as compared with controls.

Studies of growth rate and abnormalities in the F_1 and F_2 progeny of irradiated eggs and cysticercoids revealed little significant variation from controls. Apparently the inherited effects of irradiation in these tapeworms are too small to measure by the methods used in this study.

From the literature on effects of radiation on mammals, insects, and other organisms, it appears that radiation produces abnormalities chiefly by destroying or inhibiting growing cells and tissues. The effects on length of the worms may be due to reduction in cell population responsible for growth. The presence of certain morphological abnormalities which have been observed, particularly those which have been characterized by the absence of one or more reproductive organs, might also be attributed to a change in the growth or embryological apparatus and that radiation only serves to increase the frequency of these disturbances.

For further similar studies involving this species, it is recommended that all cestodes used for analysis be recovered from mice just before the cestodes begin expelling eggs, which is thirteen to fourteen days after infecting the mice with mature cysticercoids.

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APPENDIXES

TABLE I

Experiment I. Part one

EFFECTS ON LENGTH ATTAINED BY *HYMENOLEPIS MICROSTOMA* AFTER SUBJECTING CYSTICERCOIDS TO 2,000 r AND 10,000 r OF GAMMA IRRADIATION

	Control		2,000 r		10,000 r	
Mouse Number	Number of Worms	Average Length in mm.	Number of Worms	Average Length in mm.	Number of Worms	Average Length in mm.
1	5	71.0	5	82.0	5	57.0
2	5	110.0	5	74.25	5	74.0
3	5	80.0	4	51.0	5	52.0
4	5	69.0	3	73.66	5	75.0
5	5	61.0	4	70.33	4	81.0
Mean Length		78.2		70.83		67.3

TABLE II

Experiment I. Part two

EFFECTS ON LENGTH ATTAINED BY *HYMENOLEPIS MICROSTOMA* AFTER SUBJECTING CYSTICERCOIDS TO 15,000 r AND 20,000 r OF GAMMA IRRADIATION

	Control		15,000 r		20,000 r	
Mouse Number	Number of Worms	Average Length in mm.	Number of Worms	Average Length in mm.	Number of Worms	Average Length in mm.
1	4	88.0	4	38.75	0	
2	4	73.5	5	64.25	0	
3	5	79.4	4	59.66	3	13.2
4	5	79.4	3	54.64	3	37.0
5	5	79.4	5	56.20	2	38.0
Mean Length		79.87		55.43		28.32

TABLE III

Experiment II. Part one

EFFECTS ON LENGTH OF THE P_1 GENERATION OF HYMENOLEPIS MICROSTOMA AFTER SUBJECTING CYSTICERCOIDS TO 10,000 r AND 15,000 r OF GAMMA IRRADIATION

	Control		10,000 r		15,000 r	
Mouse Number	Number of Worms	Average Length in mm.	Number of Worms	Average Length in mm.	Number of Worms	Average Length in mm.
1	5	154.0	4	145.0	5	149.0
2	3	183.0	4	145.0	4	161.0
3	4	190.0	4	140.0	5	123.0
4	5	163.0	4	175.0	3	123.0
5	5	152.0	4	148.0	0	
Mean Length		166.0		150.6		139.6

TABLE IV

Experiment II. Part two

EFFECTS ON LENGTH OF THE F_1 GENERATION OF ADULT HYMENOLEPIS MICROSTOMA

Mouse Number	Control		10,000 r		15,000 r	
	Number of Worms	Average Length in mm.	Number of Worms	Average Length in mm.	Number of Worms	Average Length in mm.
1	10	115.1	9	109.0	10	76.0
2	0		9	109.3	10	94.1
3	8	114.0	8	104.5	10	106.3
4	10	123.6	9	110.2	9	95.8
5	10	115.3	10	109.4	4	87.75
Mean Length		117.13		108.62		92.5

TABLE V

Experiment II. Part three

EFFECTS ON LENGTH OF THE F_2 GENERATION OF ADULT HYMENOLEPIS MICROSTOMA

Mouse Number	Control		10,000 r		15,000 r	
	Number of Worms	Average Length in mm.	Number of Worms	Average Length in mm.	Number of Worms	Average Length in mm.
1	7	194.8	9	139.9	9	132.8
2	10	147.9	8	153.8	0	
3	9	129.8	10	149.7	4	166.5
4	9	145.7	6	168.3	10	141.9
5	Died		7	120.7	Died	
Mean Length		152.0		145.9		142.6

TABLE VI

Experiment II. Part one (Supp. 1)

EFFECTS ON LENGTH OF THE P_1 GENERATION OF HYMENOLEPIS MICROSTOMA AFTER SUBJECTING CYSTICERCOIDS TO 20,000 r OF GAMMA IRRADIATION

	Control		20,000 r	
Mouse Number	Number of Worms	Average Length in mm.	Number of Worms	Average Length in mm.
1	4	175.0	9	118.7
2	8	146.0	7	128.4
3	8	168.0	10	138.0
4	1	280.0	8	139.0
5	8	153.0	3	198.0
Mean Length		161.9		136.6

TABLE VII

Experiment II. Part two (Supp. 2)

EFFECTS ON LENGTH OF THE F_1 GENERATION OF ADULT HYMENOLEPIS MICROSTOMA

	Control		20,000 r	
Mouse Number	Number of Worms	Average Length in mm.	Number of Worms	Average Length in mm.
1	9	128.3	9	125.7
2	9	117.0	10	111.5
3	Died		2	70.5
4	Died		Died	
5	Died		Died	
Mean Length		122.7		113.7

TABLE VIII

Experiment II. Part three (Supp. 3)

EFFECTS ON LENGTH OF THE F_2 GENERATION OF ADULT HYMENOLEPIS MICROSTOMA

	Control		20,000 r	
Mouse Number	Number of Worms	Average Length in mm.	Number of Worms	Average Length in mm.
1	6	131.7	5	101.4
2	4	110.0	8	92.4
3	0		9	87.3
4	7	130.4	8	127.3
5	7	118.4	6	102.3
Mean Length		123.8		101.8

TABLE IX

Experiment III. Part one[a]

EFFECTS ON LENGTH ATTAINED BY HYMENOLEPIS MICROSTOMA AFTER SUBJECTING EGGS TO 10,000 r AND 15,000 r OF GAMMA IRRADIATION

	Control		10,000 r		15,000 r	
Mouse Number	Number of Worms	Average Length in mm.	Number of Worms	Average Length in mm.	Number of Worms	Average Length in mm.
1	9	121.0	1	115.0	1	85.0
2			3	152.0	1	185.0
Mean Length		121.0		142.75		135.0

[a]Because of the mouse population running low, only enough mice were infected to obtain material for the F_1 generation. The information particularly desired in this experiment was that secured from F_1 and F_2 infections.

TABLE X

Experiment III. Part two

EFFECTS ON LENGTH OF THE F_1 GENERATION OF ADULT HYMENOLEPIS MICROSTOMA

	Control		10,000 r		15,000 r	
Mouse Number	Number of Worms	Average Length in mm.	Number of Worms	Average Length in mm.	Number of Worms	Average Length in mm.
1	8	171.37	9	129.90	8	105.75
2	9	153.50	6	120.00	10	113.50
3	6	134.50	5	143.00	9	156.10
4	10	154.00	9	141.44	10	164.00
5	8	142.00	10	115.60	0	
Mean Length		152.10		129.00		136.10

TABLE XI

Experiment III. Part three

EFFECTS ON LENGTH OF THE F_2 GENERATION OF ADULT HYMENOLEPIS MICROSTOMA

Mouse Number	Control		10,000 r		15,000 r	
	Number of Worms	Average Length in mm.	Number of Worms	Average Length in mm.	Number of Worms	Average Length in mm.
1	10	147.8	6[b]	65.3	9	116.6
2	9	154.1	8[c]	88.6	10	140.1
3	Died[a]		8	151.2	7	115.4
4	Died[a]		9	151.1	9	118.8
5	Died[a]		10	172.0	10	113.1
Mean Length		150.8		131.5		119.9

[a]Died seven and eight days after infection.

[b]Died fourteen days after infection, but cestodes were recovered and prepared for study.

[c]Died sixteen days after infection, but cestodes were recovered and prepared for study.

TABLE XII

Experiment I. Part one and two combined

EFFECTS ON LENGTH ATTAINED BY *HYMENOLEPIS MICROSTOMA* AFTER SUBJECTING CYSTICERCOIDS TO 2,000 r TO 50,000 r OF GAMMA IRRADIATION[a]

Mouse Number	Average Lengths of Worms in mm.				
	Controls	2,000 r	10,000 r	15,000 r	20,000 r
1	78.50	82.00	57.00	38.75	Neg.
2	93.75	74.25	74.00	64.25	Neg.
3	79.70	51.00	52.00	59.66	13.20
4	74.20	73.66	75.00	54.64	37.00
5	70.20	70.33	81.00	56.20	38.00
Mean Length	79.00	70.83	67.30	55.43	28.32

[a]All mice infected with cysticercoids receiving 25,000 r, 30,000 r, 40,000 r and 50,000 r of irradiation were negative. These figures are not shown in this table.

TABLE XIII

AVERAGE LENGTH OF *HYMENOLEPIS MICROSTOMA* AFTER SUBJECTING CYSTICERCOIDS TO 10,000 r AND 15,000 r OF GAMMA IRRADIATION (Length is expressed in mm.)

PARENT GENERATION			
Mouse Number	Control	10,000 r	15,000 r
1	154.0	145.0	149.0
2	183.0	145.0	101.0
3	190.0	140.0	123.0
4	163.0	175.0	123.0
5	152.0	148.0	Negative
Mean Length	166.0	150.6	139.6
% Deviation from Control	0	-10%	-16%

F_1 GENERATION			
Mouse Number	Control	10,000 r	15,000 r
1	115.1	109.0	76.0
2	Negative	109.3	94.1
3	114.0	104.5	106.3
4	123.6	110.2	95.8
5	115.3	109.4	87.75
Mean Length	117.13	108.62	92.50
% Deviation from Control	0	-8%	-22%

F_2 GENERATION			
Mouse Number	Control	10,000 r	15,000 r
1	194.8	139.9	132.8
2	147.9	153.8	Negative
3	129.8	149.7	166.5
4	145.7	168.3	141.9
5	Died	120.7	Died
Mean Length	150.0	145.0	142.0
% Deviation from Control	0	-3.4%	-5.4%

TABLE XIV

(SAMPLE) EXPERIMENT NO. 3 F_2 RADIATION DOSE 10,000 r

Mouse Number	Worm	Abnormal Testes Development						Abnormal Cirrus Pouch			Sterility		Abnormal Segmentation							
		Testes Counted	Testes Reversal	One Testis per side	Three Testes on one side	Extra Testes	Two Testes per side	Pouches Counted	Pouches Reversed	Double Pouch	Sterility	Few and Large Embryos	Fusion of Wall	Incomplete Segmentation	Incomplete Development	Blistered Segments	Proglottids Reversed	Indistinct Cross-wall	Irregular Out-line	Total Count
1	a	582	3	0	6	1		568			1			1	1	20				766
	b	460	4	0	2	0		416			3	1		3	1	72				643
	c	201	6	0	4	0		202			8			1	1	22		155	155	384
2	a	646	5	0	1	0		763		1	1			6	4					1048
	b	556	4	1	6	2		627			3	4		3	1					823
	c	485	4	0	0	0	2	534	1	2	4	2	9	4						677
3	a	613	2	1	1	0		752			2	1		3	1	78				628
	b	657	5	0	0	0		827		1	1			4	2			12	12	1091
	c	704	6	0	9	0	1	856		1				6	2	43		45	45	1052
4	a	687	2	0	6	1		857	71	9	12	4		7	7	11				1127
	b	632	10	0	2			672	15	5	11	4		5	6					878
	c	779	4	0	1			903	1		16	1		5	1					1071
5	a	644	1	0	2			702		1	2	2		5	8					1138
	b	517	2	0	0			704		2	1			14	1	45				844
	c	679	5	0	1			903		1	1	13	2	10		34				1132

TABLE XV

FREQUENCY OF OCCURRENCE OF PROGLOTTIDS SHOWING ABNORMAL TESTES DEVELOPMENT IN CESTODES OBTAINED BY DIRECT INFECTION, IN ALBINO MICE, WITH GAMMA IRRADIATED CYSTICERCOIDS OF HYMENOLEPIS MICROSTOMA

Number of Abnormal Testes per 100 Testes Counted in each Worm

Irradiation Dose	Mouse Number	Worm Number 1	Worm Number 2	Total for Two Worms	Av. No. of Abnormalities Per Worm
Control group	1	2.28	1.29	3.59	
Total Number	2	1.05	1.12	2.17	
of	3	1.78	0.65	2.43	
Proglottids	4	1.54	1.09	2.63	
examined-4193	5	1.13	1.04	2.17	1.30
2,000 r group	1	2.33	2.37	4.70	
Total Number	2	2.57	2.75	5.32	
of	3	1.58	4.58	6.16	
Proglottids	4	3.88	2.70	6.58	
examined-3838	5	2.28	3.08	5.36	2.81
10,000 r group	1	3.45	3.02	6.47	
Total Number	2	3.77	2.71	6.48	
of	3	1.69	2.71	4.40	
Proglottids	4	1.41	4.34	5.75	
examined-4027	5	1.66	1.45	3.11	2.62
15,000 r group	1	12.28	5.91	18.19	
Total Number	2	28.50	2.71	31.21	
of	3	1.39	5.65	7.04	
Proglottids	4	3.24	5.48	8.72	
examined-3502	5	1.94	2.22	4.16	6.93
20,000 r group	1 Neg.	0.00	0.00	0.00	
Total Number	2 Neg.	0.00	0.00	0.00	
of	3[a]	4.27	0.00	4.27	
Proglottids	4	4.54	1.55	6.09	
examined-1106	5	4.35	6.25	10.60	4.19

[a]In mouse No. 3 only one whole worm was recovered. The other two worms were accidentally cut too badly to be analyzed.

TABLE XVI

FREQUENCY OF OCCURRENCE OF PROGLOTTIDS SHOWING ABNORMAL CIRRUS POUCH DEVELOPMENT IN CESTODES OBTAINED BY DIRECT INFECTION, IN ALBINO MICE, WITH GAMMA IRRADIATED CYSTICERCOIDS OF HYMENOLEPIS MICROSTOMA

Number of Abnormal Cirrus Pouches per 100 Segments Counted in Each Worm

Irradiation Dose	Mouse Number	Worm Number 1	Worm Number 2	Total for Two Worms	Av. No. of Abnormalities Per Worm
Control group	1	0.16	1.16	1.32	
Total Number	2	0.13	2.99	3.12	
of	3	0.33	0.25	0.58	
Proglottids	4	4.51	0.70	5.21	
examined-3575	5	6.78	3.49	10.27	2.05
2,000 r group	1	0.32	0.83	1.15	
Total Number	2	0.45	0.00	0.45	
of	3	6.35	0.00	6.35	
Proglottids	4	0.29	0.29	0.58	
examined-3519	5	0.00	0.00	0.00	0.85
10,000 r group	1	0.00	3.03	3.03	
Total Number	2	1.13	0.00	1.13	
of	3	0.00	0.32	0.32	
Proglottids	4	0.00	24.70	24.70	
examined-3357	5	0.66	0.30	0.96	3.01
15,000 r group	1	0.66	1.11	1.77	
Total Number	2	2.26	0.56	2.82	
of	3	6.21	5.07	11.28	
Proglottids	4	6.32	28.20	34.52	
examined-3041	5	0.32	0.54	0.86	5.12
20,000 r group	1 Neg.	0.00	0.00	0.00	
Total Number	2 Neg.	0.00	0.00	0.00	
of	3[a]	2.52	0.00	2.52	
Proglottids	4	14.76	2.33	17.09	
examined-695	5	3.65	8.74	12.39	6.40

[a]In mouse No. 3 only one whole worm was recovered. The other two worms were accidentally cut too badly to be analyzed.

TABLE XVII

FREQUENCY OF OCCURRENCE OF PROGLOTTIDS SHOWING STERILE SEGMENTS IN CESTODES OBTAINED BY DIRECT INFECTION, IN ALBINO MICE, WITH GAMMA IRRADIATED CYSTICERCOIDS OF HYMENOLEPIS MICROSTOMA

Number of Sterile Segments per 100 Segments Counted in Each Worm

Irradiation Dose	Mouse Number	Worm Number 1	Worm Number 2	Total for Two Worms	Av. No. of Abnormalities Per Worm
Control group	1	0.00	0.14	0.14	
Total Number	2	0.20	0.23	0.43	
of	3	0.31	0.13	0.44	
Proglottids	4	0.15	0.45	0.60	
checked-6857	5	0.08	0.41	0.49	0.21
2,000 r group	1	0.69	0.40	1.09	
Total Number	2	0.27	0.48	0.75	
of	3	0.42	0.00	0.42	
Proglottids	4	0.95	0.80	1.75	
checked-6715	5	0.61	0.12	0.73	0.47
10,000 r group	1	1.90	0.57	2.47	
Total Number	2	0.51	0.35	0.86	
of	3	0.83	0.32	1.15	
Proglottids	4	0.32	0.58	0.90	
checked-6831	5	0.73	0.69	1.42	0.68
15,000 r group	1	2.74	4.66	7.40	
Total Number	2	1.18	1.12	2.30	
of	3	0.90	3.52	4.42	
Proglottids	4	0.33	11.42	11.76	
checked-6061	5	1.74	3.53	5.27	3.11
20,000 r group	1 Neg.	0.00	0.00	0.00	
Total Number	2 Neg.	0.00	0.00	0.00	
of	3[a]	2.68	0.00	2.68	
Proglottids	4	4.52	1.59	6.11	
checked-1896	5	1.01	0.37	1.38	2.03

[a]In mouse No. 3 only one whole worm was recovered. The other two worms were accidentally cut too badly to be analyzed.

TABLE XVIII

FREQUENCY OF OCCURRENCE OF PROGLOTTIDS SHOWING ABNORMAL DEVELOPMENT OF SEGMENTS IN CESTODES OBTAINED BY DIRECT INFECTION, IN ALBINO MICE, WITH GAMMA IRRADIATED CYSTICERCOIDS OF HYMENOLEPIS MICROSTOMA

Number of Abnormal Segments per 100 Counted in Each Worm

Irradiation Dose	Mouse Number	Worm Number 1	Worm Number 2	Total for Two Worms	Av. No. of Abnormalities Per Worm
Control group	1	1.33	1.02	2.35	
Total Number	2	0.97	1.37	2.34	
of	3	0.86	1.91	2.77	
Proglottids	4	1.63	1.37	3.00	
checked-6857	5	1.48	1.19	2.67	1.31
2,000 r group	1	0.87	2.02	2.89	
Total Number	2	2.57	0.98	3.55	
of	3	3.39	0.88	4.27	
Proglottids	4	3.96	2.58	6.54	
checked-6715	5	0.61	2.93	3.54	2.08
10,000 r group	1	3.02	1.58	4.60	
Total Number	2	0.52	1.78	2.30	
of	3	4.04	1.78	5.82	
Proglottids	4	1.46	4.09	5.55	
checked-6831	5	1.60	1.67	3.27	2.15
15,000 r group	1	5.24	9.96	15.20	
Total Number	2	3.40	2.25	4.65	
of	3	2.34	2.01	4.35	
Proglottids	4	1.49	36.35	37.84	
checked-6061	5	5.95	7.23	13.18	7.52
20,000 r group	1 Neg.	0.00	0.00	0.00	
Total Number	2 Neg.	0.00	0.00	0.00	
of	3[a]	4.57	0.00	4.57	
Proglottids	4	3.57	3.20	6.77	
checked-1896	5	33.84	4.07	37.91	9.85

[a]In mouse No. 3 only one whole worm was recovered. The other two worms were accidentally cut too badly to be analyzed.

TABLE XIX

FREQUENCY OF OCCURRENCE OF PROGLOTTIDS SHOWING ABNORMAL TESTES DEVELOPMENT IN CESTODES OBTAINED BY INFECTION, IN ALBINO MICE, WITH GAMMA IRRADIATED CYSTICERCOIDS OF HYMENOLEPIS MICROSTOMA CARRIED TO F_2 GENERATION

Initial Irradiation Dose	Total Number of Proglottids Examined	Number of Proglottids with Abnormal Testes per 1,500 Segments
	PARENT GENERATION	
Control	9438	20.21
10,000 r	9217	17.15
15,000 r	7851	18.64
	F_1 GENERATION	
Control[a]	7234	12.06
10,000 r	8369	32.73
15,000 r	8224	26.63
	F_2 GENERATION	
Control[b]	8429	12.45
10,000 r	9351	22.74
15,000 r[a,b]	5600	9.09

[a]One mouse negative.

[b]Mouse died.

TABLE XX

FREQUENCY OF OCCURRENCE OF PROGLOTTIDS SHOWING CIRRUS POUCH ABNORMALITIES IN CESTODES OBTAINED BY INFECTION, IN ALBINO MICE, WITH GAMMA IRRADIATED CYSTICERCOIDS OF HYMENOLEPIS MICROSTOMA CARRIED TO THE F_2 GENERATION

Initial Irradiation Dose	Total Number of Proglottids Examined	Number of Proglottids with Abnormal Cirrus Pouch per 1,500 Segments
	PARENT GENERATION	
Control	11,540	13.95
10,000 r	11,491	19.08
15,000 r[a]	8,701	25.59
	F_1 GENERATION	
Control[a]	7,774	48.19
10,000 r	9,042	37.73
15,000 r	8,102	60.34
	F_2 GENERATION	
Control[b]	8,767	2.4
10,000 r	10,601	1.5
15,000 r[a,b]	7,392	2.0

[a]One mouse negative.

[b]One mouse died.

TABLE XXI

FREQUENCY OF OCCURRENCE OF PROGLOTTIDS SHOWING STERILITY AND ABNORMAL SEGMENTATION OF SEGMENTS IN CESTODES OBTAINED BY INFECTION, IN ALBINO MICE, WITH GAMMA IRRADIATED CYSTICERCOIDS OF HYMENOLEPIS MICROSTOMA CARRIED TO THE F_2 GENERATION

Initial Irradiation Dose	Total Number of Proglottids Examined	Number of Sterile Proglottids per 1,500	Number of Proglottids with Abnormal Segmentation per 1,500
	PARENT GENERATION		
Control	16,205	3.9	24.99
10,000 r	15,983	10.3	25.89
15,000 r[a]	11,878	10.8	27.05
	F_1 GENERATION		
Control[a]	11,035	12.72	22.50
10,000 r	12,406	5.67	23.13
15,000 r	12,050	2.93	41.00
	F_2 GENERATION		
Control[b]	12,276	3.04	20.88
10,000 r	14,292	3.78	11.30
15,000 r[a,b]	9,450	1.72	8.87

[a]One mouse negative.

[b]One mouse died.

TABLE XXII

FREQUENCY OF OCCURRENCE OF PROGLOTTIDS SHOWING ABNORMAL TESTES DEVELOPMENT IN CESTODES OBTAINED BY INFECTION, IN ALBINO MICE, WITH CYSTICERCOIDS DEVELOPED FROM GAMMA IRRADIATED EGGS OF HYMENOLEPIS MICROSTOMA CARRIED TO THE F_2 GENERATION

Initial Irradiation Dose	Total Number of Proglottids Examined	Number of Proglottids with Abnormal Testes per 1,500 Segments
	PARENT GENERATION	
Control	961	80
10,000 r	1,114	33
15,000 r	1,221	73
	F_1 GENERATION	
Control	10,944	23.14
10,000 r	10,036	24.24
15,000 r[a]	7,203	40.18
	F_2 GENERATION	
Control[a,b]	3,615	17.0
10,000 r[b]	8,842	19.0
15,000 r	7,612	35.0

[a]One mouse negative.

[b]Two mice died.

TABLE XXIII

FREQUENCY OF OCCURRENCE OF PROGLOTTIDS SHOWING CIRRUS POUCH ABNORMALITIES IN CESTODES OBTAINED BY INFECTION, IN ALBINO MICE, WITH CYSTICERCOIDS DEVELOPED FROM GAMMA IRRADIATED EGGS OF HYMENOLEPIS MICROSTOMA CARRIED TO THE F_2 GENERATION

Initial Irradiation Dose	Total Number of Proglottids Examined	Number of Proglottids with Abnormal Cirrus Pouch per 1,500 Segments
	PARENT GENERATION	
Control	979	21
10,000 r	1,423	1.4
15,000 r	1,149	7.0
	F_1 GENERATION	
Control	11,470	2.31
10,000 r	10,155	1.02
15,000 r[a]	7,659	0.96
	F_2 GENERATION	
Control[a,b]	4,183	3.7
10,000 r[b]	10,286	16.0
15,000 r	9,006	20.0

[a]One mouse negative.

[b]Two mice died.

TABLE XXIV

FREQUENCY OF OCCURRENCE OF PROGLOTTIDS SHOWING STERILITY AND ABNORMAL SEGMENTATION OF SEGMENTS IN CESTODES OBTAINED BY INFECTION, IN ALBINO MICE, WITH CYSTICERCOIDS DEVELOPED FROM GAMMA IRRADIATED EGGS OF HYMENOLEPIS MICROSTOMA CARRIED TO THE F_2 GENERATION

Initial Irradiation Dose	Total Number of Proglottids Examined	Number of Sterile Proglottids per 1,500	Number of Proglottids with Abnormal Segmentation per 1,500
	PARENT GENERATION		
Control	1,458	0	10
10,000 r	1,948	.2	17
15,000 r	1,839	1	5.3
	F_1 GENERATION		
Control	16,688	1.81	16.23
10,000 r	14,459	4.13	17.33
15,000 r[a]	11,116	7.69	13.75
	F_2 GENERATION		
Control[a,b]	5,563	.7	21
10,000 r[b]	12,702	7.1	65
15,000 r	12,180	4.0	61

[a]One mouse negative.

[b]Two mice died.

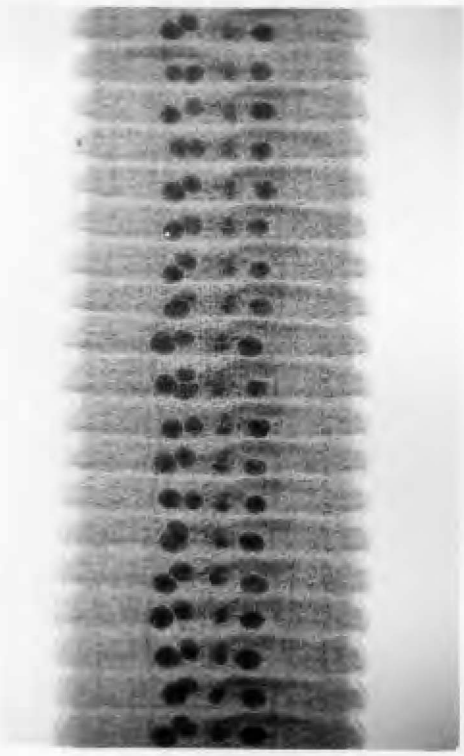

Fig. 1

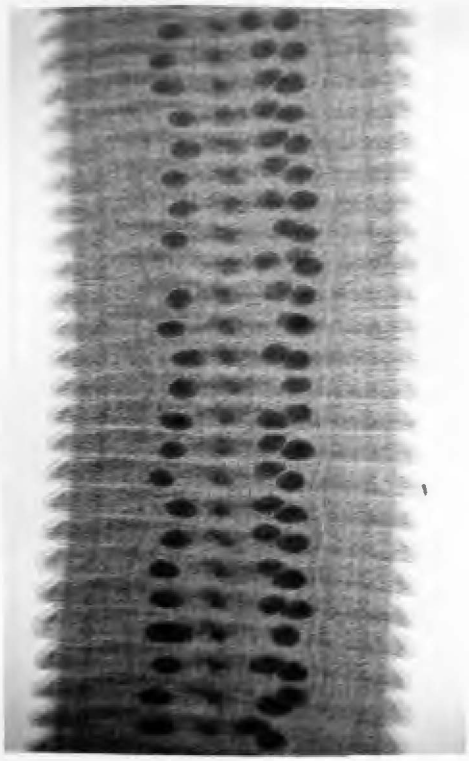

Fig. 2

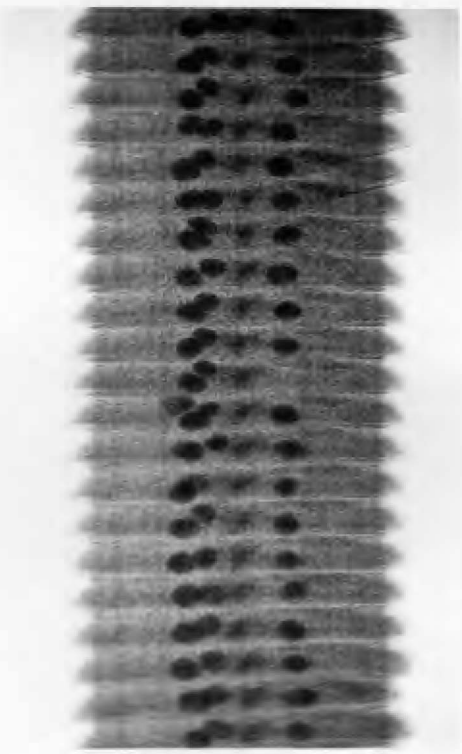

Fig. 3

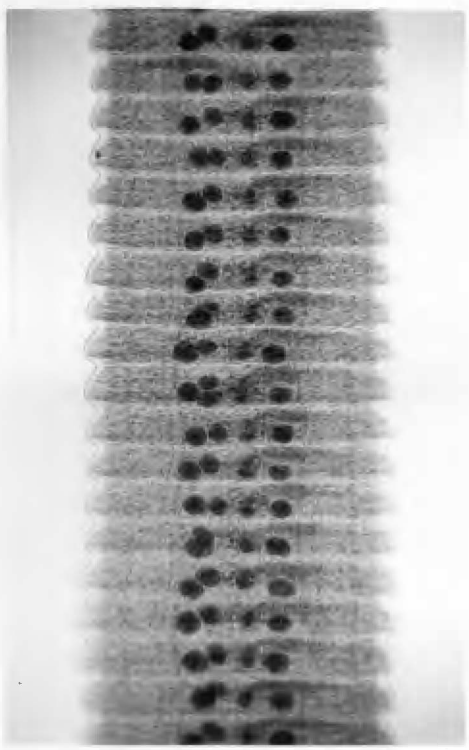

Fig. 4

PLATE I

Anomalies of testes

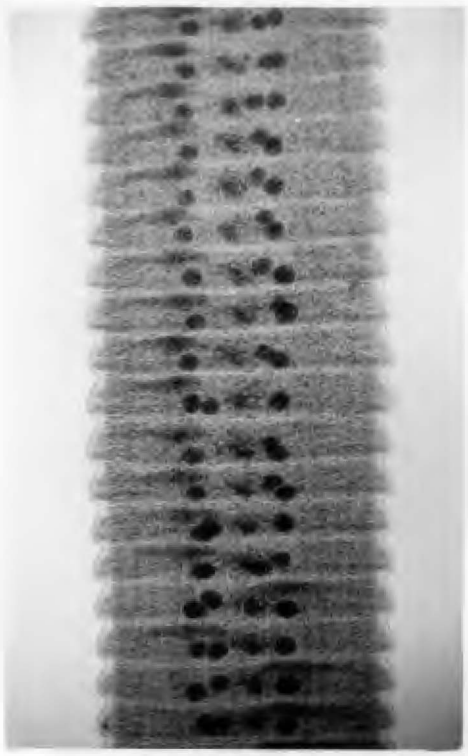

Fig. 1

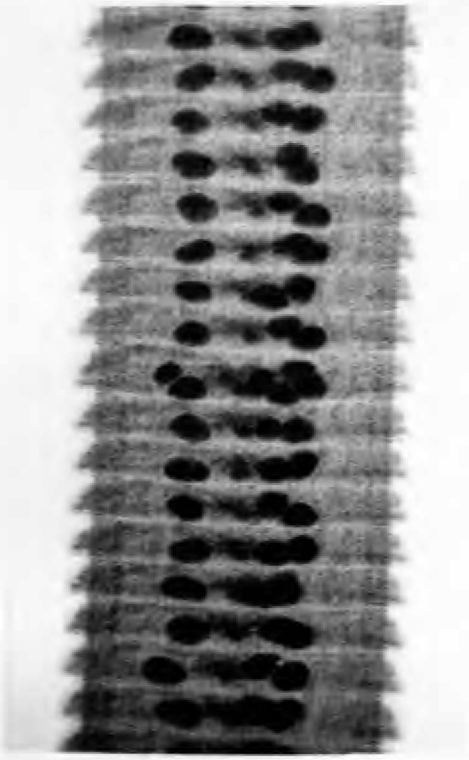

Fig. 2

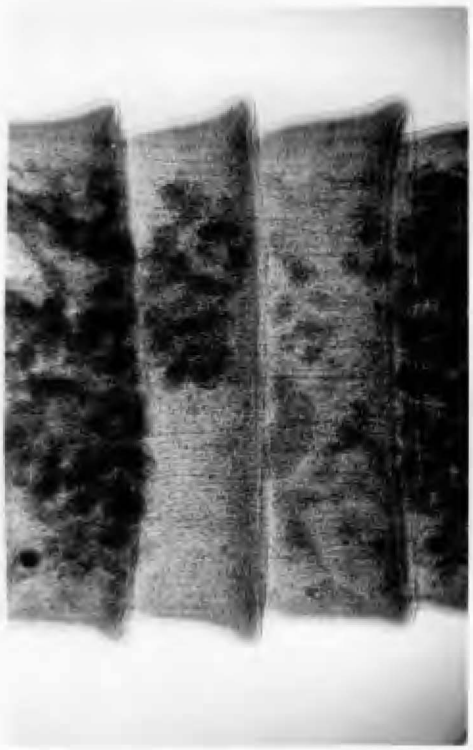

Fig. 3

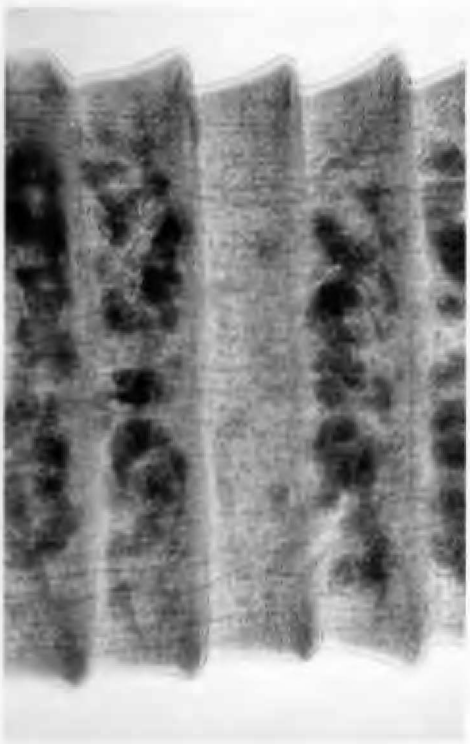

Fig. 4

PLATE II

Anomalies of organs

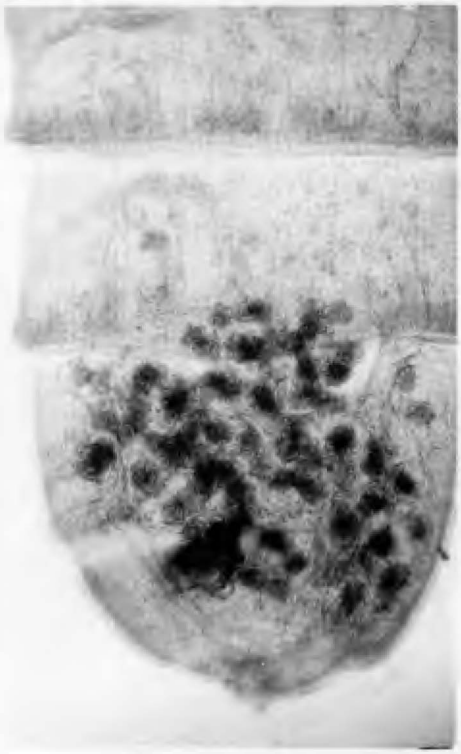

Fig. 1

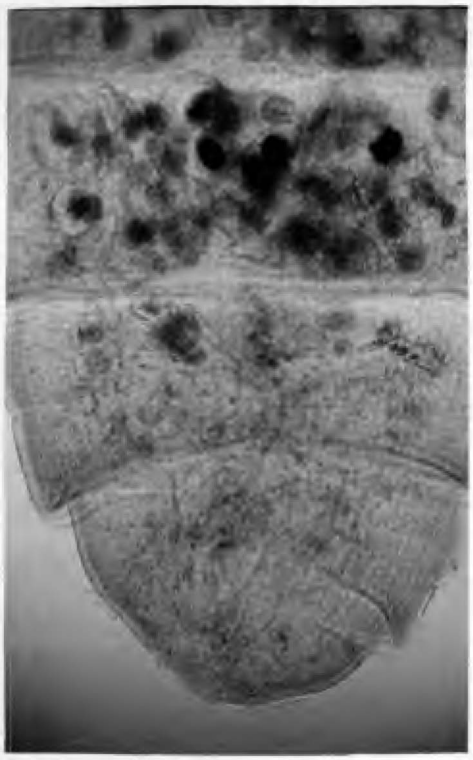

Fig. 2

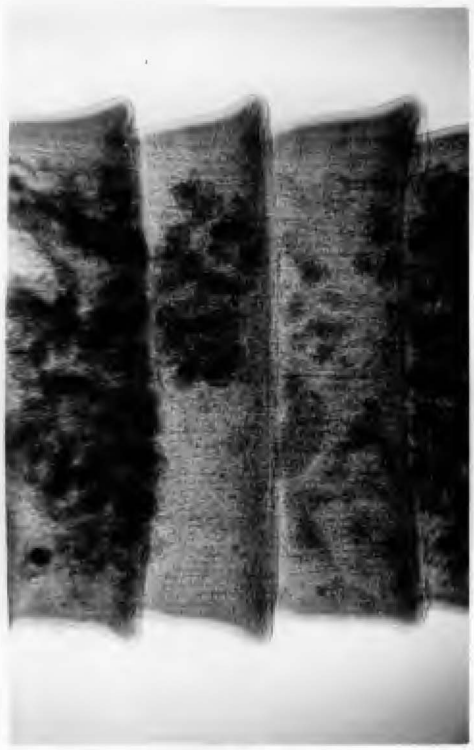

Fig. 3

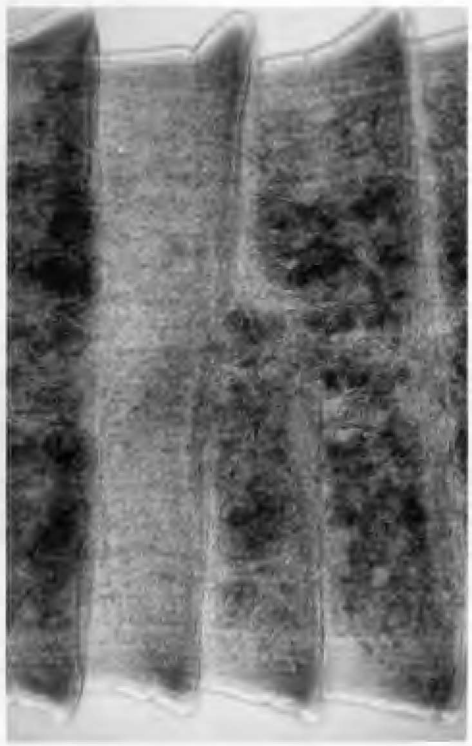

Fig. 4

PLATE III

Partial and complete sterility

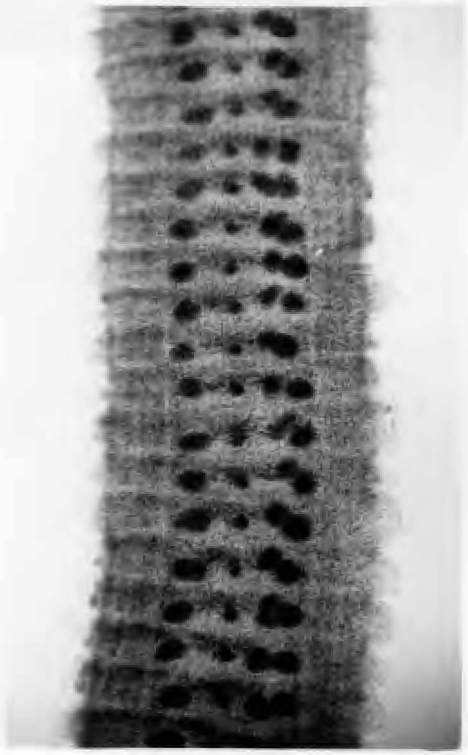

Fig. 1

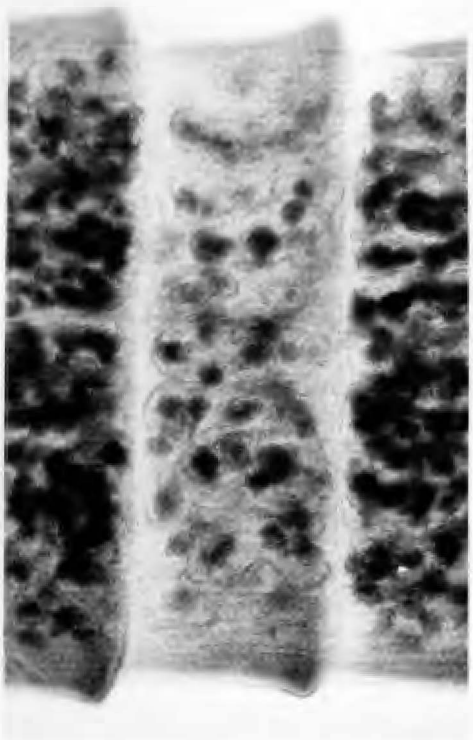

Fig. 2

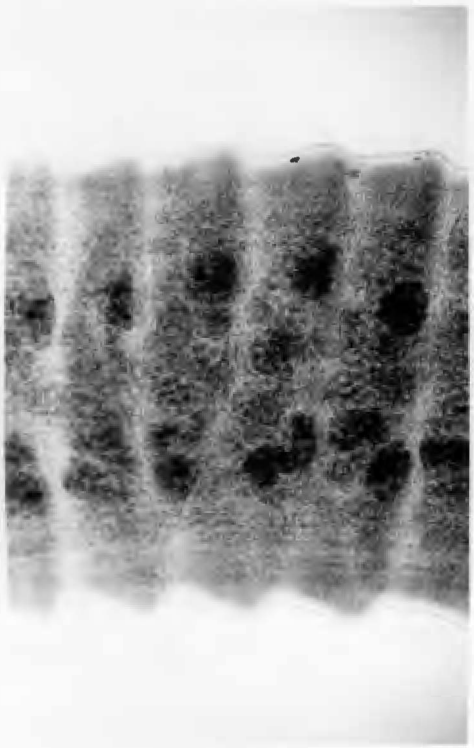

Fig. 3

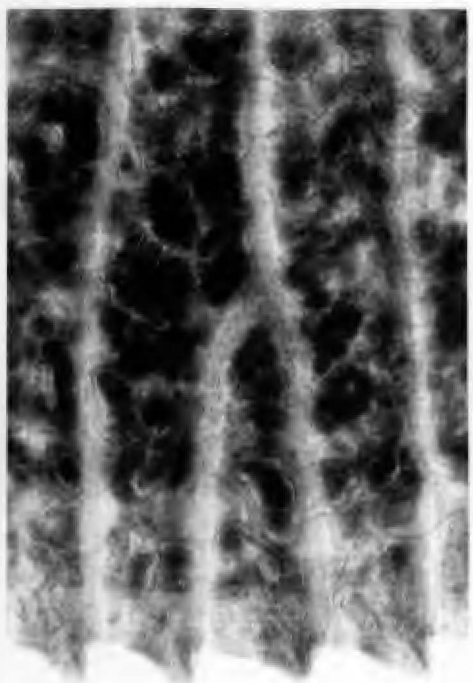

Fig. 4

PLATE IV

Anomalies of strobila and embryos

Fig. 1

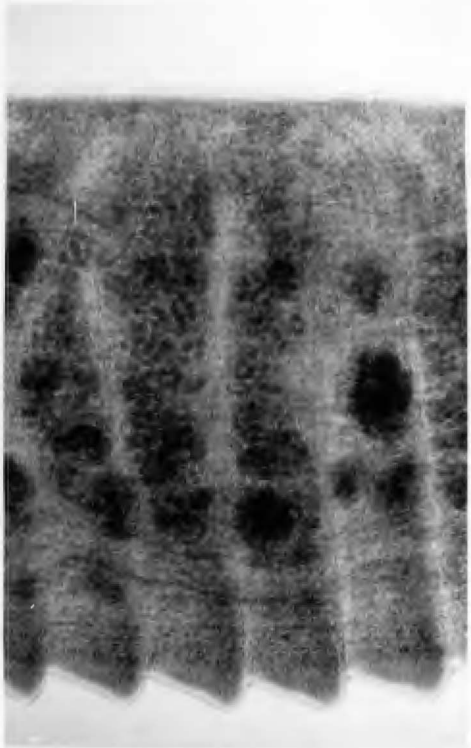

Fig. 2

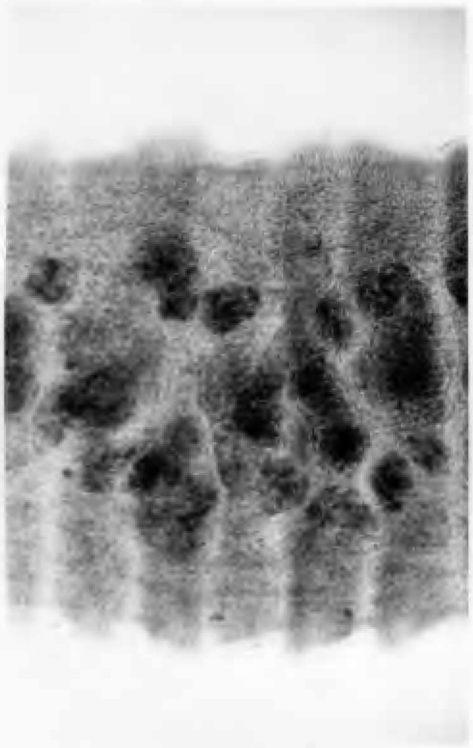

Fig. 3

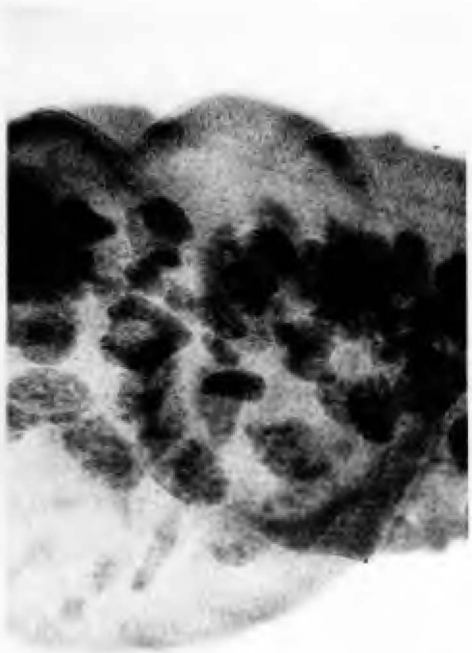

Fig. 4

PLATE V

Anomalies of segmentation