SOME ASPECTS OF FASCIOLIASIS IN UGANDA

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Dedicated to my parents whose strong hand turned me away from a road to juvenile delinquency and moulded me into a potential scholar; and especially to my mother who in later years after my father's death carried so many heavy loads of produce on her head, walked so many miles on foot and visited uncountable markets to sell her produce in order to raise money for my school fees.

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Last but by no means least, my sincere gratitude is expressed to my wife Beatrice who has always been a rich source of inspiration and encouragement all along.

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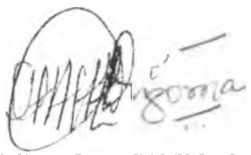
I, Apollo Hezekiah Ogambo-Ongoma

hereby declare that this work has not been submitted to any other University for the award of a degree.



APOLLO H. OGAMBO-ONGOMA

16th DECEMBER 1970.

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Chapter I : An account of a survey carried out throughout the 18 districts of Uganda to establish the spread of fascioliasis and what species of *Fasciola* is responsible for the disease in Uganda, and the findings of this survey is given.

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I N T R O D U C T I O N

Livestock farmers throughout Uganda suffer heavy annual financial losses as a result of high incidence of fascioliasis in their animals. "Wormy" livers are condemned at slaughter houses. Uganda Meat Packers abattoir has recorded a total of 209,739 cattle slaughted between May 1954 up to and including March 1969. Out of this total, 34,992 animals had their livers condemned, as a result of fascioliasis infection. Authorities of the Ankole Ranching Scheme in Western Uganda reckon that 35-40% of their cattle sent to abattoirs have their livers condemned due to fascioliasis infection (personal communication).

Coyle (1956) has stated "In assessing the economic importance of the disease (fascioliasis) to Uganda, two separate aspects must be considered; firstly, the loss of liver both to the consumer and butcher; and secondly, the quite inestimable loss due to loss of health during life (of the animal) with subsequent loss of carcass value after death. Much of this loss of health is brought about by increased susceptibility to other predisposing diseases..... Sudden deaths in Uganda sheep are common due to mass invasion of the liver by young flukes."

Work on fascioliasis has been in progress in the world and in Africa for many years. In East Africa important studies are those of Dinnik and Dinnik (1958, 1959, 1963 and 1964), Froyd (1959), Bitakaramire (1968) in Kenya and Tanzania. In Uganda, the pioneering work is that of Coyle (1956 and 1958) and more recently that of Mitchel (1968); however, much remains to be done to try and answer a number of questions that are still a mystery to veterinarians and farmers.

It was therefore decided that the following aspects of fascioliasis be investigated.

a) A survey to establish the spread of fascioliasis and the species of Fasciola that is responsible for the disease in Uganda's livestock.

b) The field epidemiology of the disease in Uganda.

c) The population dynamics of the snail host Lymnaea natalensis in relation to its habitats in Uganda.

d) The intramolluscan phase of the life cycle of the species of Fasciola responsible for fascioliasis in Uganda.

The story of fascioliasis in the world dates back many years. The earlier published work, according to Taylor (1964), goes back to Jean de Brie, who was ordered by Charles V of France to write a treatise on wool production, and who, according to some later accounts of the work (the original having been lost), made reference to the liver fluke and to the disease "liver rot". It never occured to de Brie that the disease might be caused by the fluke, but rather as the result of "a corruption of the liver through noxious substances produced by certain plants, that give rise to development of the worms."

Reinhard (1957) refers to Sir Anthony Fitsherbert's book of 1523, "A newe Tracte or Treatyse moost profitable for all Husbandmen" as giving the first recognizable description of the liver fluke. After this, very occasional accounts on the subject appeared in literature. Next came records on observations of several intermediate stages. At this time, there existed no idea of their connection with one another or that they might represent various stages in the growth of one and the same organism. For 40 or 50 years cercariae swimming in pond water were written about without their connection with the liver fluke being suspected. An intervening period of 115 years elapsed between the first recorded observation of a cercaria and the proof that cercariae represent an earlier stage in the development of a fluke.

Prior to the important observations made by Thomas (1882) and Leuckart (1882) there were isolated observations and recognition of connections between the different stages.

In 1737 Swammerdam observed while dissecting a snail some separate living things which he recognised as not being part of snail; he recognised these were parasites, which he called "worms" (a popular term in those days referring to any creature that was long and thin, without legs and which wriggled). According to Taylor (1964) Swammerdam's illustrations of the "worms" are very easily recognizable as the cercariae of trematodes. It took 145 years after this first record of the intramolluscan stage before the life cycle of the liver fluke was worked out.

In 1773, Muller reported his discovery of a microscopic tadpole-like creature that swam about in pond water. Muller named this creature "Cercaria". In 1783 Hermann noticed similar creatures and named them Vibrio; however, these were of more elongated shape. Other new names appeared e.g. Furcocera (for cercariae with forked tails). According to Schmid (1929) Weinland in 1875 described a cercaria that later proved to be identical to that of Fasciola hepatica.

In 1800, Zeder recorded the process of hatching of a trematode egg and the subsquent escape of the miracidium into the water. In 1837 Creplin observed this process in the egg of the liver fluke itself. After Zeder's observation, Nitzsch in 1807 reported the process of encystment of a cercaria; he interpreted this process as "an unusual kind of death" with no notion that he had observed the encystment of the infective stage of a trematode. In 1816, Nitzsch went further and pointed out the remarkable similarity between the cercariae and the flukes, although he was still of the opinion that the two were independent. He wrote, "the tail of the cercaria clearly distinguishes it from flukes and from all other forms of pond life".

Up to this time, therefore, the only thing that was definitely known about the life history of the liver fluke, or any other fluke for that matter, was that eggs were laid. All of the many observations on cercariae and rediae, leading up to Nitzsch's observed connection between the cercaria and the metacercaria, still remained isolated from one another. The nature of the creature hidden under the disguise was still to be revealed.

In 1818 Bojanus reported the discovery of what he called the royal-yallows worms while dissecting some pond snails. These "royal-yallows worms" contained cercariae, and that these

enventually emerged from the nurse worm and swam off, tadpole-fashion, into the water.

In 1813, Mehlis made the next significant contribution. He reported how he had witnessed the minute, ciliated embryo pushing off the end of the fluke's egg and swimming rapidly away in water. He therefore suggested that its energetic movements might represent a search for something that could enable it to grow to a more advanced stage of development at which it could preceed to infect the final host. Earlier on, it had come as a surprise to Biologists that the young fluke should be so different from the adult which had laid the egg, and this suggestion of Mehlis' helped to form the earlier idea that sheep and cattle do not become infected through swallowing the fluke's egg but some other stage in its complicated development. Despite this suggestion by Mehlis, the popular theory still remained, that in some way the swallowing of eggs with the food must give rise to the appearance of flukes in the liver. As late as 1862 when Simmonds reported a negative result having "exhibited" thousands of eggs to an experimental sheep, which was killed some six months later. Similar negative results had been realised in Germany and elsewhere.

In 1837, van Siebold noticed that a miracidium of a certain fluke contained an embryo which in general form resembled the rediae that had for so long been known in the internal organs of snails.

In 1842, Steenstrup, professor of Zoology at Copenhagen published his "principle of metagenesis or the alternation of generations". He defined his theory "the remarkable phenomenon of an animal producing an offspring which at no time resembles its parent, but which, on the other hand, itself brings forth a progeny, which returns in its form and nature to the parent animal, so that the maternal animal does not meet with its resemblance in its own brood but in its descendants of the second, third, or fourth degree or generation; and this always takes place in the different animals which exhibit the phenomenon, in a determinate number of generations." He fitted the theory to the life history of the trematodes among other creatures, and expressed the opinion that the whole established divisions of families of animals must now be abolished, since they included only undeveloped forms and that several forms which had been regarded as of different species and genera would be found to be stages in the development of one and the same animal.

Steenstrup's application of this principle to the life history of trematodes contained several fallacies: the redia was regarded as the "wet nurse" of the cercariae which, in their turn, were thought to be essential to the existence of the rediae; the cercariae were regarded as "trying to return to the snail in order to pupate because they had lost their tails," and Steenstrup considered that the sexually mature fluke must exist in the snail which becomes infected through the cercariae. The theory of alternation of generations made the specific identity of these many varied forms seem possible, however, and offered a solution to what had previously been inexplicable.

In 1852, Leuckart advanced the idea that a certain fluke found in the intestine of predaceous fishes was specifically identical with an encapsulated cercaria found in the gills of certain other smaller fishes on which the final host feed. This idea was later shown to be true.

In 1855 La Valette de St. George published results of feeding experiments demonstrating that certain encysted cercariae from water snails developed into sexually mature flukes in birds. He also observed that only the encysted cercariae would do this, and that the tailed cercariae which swam actively in the water and had not yet become encysted were digested in

the intestines of an experimental host.

In 1854 de Filippi studied and described a new trematode, Distoma paludinae impurae. During this study he showed, for the first time, the difference between the sporocyst and the redia of this parasite. He came to understand that a parasite he had earlier described as Redia gracilis, in 1837 was an ealier stage in the development of an amphistome and that similar stages occur in other species. He, therefore, went on to conclude that Redia is not a generic name for an adult trematode, but like the genus Cercaria. Muller, 1773, is merely a stage in the life cycle of certain trematodes. de Filippi described sporocysts as being simple membranous sacs, without internal organization, whereas rediae are provided with a mouth, muscular pharynx and an intestine.

In 1857 Wagener observed a miracidium penetrate a snail and its subsequent development into the redia. This last link in the whole chain in the life cycle of the liver fluke took 120 years after the publication of drawings of cercariae by Swammerdam.

Between 1880 and 1883 Thomas and Leuckart working independently elucidated the life cycle of Fasciola hepatica by showing all its stages plus the snail carrier Lymnaea truncatula. This was then the pleasant conclusion of 145 years of many observations and hard work by many biologists.

Although as early as 1807 Nitzsch had noticed the encystment of a cercaria it never occured to him that he had witnessed the encystment of the infective stage of a trematode. Strangely enough he interpreted the process as "unusual kind of death." It was not until 1913 that the term metacercaria came into being. Dollfus (1912) proposed the term "meta-cercaire" to designate the immediate post-cercarial stage, whether encysted or not, and in later publications wrote the term as a single word, "metacercaire."

Most of the early work described dealt with Fasciola hepatica. At this time other workers were engaged in taxonomical work. In 1856, Cobbold described a liver fluke he recovered from a giraffe belonging to a travelling menagerie in England. To this fluke Cobbold gave the name Fasciola gigantica. Later in 1895 from cattle at a St. Louis abattoir in Senagal Railliet described a new liver fluke which he named Fasciola hepatica var. angusta. From a slaughter house in Egypt, Loos (1896) described a new liver fluke he recovered from cattle; he called

this parasite Fasciola hepatica var. aegyptiaca. Both of these were later to be classified together with Cobbold's species Fasciola gigantica.

In South Africa Porter (1920) elucidated the life history of F. gigantica. She used rats, rabbits, guinea-pigs and sheep as definitive hosts and the snail Lymnaea natalensis as the intermediate host. Alicata (1938) repeated the cycle using rabbits and the snail host Fossaria ollula in Hawaii.

Although Porter (1920) and other workers refer to F. gigantica as the African liver fluke, its geographical distribution is quite wide. This wide geographical distribution has necessitated the use of other species of snails in different parts of the world. In Africa Monning (1934) reports that Lymnaea natalensis and Physopsis africana are the two snail hosts of the fluke. Khalerao (1933) points out that Lymnaea acuminata is the host in India. From the Philippines, Manipol (1936) reports Lymnaea philippinensis, L. swinhoei and Amphipeplea cumingiana as the hosts, while Alicata and Swanson (1937) have found Fossaria ollula and Pseudosuccinea columella Alicata (1953b) to be the hosts in Hawaii. According to Pantelouris (1965), Kibakin (1960) has reported Radix lagotis, R. Ovata, R. peregor, Galba truncatula, and Physa acuta to be the hosts in Turkmen, Russia.

C H A P T E T I

1. FASCIOLIASIS SURVEY

Fascioliasis as a disease of livestock has been recognized in East Africa for a long time. Records received from Uganda Meat Packers Ltd. abattoir show that the condemnation rate of livers infected with Fasciola is on the increase over the last ten years. Coyle (1956, 1958) has reported his work on fascioliasis in Uganda where he says no Fasciola hepatica had been reported up to 1956. Mitchel (1968) has stated that Kenya and Uganda are inhabited solely by Fasciola hepatica while Tanzania is exclusively F. gigantica territory. Bitakaramire (1968) has reported that in Kenya, fascioliasis of cattle is mainly due to F. gigantica. Ogambo-Ongoma (1969) has reported the incidence of F. hepatica in Kenya cattle as being very slight and confined to the highlands while the vast majority of cases of fascioliasis, whether in the highlands or at lower altitudes are due to F. gigantica.

It was therefore decided that a detailed survey of the disease be carried out throughout Uganda to establish the following facts:

a) The spread of fascioliasis in Uganda.

b) What species of Fasciola is responsible for the disease in Uganda cattle.

1.1 MATERIALS AND METHODS

Over a period of 25 months various places thoughout the eighteen districts of Uganda were visited; these included big towns and small villages; lakes, streams, rivers, ponds, swamps and small ditches or holes that had water in them. At abattoirs and slaughter sites, livers of slaughtered animals were examined; two transverse incisions were made across the main bile ducts visible on the surface of the liver lobe. The lobe was squeezed to see if flukes would come out through the cuts. A third incision was made longitudinally through the lobes to expose ducts in the center. Flukes, when present, were examined visually and some of them put into plastic bottles with 70% alcohol. Name of the slaughter site or abattoir, the area from where the animal was raised and the number of livers examined and those infected with Fasciola were recorded. The flukes thus preserved in alcohol were taken back to the laboratory at Makerere University where they were fixed in AFA, stained in acetocarmine and mounted in Canadian balsam for identification.

From various bodies of water snails were collected using a net. Whenever the right snail host, Lymnaea natalensis, was found the snails were placed in a petri dish with tap water brought from the laboratory, dissected under a dissecting microscope in the field, and examined for the intramolluscan stages of Fasciola. The number of snails examined, and those infected with Fasciola, and the district where they were found were recorded. See Table II.

From the following centers, listed according to districts livers infected with Fasciola were found. See appendix I. Table I shows the number of cattle livers examined and those infected in every district. Most of the centers were visited by the author; however, a few of the centers are listed because some of the infected animals examined at some of the visited centers were raised in different localities. Such animals are recorded as coming from the place where they were raised and not where they were slaughtered. Names of a number of smaller villages which were visited cannot be located on the map, such places are regarded as being part of the larger centers and are therefore recorded as such. Places where Fasciola was found are represented on the Uganda map by dots. Each dot represents a single known center including smaller villages immediately surrounding it. See figure 1.

1.2 RESULTS:

Over a period of 25 months -- September 1968 to September 1970 - a total of 11,433 cattle livers were examined throughout Uganda; of this total 6,144 were infected with Fasciola. Full results based on districts is shown in Table I. All species of flukes recovered and identified turned out to be Fasciola gigantica Cobbold, 1856.

Over the same period, a total of 8,311 Lymnaea natalensis were examined, and out of this total 981 were infected with intramolluscan stages of Fasciola. See Table II for full results based on districts.

Figure 1. A map of Uganda showing the distribution of fascioliasis.

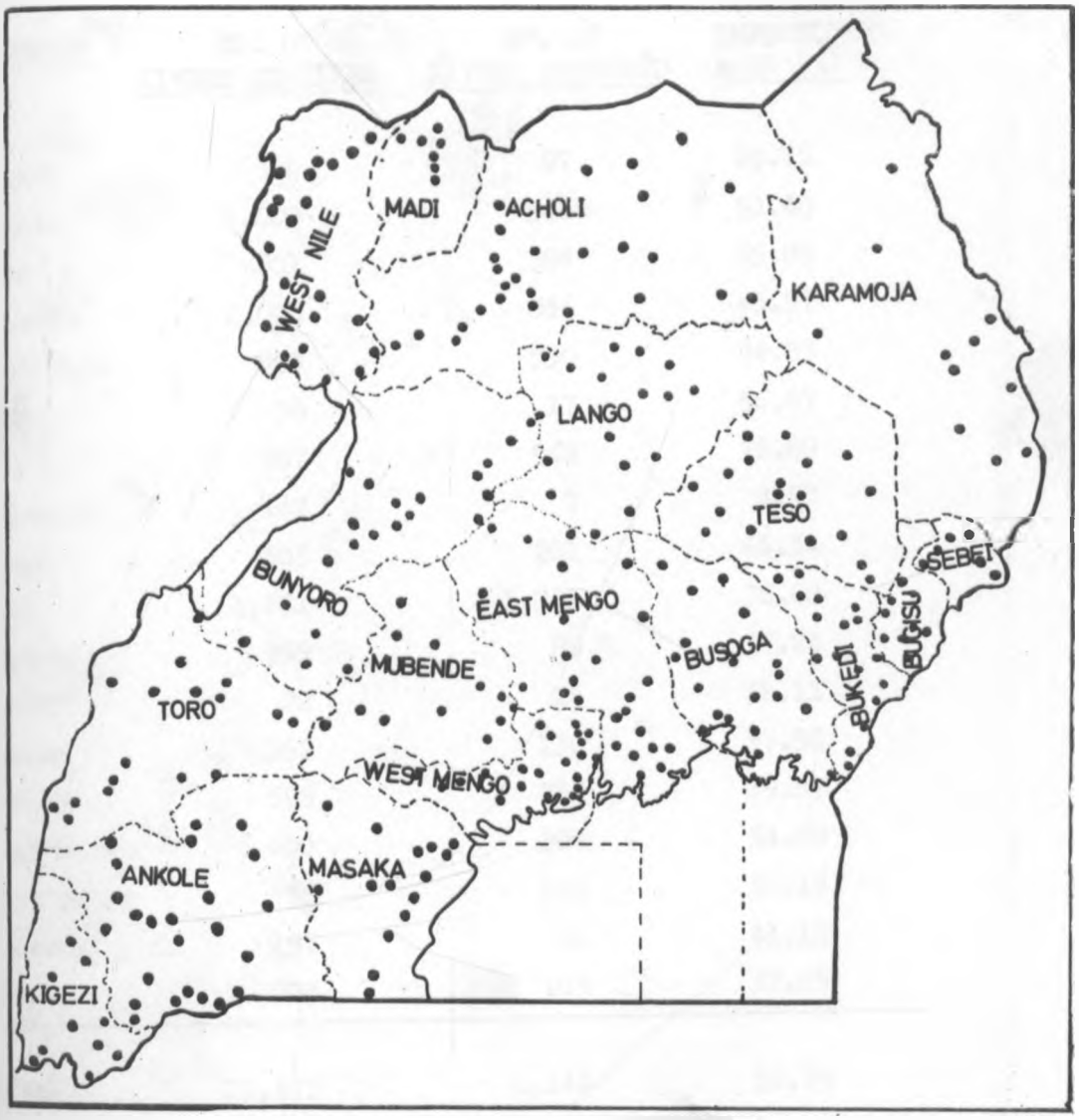
WEST NILE
MADI
ACHOLI
KARAMOJA
LANGO
TESO
SEBEI
BUNYORO
EAST MENGO
BUGISU
BUSOGA
BUKEDI
MUBENDE
TORO
WEST MENGO
ANKOLE
MASAKA
KIGEZI

Table I. CATTLE LIVERS EXAMINED FOR FASCIOLA INFECTION IN VARIOUS DISTRICTS

DISTRICT	NO. OF LIVERS EXAMINED	No. OF LIVERS INFECTED	INFECTION RATE (%)
Kigezi	326	97	29.75
Ankole	1,809	966	53.40
Toro	601	396	65.89
Bunyoro	2,036	936	45.97
West Nile	753	550	74.83
Madi	30	17	56.67
Acholi	687	542	78.89
Karamoja	142	7	4.93
Lango	303	201	66.34
Teso	1,651	1,301	78.80
Bugisu	239	89	37.24
Sebei	72	26	36.11
Bukedi	352	132	37.50
Busoga	599	237	39.57
East Mengo	6,600	204	34.00
West Mengo	758	259	34.16
Mubende	197	81	41.12
Masaka	278	103	37.05
Total	11,433	6,144	53,74

Table II. SNAILS EXAMINED FOR INTERMOLLUSCAN STAGES OF BASCIOLA IN VARIOUS DISTRICTS.

DISTRICT	NO. OF SNAILS EXAMINED	NO. OF SNAILS INFECTED	INFECTION RATE (%)
Kigezi	201	30	14.93
Ankole	123	21	17.07
Toro	250	42	16.80
Bunyoro	245	36	14.69
West Nile	172	18	10.47
Madi	96	12	12.50
Acholi	203	27	13.30
Karamoja	47	6	12.77
Lango	306	45	14.71
Teso	331	51	15.39
Bugisu	115	17	14.78
Sebei	104	8	7.67
Bukedi	217	23	10.60
Busoga	388	41	10.57
East Mengo	200	19	9.50
West Mengo	4806+	530	11.03
Mubende	146	18	12.33
Masaka	311	37	11.90
Total	8311	981	

+The number of snails examined in West Mengo District is much higher than the rest of the districts because of snails which were collected bi-weekly for 24 months from Port Bell and used in another experiment.

1.3 DISCUSSION:

This survey reveals the existence of fascioliasis in all districts of Uganda, even though some districts have a lower incidence than the others. The average liver infection rate of 53.74% throughout the nation is a very costly loss in revenue not only to the farmer and butcher, but to the nation as well. Some figures, like those recorded by the Uganda Meat Packers abattoir show an average liver infection rate of 31.4% over the last 10 years, this is much lower than the national average liver infection rate of 53.74% revealed by this survey. This could be attributed to two main factors:

a) Cattle going through the Uganda Meat Packers abattoir come in from Uganda, Tanzania and Kenya, therefore, figures recorded do not necessarily reveal the true picture of fascioliasis in Uganda, but rather that of East Africa.

b) Most cattle slaughtered at this abattoir come in mainly from well maintained ranches like those of the Uganda Livestock Industries Corporation, and various Co-operative Ranches throughout the country. Cattle from these areas, even though they are still infected, have a greater protection against fascioliasis through proper

grazing and watering practices and to some extent through the use of drugs. On the other hand, the villagers who practice communal grazing and watering do expose their cattle much more to the disease than the ranches do.

Animals that are raised along rivers and swamps covered with papyrus do seem to be free from fascioliasis to a certain extent. This is evidenced by cattle raised along river Nile in West Nile, and Madi and some of the papyrus swamps in East and West Mengo. It is also interesting to note that on many occasions careful search for snails in these papyrus-covered swamps and rivers have failed to reveal the presence of any lymnaeid snails in several areas throughout Uganda. The high dissolved oxygen concentration in water required by lymnaeid snails Pennak (1953) is not found in these habitats. The papyrus plants do form a kind of mat with its roots which spread over the water surface and hence does not allow free access of atmospheric oxygen to the water. The dying and decaying bits of plant material also deplete the water of oxygen and adds more carbon dioxide. This situation renders the habitat completely unsuitable for Lymnaea natalensis. It is, therefore, not by accident that cattle grazing in such habitats are relatively free of fascioliasis, since the intermediate host of the parasite is very rare in such habitats.

In the drier districts like Karamoja, the incidence of fascioliasis in cattle is low; however, it is important to note that the infection rate in snails compares very favourably with that of other districts in the nation. One can, therefore, explain this situation in terms of availability of snail habitats. Due to dryness in the district there are relatively fewer snail habitats and therefore, not all cattle are exposed to them. The disease could increase very easily with the introduction of new snail habitats through human activities, such as irrigation and bore holes.

Certain districts like Teso, Lango, West Nile and Bunyoro have a high rate of infection among cattle, yet the infection rate in snails remains almost the same as in other districts. This situation exists because snail habitats are widespread throughout these districts; and therefore cattle are exposed to the disease over a wide area.

Ankole District which is relatively dry in some areas is becoming a district with many snail habitats through dams which are being built to hold and store rain water for cattle in most of the ranches.

These dams very soon harbour lots of lymnaied snails; as watering points, they are focal points for all cattle in a particular locality, the incidence of the disease is therefore bound to increase in future.

Overall, one can only forecast an increase in the incidence of fascioliasis throughout the country in future. Uganda Government has already started some irrigation schemes such as the Mubuku Irrigation Scheme. As much as these schemes are going to introduce water into hitherto dry areas and therefore, bring these areas under agricultural production, they are also going to introduce more snail habitats. Irrigation channels that are kept without too much vegetation to 'choke' channels are very favourable breeding grounds for Lymnaea natalensis, Ogambo-Ongoma (1970). Therefore, as we introduce fresh snail habitats in the country, we are in effect increasing the incidence of fascioliasis throughout the country.

All trematodes recovered and identified from all the 18 districts of Uganda proved to be Fasciola gigantica Cobbold, 1856. Contrary to what Mitchel (1968) reported, the author could not find a single species of F. hepatica. Granted that by strange coincidence the author missed just the cattle that had....

had F. hepatica, still it doesn't agree with Mitchel's findings, for what he reported is that the whole of Uganda is exclusively F. hepatica territory. It is, therefore, evident, based on this survey, that Fasciola gigantica is responsible for fascioliasis and not F. hepatica in Uganda; this is in agreement with Coyle (1956) who reported that up to that year no F. hepatica had been reported in Uganda.

The intramolluscan stages found in snails were identified as those of Fasciola. It was not possible to tell whether they were Fasciola gigantica or F. hepatica as it is difficult to distinguish the intramolluscan stages of the two species. Since all adult flukes recovered from cattle which grazed in the same habitats from which snails were collected, were identified as F. gigantica it is therefore, concluded that the intramolluscan stages were those of Fasciola gigantica Cobbold, 1856.

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C H A P T E R II

2. FIELD EPIDEMIOLOGY OF FASCIOLIASIS

Fascioliasis is an important disease among cattle in Uganda. Ogambo-Ongoma (1970) found the incidence of the disease in all districts of Uganda. Coyle (1956) has reported that up to 1956 no Fasciola hepatica had been recorded in Uganda. Ogambo-Ongoma (1970) examined flukes recovered from cattle livers from all districts in Uganda and found all of them to be Fasciola gigantica. It is therefore evident that based on data so far collected, fascioliasis in Uganda's livestock is caused by F. gigantica. The occurence of this trematode is determined by the presence of the intermediate snail host, Lymnaea natalensis; the incidence of the disease, however, is influenced by several factors, seasonal ones included. Ingestion of the metacercariae by the grazing livestock is dependent on the availability of the former on pastures. This in turn depends on the presence of mature cercariae in the intermediate host. The quantity of these mature cercariae in the snails will determine whether clinical or sub-clinical disease will result in a given habitat.

The epidemiology of fascioliasis and the factors involved have been discussed by Ollerenshaw (1959) in Britain; and by Gordon (1955), Boray (1963), and Boray, Happich and Andrew (1969) in Australia.

The effective control of fascioliasis depends on the application of both curative and preventive measures, combined with proper livestock management. Any effective preventive measures must therefore be based on information concerning the intermediate snail host and its habitat, including seasonal fluctuation, if any, in the proportion of infected snails in a given population, and the proportion of the infected ones that actually harbour mature cercariae, since the infective metacercaria can only develop from the former.

It was therefore decided to investigate whether certain seasons of the year favour a higher incidence of the infective stage of Fasciola gigantica in the snail than other seasons.

2.1 MATERIALS AND METHODS

A well known fascioliasis endemic area at Port Bell near the shores of Lake Victoria was chosen for investigation. This area is used as a communal grazing ground for cattle owned by villagers living in the sorrounding villages. In addition, the

area is used as a holding ground for cattle shipped across the lake from Tanzania and destined for slaughter houses in Kampala. There are therefore, cattle present in this habitat every day of the year. The whole area is a flat grassland with isolated bushes on scattered termite mounts, and uneven depressions (that hold rain water for a few days following heavy rains) scattered throughout. Several channels drain used water from nearby factories and Luzira Prison into the lake. One of the channels, however, drains water into the lake from an underground spring used as a source of drinking water by nearby villagers. All channels are occasionally cleared of their vegetation by factory owners and prison authorities.

The channel originating from the spring was chosen for its fresh water and high snail population as a snail habitat for investigation; it is roughly 2½ feet deep by 2½ feet wide with steep banks and sandy bottom. The water depth varies from 6 inches in the drier part of the year to about 12 to 18 inches during the wet season. The water flow is generally sluggish and because of its steep banks water does not overflow from the channel into the sorrounding flat ground at any time. The snail population in this channel is predominantly Lymnaea natalensis, although a few Biomphalaria sudanica and very rarely Bulinus sp. occur.

Lymnaea natalensis were collected from this habitat twice every month for a period of 24 months from November 1968 up to and including October 1970. The first collecting was done within the first week and the second collecting done within the last week of every month throughout the study period. Snails were picked by hand using a long pair of forceps along the entire length of the channel and an average of 183 snails were collected every month.

These snails were taken to the laboratory where they were placed in a plastic basin with tap water. The snails were dissected individually and the intramolluscan stages of Fasciola gigantica in them counted. Each snail was placed in a petri dish with tap water and the length of its shell measured in millimetres, after which the dish was put on a dissecting microscope and the shell carefully removed using finely pointed forceps. The snail tissues were macerated and the intramolluscan stages released in the process were uniformly spread throughout the petri dish. Using the same dissecting microscope five different fields were examined; all cercariae seen in each field were counted using a hand tally counter and the average number of these per field recorded. Rediae were similarly counted and recorded. The area of the petri dish

seen....

seen in one field at a particular magnification was determined by measuring the diameter of the field and then calculate the total area in one field. The diameter of the petri dish was also measured and its total area calculated. The total number of parasites (P) in the whole petri dish was calculated by dividing the total area of the petri dish (D) by the area of each field (d) and multiplied by the number of parasites in each field (F). Thus the formula for calculating the total number of parasites in a whole petri dish is: $\frac{D\ F}{d} = P$.

Records of all these were kept for every snail dissected throughout the 24 months period.

To confirm that the intramolluscan stages dissected out of the wild snails were those of Fasciola gigantica, two methods were employed: a) Infected wild snails collected from the same habitat were kept singly in petri dishes with tap water over night. The following morning those that were shedding cercariae were removed and placed in different petri dishes containing tap water and sweet potato leaves; snails were left in there until they shed cercariae which encysted on the potato leaves as metacercariae. (The potato leaves used were grown far from any aquatic environment, nor was irrigation used in raising them

and....

and therefore there is no possibility that the leaves had metacercariae encysted on them while in the garden.) The potato leaves with metacercariae encysted on them were fed to 'fluke-free' rabbits. The rabbits were later autopsied and adult trematodes recovered; these were later identified.

b) F. gigantica eggs were recovered from gall bladders of infected cattle and taken to the laboratory where they were incubated at room temperature till miracidia hatched. Laboratory raised snails were exposed to these miracidia and then kept in aquarium tanks. Later the exposed snails were dissected and the intramolluscan stages of F. gigantica in them were recovered and compared to those dissected out of wild snails.

For the purpose of this study any fully formed cercaria, even though it was still inside the redia was regarded as a mature cercaria and was therefore counted as such. Any redia that had even one fully formed cercaria was not counted; only redia that had not developed any cercariae were regarded as such.

2.2 RESULTS:

Metercercariae from wild snails fed to 'fluke free' rabbits developed into adult trematodes which were identified as Fasciola gigantica Cobbold, 1856. Both rediae and cercariae

from.....

from wild snails were similar to those dissected from laboratory raised snails which had been exposed to miracidia hatched out of eggs that were recovered from gall bladders of cattle infected with F. gigantica.

During the two year period of study, 4,932 snails were dissected and examined for the intramolluscan stages of Fasciola gigantica; out of this total 512 were infected, giving an infection rate of 11.[illegible]%. The rate of infection varies from one month to another, and this seems to fluctuate with rainfall so that the incidence of infected snails increase as rainfall increases. See Table III for monthly variations, Figure II for graphical comparison of rates of infection with Rainfall, and Figure III for the correlation-regression diagram.

Table III: SHOWING MONTHLY RAINFALL AND INFECTION RATES AMONG SNAILS DISSECTED

MONTH	No. DISSECTED	No. INFECTED	(%) INFECTION RATE	RAINFALL IN CM.	RAIN DAYS
November 1968	90	18	20.00	18.04	19
December 1968	35	8	22.86	14.05	10
January 1969	96	15	15.63	8.34	9
February 1969	84	7	8.33	15.22	8
March 1969	92	21	22.83	12.44	11
April 1969	124	20	16.13	17.30	16
May 1969	100	5	5.00	7.81	9
June 1969	98	15	15.31	3.01	7
July 1969	100	5	5.00	3.22	4
August 1969	95	7	7.37	3.70	5
September 1969	21	1	4.76	16.98	12
October 1969	173	3	1.73	6.49	7
November 1969	138	4	2.90	14.42	14
December 1969	202	32	15.84	2.71	6
January 1970	161	41	25.47	22.37	10
February 1970	291	43	14.78	5.18	5
March 1970	314	58	18.47	14.83	18
April 1970	678	90	13.27	18.30	16
May 1970	566	32	5.65	7.36	14
June 1970	251	40	15.94	1.19	3
July 1970	249	4	1.61	7.70	6
August 1970	118	3	2.54	15.95	8
September 1970	156	23	14.74	5.64	10
October 1970	160	17	10.63	9.95	14

Figure 2. Graph showing monthly variations in fascioliasis infection rate among snails.

LEGEND:

INFECTION RATE.

RAINFALL.

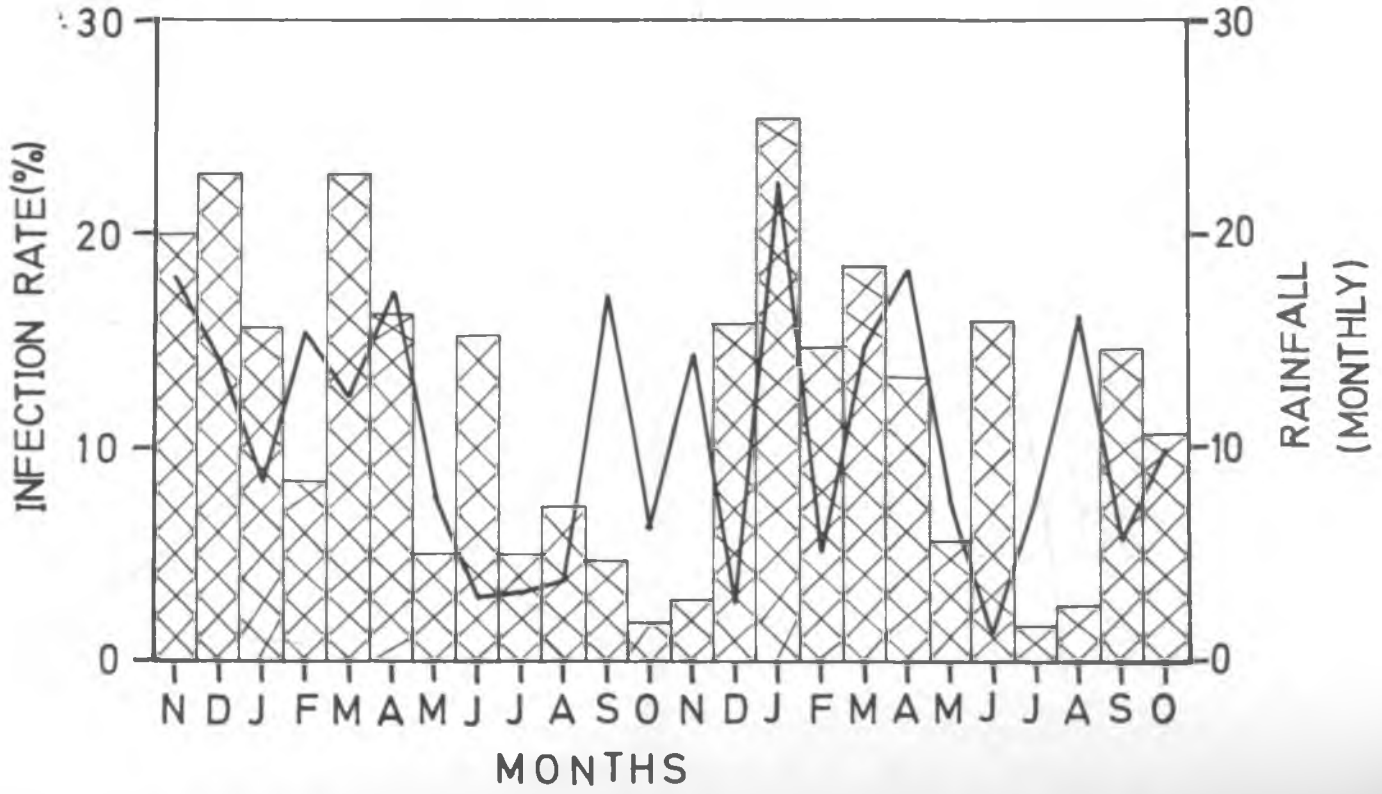
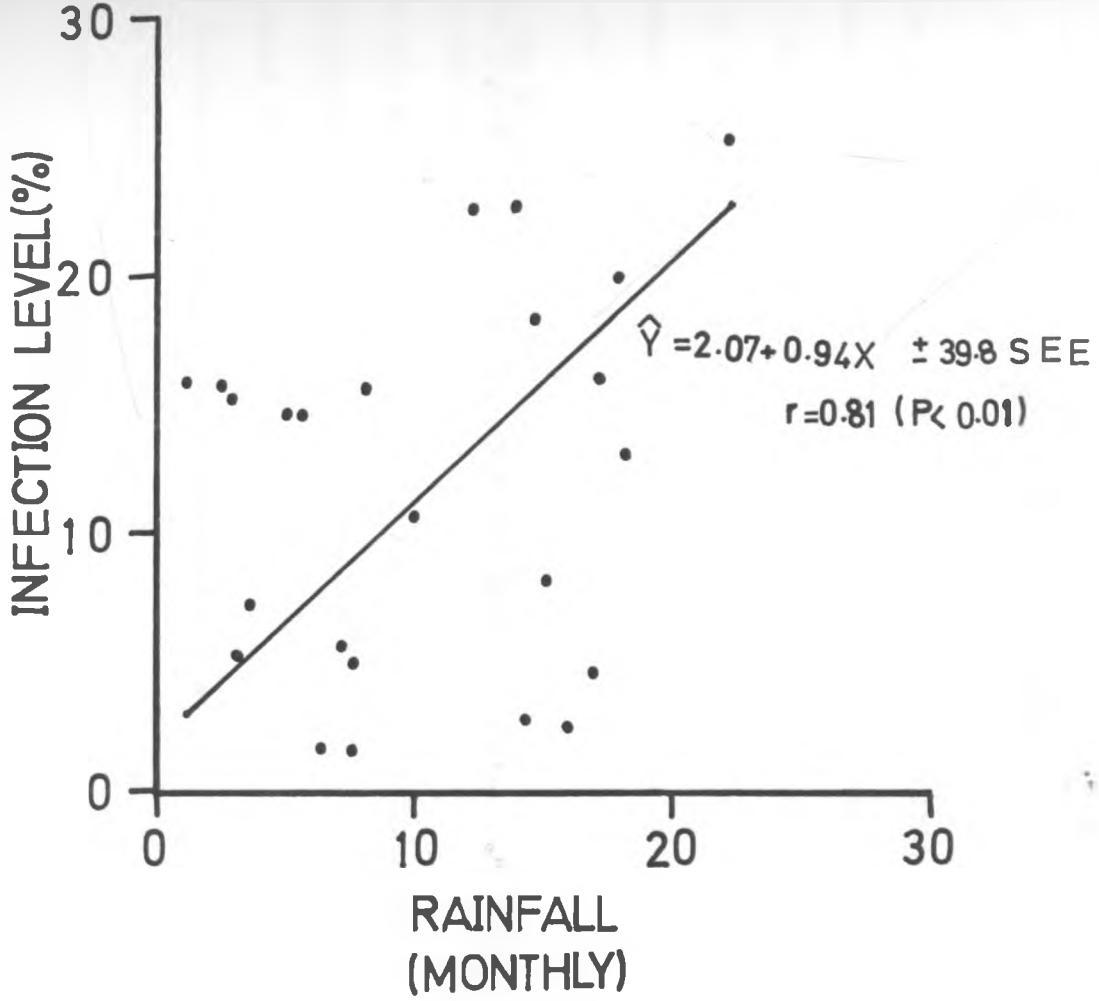

30
20
10
0
INFECTION RATE(%)
RAINFALL
(MONTHLY)
N D J F M A M J J A S O N D J F M A M J J A S O
MONTHS

Figure 3. Diagram showing the correlation between rainfall and infection rate, and the regression line.


30
20
10
0
INFECTION LEVEL(%)
0
10
20
30
RAINFALL
(MONTHLY)
Ŷ =2·07+0·94X ± 39·8 SEE
r=0·81 (P< 0·01)

Of the 512 infected snails 236 or 51.37% had mature cercariae in them and 249 or 48.63% had only rediae with no cercariae at all. All the 512 infected snails had rediae in them although snails that had cercariae in them contained very few rediae compared to those with rediae only. The incidence of snails infected with both cercariae and rediae, and those infected with the latter only varied from one month to another. The variation follows rainfall fluctuation so that the incidence of snails harbouring cercariae increases as rainfall decreases, and decreases as rainfall increases. On the contrary, the incidence of snails harbouring rediae only increases as rainfall increases and decreases as rainfall decreases. See Table IV for variation in monthly infection rates and Figure IV for a comparison of monthly variations with rainfall, and Figure V for the correlation-regression diagram.

Table IV: SHOWING MONTHLY INFECTION WITH REDIAE AND WITH BOTH REDIAE AND CERCARIAE

MONTH	NO. INFECTED	NO. WITH REDIAE ONLY	% AGE WITH REDIAE ONLY	NO. WITH CERCARIAE	% AGE WITH CERCARIAE
November 1968	18	8	44.44	10	55.56
December 1968	8	1	12.50	7	87.50
January 1969	15	3	20.00	12	80.00
February 1969	7	4	57.14	3	42.86
March 1969	21	15	71.43	6	28.57
April 1969	20	11	55.00	9	45.00
May 1969	5	4	80.00	1	20.00
June 1969	15	9	60.00	6	40.00
July 1969	5	1	20.00	4	80.00
August 1969	7	3	42.86	4	57.14
September 1969	1	0	0.00	1	100.00
October 1969	3	2	66.67	1	33.33
November 1969	4	2	50.00	2	50.00
December 1969	32	14	43.75	18	56.25
January 1970	41	7	17.07	34	82.93
February 1970	43	7	16.28	36	83.72
March 1970	58	38	65.52	20	34.48
April 1970	90	58	64.44	32	35.56
May 1970	32	29	90.63	3	9.37
June 1970	40	19	47.50	21	52.50
July 1970	4	1	25.00	3	75.00
August 1970	3	0	0.00	3	100.00
September 1970	23	10	43.48	13	56.52
October 1970	17	3	17.65	14	82.35

Figure 4. Graph showing monthly variations in the percentage of the infected snails habouring rediae only and those habouring both rediae and mature cercariae.

LEGEND:

 % OF INFECTED SNAILS HABOURING BOTH REDIAE AND MATURE CERCARIAE.

 % OF INFECTED SNAILS HABOURING REDIAE ONLY.

MONTHLY RAINFALL.

C & R: CERCARIAE AND REDIAE.

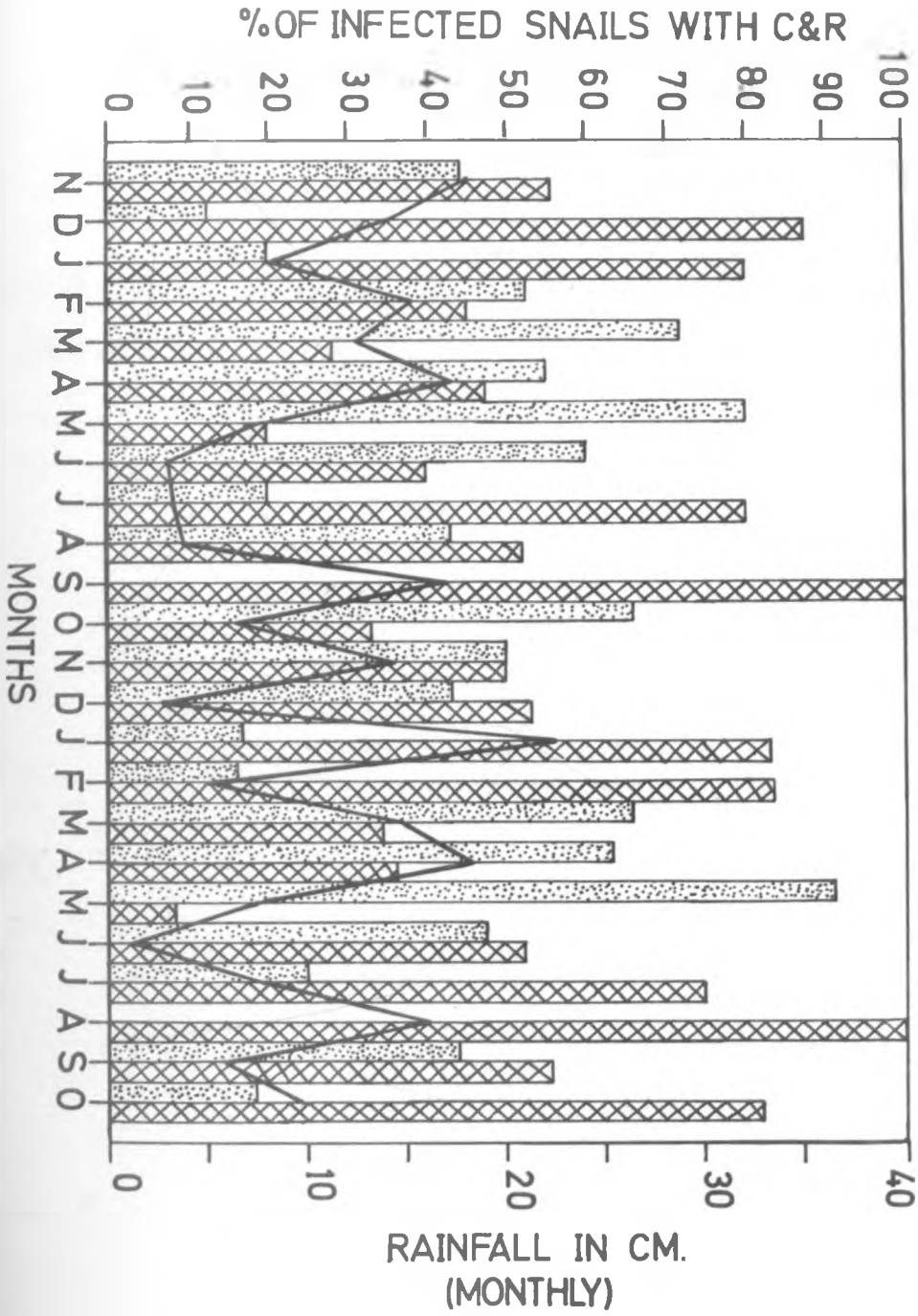
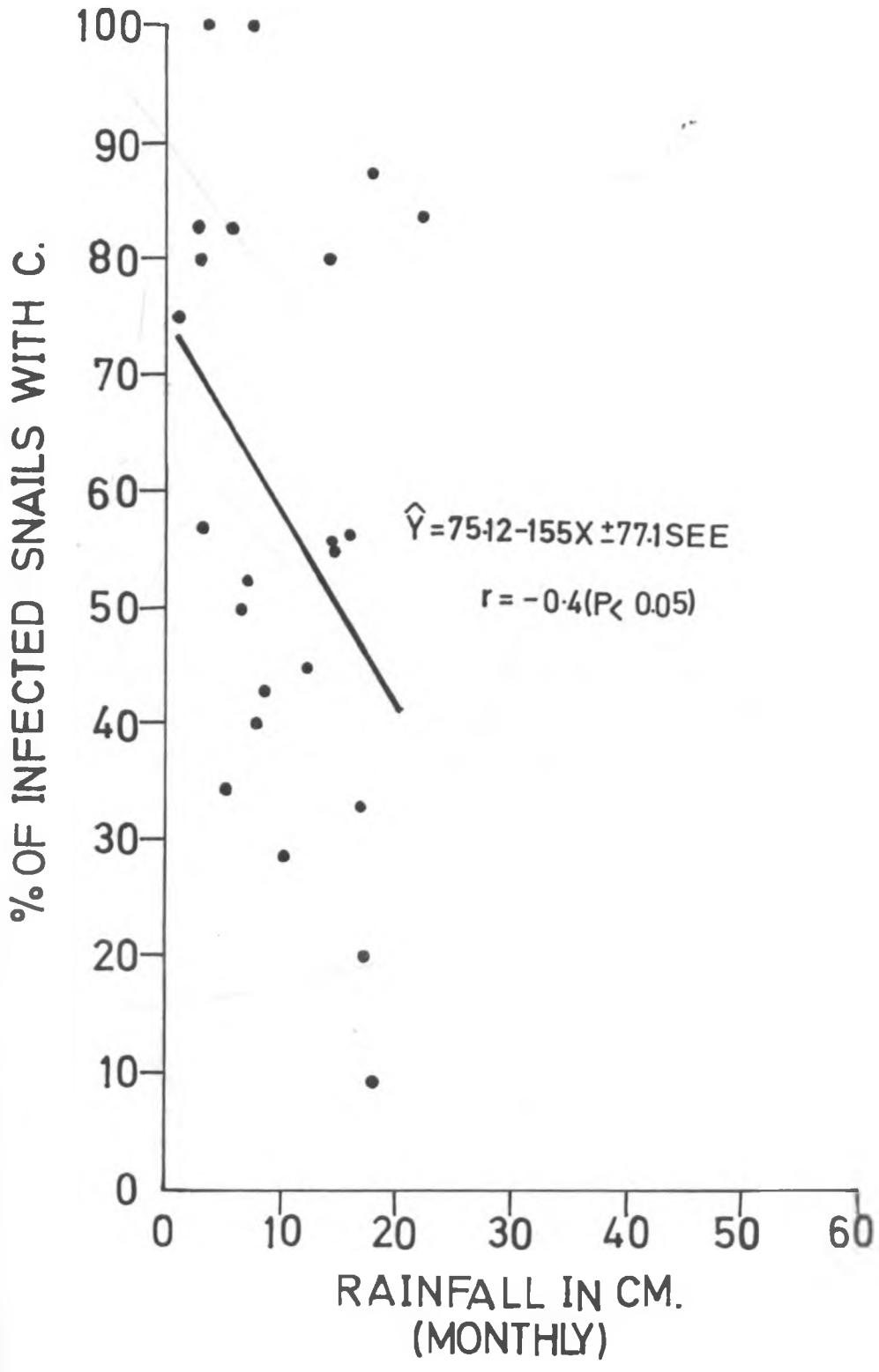

%OF INFECTED SNAILS WITH C&R
0
10
20
30
40
50
60
70
80
90
100
N D J F M A M J J A S O N D J F M A M J J A S O
MONTHS
0
10
20
30
40
RAINFALL IN CM.
(MONTHLY)

Figure 5. Diagram showing the correlation between rainfall and the percentage of infected snails habouring mature cercariae.

LEGEND

C: CERCARIAE.


100
90
80
70
60
50
40
30
20
10
0
% OF INFECTED SNAILS WITH C.
$\hat{Y}$ = 75·12 − 1·55X ± 77·1 SEE
r = − 0·4 (P < 0·05)
0
10
20
30
40
50
60
RAINFALL IN CM.
(MONTHLY)

A total of 71,141 rediae were counted from the 512 infected snails, giving an average infection of 138.95 rediae per snail. Out of the 263 snails which had cercariae in them, a total of 304,001 cercariae were counted giving an average infection of 1,155.9 cercariae per snail.

The 4,392 snails dissected varied in sizes and various sizes had varrying rates of infection with the intramolluscan stages of Fasciola gigantica. Table V shows these variations.

Table V : SNAIL SIZES AND THEIR RESPECTIVE INFECTION RATES:

	< 10 mm	10-15 mm	16-20 mm	> 20 mm	Total
No. of snails dissected	49	2645	1665	33	4392
No. of snails infected	0	262	246	4	512
Rate of infection (%)	0.00	9.91	14.77	12.12	11.66

2.3 DISCUSSION

The epidemiology of fascioliasis in Britain was discussed by Ollerenshaw (1959). Boray et al. (1969) refers to Boray's work of 1967 in which he concluded that it was relatively easier to make general recommendations in Britain and Europe for strategic application of control measures based on temperature and rainfall than in Australia where temperatures are much more favourable for propagation of Fasciola hepatica. He stated that the development of eggs on pastures and of larval stages in the snails may continue for 7 to 9 months, and in addition snails may reproduce throughout the year. Boray et al (1969) has shown that in Australia where irrigation was used the infection rate among Lymnaea tomentosa increased whenever water was applied to the pastures. They state, ".....alternating wet and dry periods in irrigation area facilitate the accumulation and periodic massive emission of cercariae and dispercal, resulting in heavy infections to animals."

In a tropical environment like that in Uganda, temperatures are favourable for the development of both the snail host and the parasite throughout the year. Looking at results obtained in this investigation, it is realised that in a given habitat the percentage of Lymnaea natalensis infected with Fasciola gigantica ....

gigantica increases with increase in rainfall. See figure II. Statistical analysis shows that rainfall is 65.6% accountable for this fluctuation in the infection rate among snails; the other 34.4% is due to other factors which could not be determined. The correlation (which is positive) between rainfall and infection rate among snails is highly significant at 1% level of confidence. The regression line shows that for every centimetre increase in rainfall there is an increase of 6.56% among snails infected with F. gigantica. See figure III. This phenomenon may be explained as follows: F. gigantica eggs passed out with dung of infected cattle depend on water for their hatching. During rainy seasons, there is plenty of water available and therefore all dung that is dropped on pastures comes in contact with water resulting in almost all fluke egg getting a chance to develop and hatch into miracidia which find and penetrate snails in the habitat. The snails that become infected during the wet season are not likely to die from heavy parasite load because after penetration has occured these snails will for sometime habour only rediae, which are usually few in number and small in size compared to later on when they will be many mature rediae and cercariae, therefore doing maximum damage to snail tissues. When dry weather sets in, the snails

infected.....

infected earlier contain many rediae that have developed or now develop rapidly to produce many thousands of cercariae. Some of the infected snails die as a result of this heavy parasitization, since numerous cercariae damage snail tissues. Another factor is that at this time the number of miracidia available for snail penetration is limited since a large proportion of dung dropped in the pastures does not come in contact with water. This situation leads to a drop in the percentage of infected snails during the dry season.

Of the Lymnaea natalensis infected with Fasciola gigantica, the percentage of those carrying mature cercariae increases as rainfall decreases. Because of the lag in time between the period of miracidial penetration and when cercariae mature, the fluctuation is noticed in the subsequent months following rainfall. See figure IV. Statistical analysis based on infection rates in subsequent months following rainfall shows that rainfall is 16% accountable for this fluctuation while the other 84% is due to other undetermined factors. The correlation (which is negative) between rainfall and percentage of infected snails carrying mature cercariae in subsequent months following rainfall is significant at 5% level of confidence.

The regression line shows that for every centimetre increase in rainfall, there is a decrease of 1.6% in the number of infected snails carrying mature cercariae. See figure 5. Boray (Personal communication) has pointed out that the high percentage of snails habouring mature cercariae during dry season is due to the fact that snails are in the process of aestivating and that cercariae in the tissues of aestivating snails do not escape from the tissue; this therefore leads to accumulation of cercariae in snail tissues, he concluded. On the other hand, he maintains that during the wet season snails are active and mature cercariae in snail tissue escape into the water as soon as they are mature, leading to a lesser concentration of cercariae in snail tissues. The present writer disagrees with Boray on this hypothesis; for if Boray's contention is true, then one would expect to find many developing cercariae in rediae during this season. On the contrary very few if any developing cercariae were observed in the rediae during wet season. The phenomenon can not therefore be reasonably explained by Boray's theory. As seen a little earlier, many snails get infected during wet seasons; since there is a lag of approximately 50 days between the time when the snail is penetrated by the miracidium and when mature cercariae appear, it is .....

to be expected that most of the snails which were penetrated during the wet season will harbour mature cercariae by the time the dry season sets in. We can therefore expect a higher percentage of infected snails harbouring mature cercariae during this (dry) season.

Results of this investigation show that the longer the snail stays in a habitat the greater is its chance of becoming infected with F. gigantica. Those that stay too long eventually die of old age or as a result of parasite load with the result that few very old snails are present. Very young snails, because they have been in a habitat for a short time, seldom if ever have F. gigantica in them; however, a few that get infected probably die and so none of the yong snails are found to harbour F. gigantica. See Table V.

From results obtained from this investigation it may be advisable to apply molluscicides during the middle of the wet season when the highest number of infected snails and miracidia are available in the habitat.

C H A P T E R III

3. SNAIL POPULATION DYNAMICS

For many years the control of snail cariers of diseases to humans has been studied. Literature of work done on the control of schistosomiasis-carrying snails is plentiful. Barlow (1937) has reported his work on the canal clearance as a means of schistosomiasis control in Egypt. Berg (1953); Chernin, Michelson and Augustine (1956); Chernin (1957); Dias and Dawood (1955); Ferguson, Oliver-Gonzales and Palmer (1958); Malek (1956); Michelson (1957); Oliver-Gonzales (1946); Oliver-Gonzalez Ferguson (1959); Radke, Ritchie and Ferguson (1961); and Wager (1936) have reported their respective work on attempts to control schistonomiasis-transmitting snails. Nagar (1958) reports his work on the use of chemical barriers along 5000 kms. of irrigation canals as a means of snail control.

Control measures against fascioliasis-carrying snails, have also been studied although not as widely as the former. Alicata (1941) tried the use of predators-the crayfish- Astacus nigrescenes and the Japanese fireflies- Lucida lateralis and L. cruciate; however, this has not proved an effective method of snail control. The Fisheries Department in Uganda has

effectively...

effectively used ducks to eradicate snails at their Kajansi Fish Ponds, but at the expense of polluting the water (Personal communication); however, such habitat is good for their fish. Boray (1963) on reporting on problems associated with snail control states, "Lymnaea tomentosa, the Australian intermediate host of Fasciola hepatica, has a wide distribution and a capacity for fast reproduction. One snail can produce 1000 eggs each month and one generation takes one month. Snails survive almost a year in dry mud and for the same time at low temperatures. Intensive active and passive migration takes place.... The high rate of reproduction of L. tomentosa, especially in new environments, shows that only 100% eradication of the snails would lead to permanent control. The dry edges of habitat should also be treated with molluscicides because of the amphibious nature of the snails. Land connected with other properties by permanent creeks and channels are considered to be unsuitable for chemical control unless co-operation treatment is possible."

Snails are closely associated with their environment and should, therefore, respond to changes in the environment. Since one of the major variables in a lotic snail habitat is vegetation, it was decided to study the effect of vegetation clearance out of a channel as a possible means of snail control.

3.1 METHODS AND PROCEDURES.

A well-known all year round snail habitat at Port Bell on the shores of Lake Victoria was chosen for study. The habitat is a channel draining water from a nearby underground spring into the lake. A portion of this channel 192 metres long was used for the experiment. The channel has a sandy bottom with some muddy silt towards the outlet into the lake. There are large patches of green algae throughout the entire length of the channel. Sedges are the dominant vegetation with some Commelina diffusa, Centella asiatica and Asystasia gangetica. The land immediately surrounding the channel is a grazing area and the channel itself is used as a drinking place for the cattle. Common birds in the area are cattle egrets (Bubulcus ibis), hadada ibis (Hegedashia hagedash), hammerhead Scopus umbretta, black-necked heron (Ardea melanocephala) and the african jacana (Actophilornis africanus).

The fish population is almost entirely guppies (Lebistes sp.) and the frog population is composed of Ptychodera sp. and Xenopus sp. The snail population in this channel is predominantly Lymnaea natalensis, although Biomphalaria sudanica and a few Bulinus sp. also occur.

The portion of the channel 192 metres long selected for study was divided into 64 equal blocks, each one of them three metres long using wooden pegs driven into the ground along the channel. The blocks were numbered 1 through 64 beginning with the block nearest to the head-water and ending with the one nearest to the outlet into the lake. Twenty blocks were picked at random and snails in them counted using a hand tally counter. This was repeated every day for seven days. The highest, lowest and average daily snail counts for the seven days were recorded. This week is later on referred to as week 0 in paragraphs that follow. On the seventh day immediately after counting for the day had been done, the channel was cleared by cutting down all vegetation and removing it from the channel. The snails and egg masses on the vegetation dried and thus died through dessication. The cleared channel was then left overnight for the muddy water to clear up and the following day counting resumed. Every day for the next 72 days twenty blocks were picked at random, snails in each of them counted and records kept.

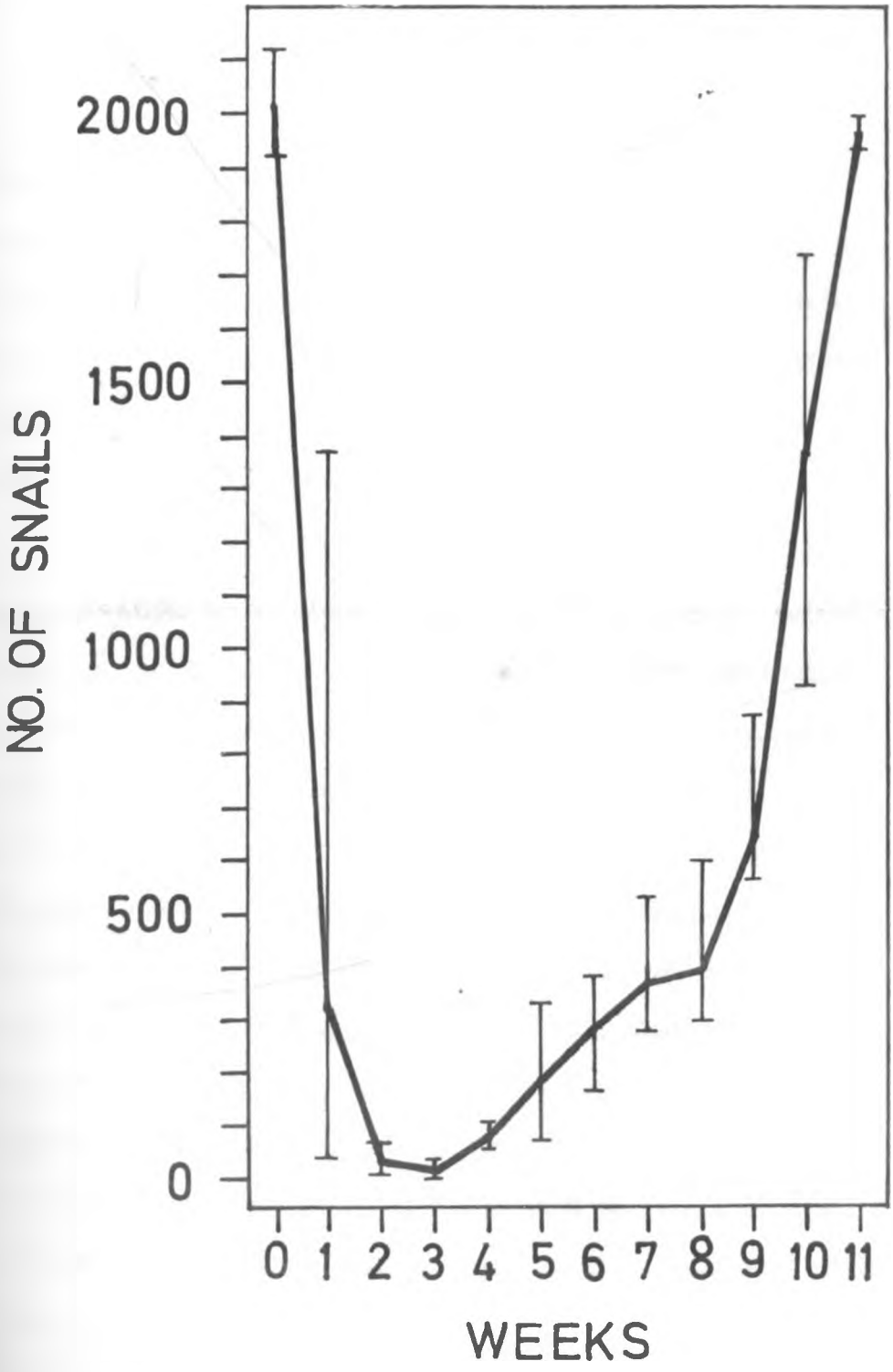
3.2 RESULTS.

As soon as clearing had been done and water cleared, hadada ibis (Hagedashia hagedash) and the african jacana (Actophilormis africanus) moved in and began to eat up exposed snails. Frogs also became active in their search for snails. Because these snails are not only exposed, but have nothing solid to hang on to, some of them are swept by the water current into the lake, while some are burried under the sand whenever there is heavy rain. A summary of results are shown in Table VI and Figure VI; full results of daily counts after the channel had been cleared is shown in the Appendix II.

Table VI : SHOWING HIGHEST, LOWEST AND AVERAGE DAILY SNAIL COUNTS PER WEEK.

WEEK	HIGHEST NO. SNAILS	LOWEST NO. SNAILS	AVERAGE NO. SNAILS
0	2117	1921	2019.00
1	1369	48	325.86
2	70	14	37.14
3	39	8	21.14
4	109	70	85.71
5	332	67	195.43
6	379	167	285.71
7	539	280	368.43
8	599	301	392.71
9	869	566	645.86
10	1741	935	1365.00
11	1996	1937	1966.50

Figure 6. Graph showing snail population re-establishment based on weekly counts.


NO. OF SNAILS
2000
1500
1000
500
0
0 1 2 3 4 5 6 7 8 9 10 11
WEEKS

After the snail population was almost completely depleted - i.e. during the second and third week following channel clearance, the few snails found were attached to stones, sticks or other objects that were heavy enough to stand the sweeping action of the water flow. It is on these solid surfaces that the snails begin to lay eggs and the population begins to re-establish itself.

Vegetation begins to re-appear in isolated small patches of green algae to be found on solid surfaces that are being swept by the flow of water. These small patches eventually grow to form larger patches. As the initial establishment of both snail population and the algae is governed by the rate of water flow, the portion of the channel near the outlet into the lake where the flow of water is rather sluggish is where the snails and algae first begin to re-establish and then spread to the entire length of the channel. During the 4th week after channel clearance, the snail population begins to increase and one finds this very gradual increase going on up to the eighth week. From the ninth week on, the increase in snail population per week is very rapid. The snail population almost corresponds with the availability of vegetation; from the fifth week, vegetation begins to come back, and by the

eighth .....

eighth week there is a lot of it and by the ninth week, it is almost back to the original size. From the tenth week on, both the green algae and the bigger plants are plentiful and during the 11th week the channel is completely back to what it was before it was cleared.

3.3 DISCUSSION:

The ecology of Lymnaea natalensis seems to go hand in hand with human activities. Mozley (1960) says that he was unable to get any good snail habitat in places completely undisturbed by human activities. Irrigation of hitherto dry areas has witnessed the creation of new habitats and eventually the establishment of populations of aquatic snails into these areas. As seen from this study, it is quite evident that human activity in the form of channel clearance does destroy snail populations especially during the 2nd, 3rd and 4th weeks following channel clearance; but it is equally true that it also serves as a stimulant to a younger and therefore a more energetic generation of snails. It is quite common knowledge that the older snails with tougher tissue are resistant to miracidial penetration to a certain degree. The killing of old snails through channel clearance does not only produce more snails, but snails that

are very succeptible to penetration by miracidia of Fasciola gigantica and therefore poses a greater danger to cattle that drink water and graze in this area.

In completely untouched habitats one finds many factors working against snail populations. In working with snails for the last four years the writer has noticed a number of things associated with snail populations. It is very interesting to note that one place might be a very good snail habitat today, but a couple of weeks later the same habitat will have no snails at all, the reverse is also true. Flooding and overgrowth of vegetation are well known limiting factors to snail populations. Goodman (Personal Communication) mentioned revisiting certain hippo pools in the Queen Elizabeth National Park where only two weeks before many snails were found only to find no snails at all. Porter (1920) in reporting her work in South Africa points out how flooding kills snails. Deposition of excessive amounts of birds' (such as ducks) droppings in water tends to kill snails. The Uganda Fisheries Department (Personal Communication) reports that their fish ponds at Kajansi have been completely depleted of snail population through being eaten by ducks, and the latter depositing their droppings into the ponds making the water unsuitable for snails; this.....

this however, was good for the fish as these droppings fertilize the pond flora. Excessive growth of vegetation in channels do 'choke' these channels and eventually render the habitat unsuitable for snails.

In his attempt to keep channels clean either for irrigation purposes or purely to open them up for watering his livestock, man has whether unknowingly or out of sheer necessity encouraged the constant production of vigorous snail populations that are a potential danger to his livestock. Through channel clearing the old snails crowding the habitat are killed; a young fresher algal flora on which snails feed Pennak (1953) is produced, the water is very well aerated, the shade provided by the young vegetation is at an optimum so that as a result one witnesses a rapidly increasing snail population. The fecundity of snails is a problem to reckon with; Boray (1963) reports that among Lymnaea tomentosa one snail produced up to 1000 eggs every month and that one generation takes one month. It is therefore evident that if even few snails escape death these are capable of completely re-establishing the population in a very short time. The use of molluscicides in snail control may not be very effective, especially in a lotic environment.

The molluscicides placed at the headwaters may be washed through to the outlet over a short period of time. Although many snails are killed, the few that escaped begin to multiply almost immediately.

From this study, however, it seems that there is a period when the snail population is at its minimum; this is during the second, third and fourth weeks following channel clearance, when snails that remained in the channel are exposed and therefore preyed upon by birds and frogs. It should be therefore feasible that a molluscicide applied to the habitat between the second and fifth week following channel clearance could be an effective technique of snail control.

C H A P T E R IV

4. THE INTRAMOLLUSCAN PHASE OF THE LIFE CYCLE OF FASCIOLA GIGANTICA COBBOLD, 1856

The life cycle of Fasciola, although elucidated during the later years of the 19th century, can be related back to the work of Swammerdam in 1737. His illustration of "worms" are very easily recognizable as the cercariae of trematodes. Since this initial finding by Swammerdam, other workers through the years have reported their findings and each time, in most cases, new information has been added to the previous knowledge. Muller in 1773, Zeder in 1800, Nitzsch in 1807 and 1816; Bojanus in 1818; von Siebold in 1837; Steenstrup in 1842; Leuckart in 1852; La Vallete de St. George in 1855; and Wagener in 1857 reported various stages in the life cycle of trematodes, Fasciola included. It was not until 1882 that Leuckart and Thomas, working independently, elucidated the life cycle of Fasciola hepatica. Thomas (1882) in his report on the complete life cycle of Fasciola hepatica states "..... The eye spots (these are found originally on the miracidium) usually become detached, but they as well as the head papilla persist,

showing.....

showing the identity of the young sporocyst - for so it must now be called ..... The active embryo (miracidium) has degenerated into a mere brood-sac, in which the next generation is produced." He observed that this sporocyst stage can constrict and therefore divide the original sporocyst into two smaller ones, although he warns that this method of multiplication is not very common in F. hepatica. In his further observation he states, "When the redia is ready to come forth, it breaks through the wall of the sporocyst and the wound caused by its forcible exit immediately closes up, and the remaining germs continue to develop." Since this report was published more work has been done on the life cycle of not only Fasciola hepatica but F. gigantica also. Porter (1920) in her description of the life history of F. gigantica from South Africa states, "..... Daughter rediae appear to be formed only towards the end of the life of the parent redia. The parent redia produces several cercariae which are active organisms, and vary in appearance according to their degree of activity....." This can be interpreted as meaning that the mother redia matures, produces cercariae and then towards the end of its (mother redia) life it begins to produce daughter rediae. Alicata (1938) in his report on the observations on the life history of F. gigantica in Hawaii.....

in Hawaii states ".....Snails dissected one day after experimental infestation showed several early-forming sporocysts in various parts of the body; at this stage the sporocysts were somewhat spherical and about 80 microns in diameter. In most cases the eye spots and germinal cells were clearly visible... A sporocyst 4 days old was 610 microns long by 190 microns wide and encloses developing mother rediae showing well formed pharynges." He reported having seen cercariae first 39 days after the initial exposure.

Kendall and Parfitt (1953) worked out the life cycle of F. gigantica at Wybridge, England using snails and flukes obtained from Pakistan. At laboratory temperatures they observed emergence of mature cercariae 78 days after infection. Dinnik (1958) reported that F. gigantica eggs do not develop while in the intestine, probably due to the shortage of oxygen. Dinnik and Dinnik (1959) have shown that hatching of miracidia from eggs begins at about 17 days after initial exposure and spreads over a long period, some take as long as 90 days from the time of incubation to hatch depending on temperature. Dinnik and Dinnik (1963) have stated, "After penetrating a snail and casting off its ciliated epithelial coat, the miracidia were transformed into sporocysts containing up to

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six embryo balls. The largest of these embryos developed into the first redia, which ruptured the wall and escaped from the sporocyst. The remaining embryos continue their development into rediae, either in the ruptured body of the sporocyst or lying outside it in surrounding tissues of the snails." The two authors continue to state, "Rediae of the 1st generation (rediae which had developed and left the sporocyst) appeared in the snail six to eight days after the penetration of a miracidium. On the 20th to 22nd day, daughter rediae develop in the 1st generation rediae and from 22nd day onwards these daughter rediae begun to leave the mother rediae." They went on to say, "After producing daughter rediae, the 1st generation rediae began to produce cercariae 26 days after infection. The 1st cercariae escaped from the parent rediae via the birth pore on the 30th - 33rd day and snails began emitting cercariae 36-40 days after the penetration of the miracidia." In the same investigation, the two authors report a small number of malformed rediae amongst the 1st generation rediae during the cooler weeks before and after the cold seasons of the year. Dinnik and Dinnik (1964) have stated, ".....It was interesting to note that in both experiments cercarial embryos appeared in the rediae of all generations at one and the same time, at the beginning of September, when the cold season of the year

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had passed and the water temperature in the acquaria rose in the afternoons from 15-16° to 21°C." They continue to say, ".....Each first generation redia produces daughter rediae initially, then cercariae. It then repeats both phases of production alternately throughout its life .....Rediae of subsequent generations also show this alternating development of rediae and cercariae in their body cavities." The two authors point out that during cold season a miracidium develops into a sporocyst which develops into rediae but no cercariae are formed; contrary to this, in the warm season the miracidium develops into a sporocyst and then rediae and cercariae alternately.

Dawes (1959) has stated, ".....The larva which enters the snail is certainly not a miracidium, although it retains the eyes the gut and other organs, and also the germinal cells; it is a young sporocyst covered by what was formerly sub-epithelial tissue, carrying with it into the snail some epithelial and other debris. The miracidium may be regarded as a form which serves to implant the sporocyst in the body of the snail host by what appears to be an elaborated process of external digestion." Dawes (1960) showed that periods of 30, 60 and 120 minutes were adequate for the miracidia of F. gigantica

to penetrate snails. He states, ".....In both species (F. hepatica and F. gigantica) of miracidia the gut comprises a syncytium having a typical cluster of 4 nuclei, and both a mouth and a lumen can be seen in some section .....In both species, therefore it is a young sporocyst which enters the snail, although it is the miracidium which first adheres to the snail's integument and then cytolyses it to the ultimate perforation."

From reports so far published, it seems that there is no unanimous agreement on the sequence of events occuring in the development of the intramolluscan stages of Fasciola gigantica. It was therefore decided that a careful study be carried out in an attempt to elucidate this phase of the life cycle of F. gigantica. This part of the thesis is an account of the work done to show the various intramolluscan stages of F. gigantica Cobbold, 1856.

4.1 MATERIALS AND METHODS

Two local abattoirs in Kampala were the sources of eggs present in bile from livers infected with Fasciola gigantica. The bile was collected and taken to the laboratory where it was diluted with tap water and was set aside for 30 minutes to allow.....

allow the eggs to settle. The diluted bile was then carefully decanted leaving fluke eggs at the bottom of the beaker; the beaker was again filled with fresh tap water and was throughly shaken to wash the eggs. The eggs were again allowed to settle for 30 minutes and this water was carefully decanted. This was repeated several times until the eggs were nearly free of bile. The clean eggs were then put in a beaker with tap water and incubated at room temperature until miracidia hatched from the eggs. During the whole period of incubation, water in the incubating beaker was carefully decanted daily and fresh tap water added. The miracidia, which swim actively in water, were carefully decanted leaving unhatched eggs in the beaker. This was gain filled with fresh tap water and kept until the next day when newly hatched miracidia were again recovered. The eggs and miracidia were pipetted on to a slide, mounted under a cover glass and studied on a compund microscope. Measurements of both the eggs and miracidia were taken and drawings made.

Wild Lymnaea natalensis were collected from Port Bell near Kampala on the shores of Lake Victoria, and brought to the laboratory where they were put in a large glass aquarium tank. The tank had earlier been prepared by putting gravel, sand and tap water and the pond weed Egeria densa planted into it.

The wild snails were left in the tank for one week after which time many egg masses were found in the tank. The adult snails were then removed and the tank drained, after which the tank was filled with fresh tap water and kept at room temperature. The water level in the tank was maintained by the addition of sufficient tap water to compensate for any evaporation. The egg masses in the tank were examined daily until young snails began to hatch out of them. Powdered fish food purchased at a local pet shop in Kampala was sprinkled into the tank as additional food for the snails. These were the snails used in all later experiments.

Twelve small glass aquarium tanks were prepared for the young snails by putting in them gravel, sand, tap water and the pond weed Egeria densa. Periodically it was necessary to add fresh tap water to compensate for evaporation. The twelve tanks were divided into four groups A, B, C and D, and numbered 1, 2 and 3 in each group. Beginning with the first day when miracidia began to hatch and every three days thereafter, were recovered and divided into two beakers. Many snails were put in one of the beakers and left with the miracidia for one hour, after which all the contents of that beaker were decanted into tank number one of group A.

From the second beaker containing miracidia, ten miracidia were pipetted into a petri dish and a single snail introduced into the dish at a time. This was then put on to a dissecting microscope and examined until miracidia were seen penetrating the snail. After all miracidia had penetrated, the snail was transferred into tank number two of group A. This was continued until there were many individually exposed snails in the tank. The same procedure was repeated, but this time using five miracidia instead of ten per snail and these were put into tank number three of the same group. On the 4th day of hatching, the entire process as described above was repeated exactly as on the 1st day, except that the tanks used were those of group B. On the 8th and 12th days of hatching, miracidia were treated as in the previous two groups using tanks of groups C and D respectively. All tanks were labelled with the time and date when snails were exposed and then kept at room temperature, and tap water was occasionally added to the tank to compensate for evaporation. If the water in any of the tanks became foul, that tank would be drained and filled with fresh tap water as soon as this was observed.

Snails were then removed and examined daily. Each snail was placed under a dissecting microscope the shell carefully removed and the snail body divided into three portions - the foot, the mantle and the visceral portion. Each portion was placed on a slide with a drop of water, pressed flat under a cover glass and carefully examined using a compound microscope. The various stages of the parasite when found in the snail were very carefully drawn and measured. Later on, usually by the 16th day, the stages of the parasite had become large enough to be seen under a dissecting microscope when each portion of the snail body would be put in a petri dish with tap water and the parasite removed from the snail tissue with a finely pointed forceps while using a dissecting microscope. The parasite would then be placed on a slide in a drop of water, pressed flat under a cover glass and studied at higher magnifications of the compound microscope and drawn and measured as described above. This was continued until the time when actively swimming cercariae were observed.

4.2 RESULTS:

4.2.1. Egg development (Figs. 7 - 15).

Eggs are passed into the gall bladder of the mammalian host by the adult fluke, and remain undeveloped while in the bile.

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Development begins only after the eggs have been repeatedly washed in tap water and incubated in tap water at room temperature. Eggs vary in size but average 161 by 75.9 microns; they are yellowish brown in colour and are operculate with a clear nucleus area situated near the opercular end. The darker granular egg contents are aggregated into uneven masses without definite shape and completely surround the clear area. This is shown in figure 7. After being incubated for 48 hours, the egg shows progressive development; at this time protoplasmic granules become organised into larger elements each surrounded by a membrane as shown in figure 8.

When incubated for 96 hours the protoplasmic aggregates, still surrounded by a membrane become more clearly nucleated. These aggregates are of two sizes; the larger ones occur on the periphery while smaller ones are found nearer the middle of the egg near the anterio - central clear area except at the opercular end where only larger aggregates occur. This is shown in figure 9. After 144 hours of incubation, the anterio-central clear area increases in size and the elemental form of an embryo begins to take shape. However, the embryo still occupies the area near the opercular end of the egg, and measures 57.7 by 46 microns. This is shown in figure 10.

The embryo elongates and measures 69 by 46 microns after 192 hours of incubation. The cell aggregates surrounding the embryo are considerably reduced on the polar ends of the egg, leaving large empty spaces at both ends. See figure 11. The embryo now begins to take the definite shape of a miracidium; however, no structures, external or internal, are evident at this time, after 240 hours of incubation. The embryo now measures about 92 by 50.6 microns. The majority of the cell aggregates surrounding the embryo have disappeared and the few remaining cells are restricted to the sides, leaving large empty spaces at the ends of the egg. This is whown in figure 12. Cilia are visible on the miracidium 264 hours after incubation and at about this period the miracidium first begins to move within the egg shell. At 288 hours of incubation the miracidium measures 110.4 by 57.5 microns and appears to be completely developed, with both internal and external structures present. Cilia, primitive gut, a pair of penetration glands, paired eye spots which are attached to each other, a pair of ampulae, a pair of flame cells with excretory ducts, and a cluster of germinal cells are all clearly visible through the egg shell. The very few surrounding cell aggregates are confined to the antero-lateral sides of the egg as shown in figure 13.

The miracidium continues to grow and at 366 hours of incubation it measures 126.5 by 57.5 microns. By now all surrounding cell aggregates have disappeared. This is shown in figure 14. After 384 hours of incubation the miracidium increases in size to 154.1 by 57.4 microns and almost fills up the egg shell. See figure 15. At this time the miracidium is getting ready to escape from the egg shell. The first hatching began 408 hours after incubation started. The hatched miracidia, like the eggs, vary in size, but average 155 by 58 microns. Once hatching begins this goes on continuously, so that 111 days after the fist miracidia appeared some miracidia were still hatching.

Figure 7. The egg at the time of recovery from bile.

" 8. The egg at 48 hours incubation.

" 9. The egg at 96 hours of incubation.

" 10. The egg at 144 hours of incubation.

" 11. The egg at 192 hours of incubation.

" 12. The egg at 240 hours of incubation.

" 13. The egg at 288 hours of incubation.

" 14. The egg at 366 hours of incubation.

" 15. The egg at 384 hours of incubation.

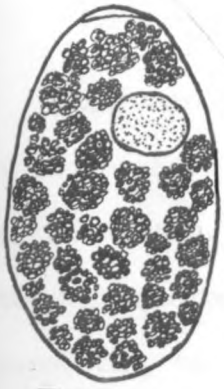


FIG. 7

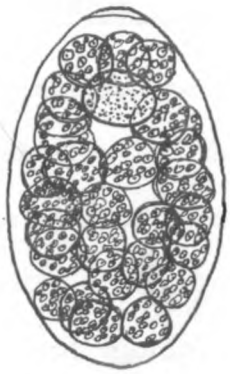


FIG. 8

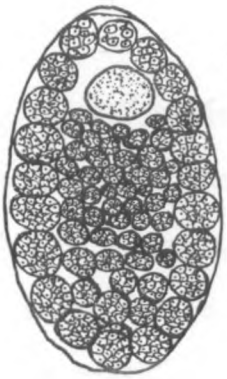


FIG. 9

FIG. 10

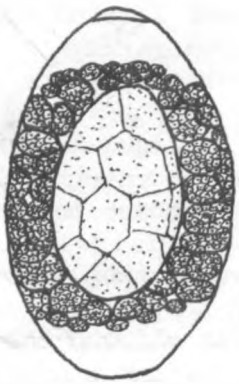


FIG. 11

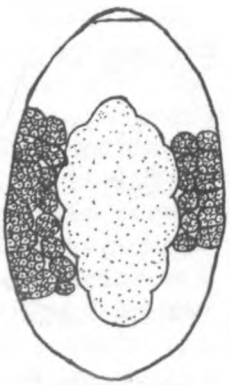


FIG. 12

FIG. 13

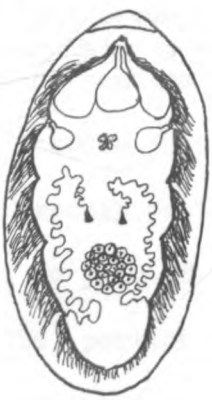


FIG. 14

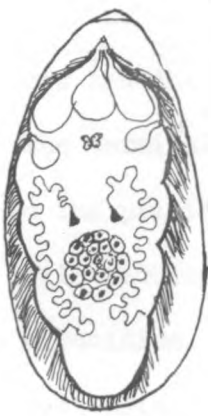


FIG. 15.

4.2.2 The miracidium (Fig. 16).

The miracidium appears as a tiny densely ciliated organism which hatches out of the egg after at least 17 days of incubation. The body measures 156 X 58 microns. The cilia are arranged on plates in whorls with narrow naked spaces separating two adjacent whorls. Because cilia are long those on anterior plates overlap on those of the next posterior plates and as a result conceal the narrow naked space in between. Cilia on the anterior plates originate at the base of the thinly pointed apical papilla. At the junction of the first (anterior-most) and second row of plates occurs a pair of ampulae placed laterally just below the posterior end of each penetration gland. The ampulae are blind pockets opening on the outside. A primitive central gut and two penetration glands situated laterally to the gut occur in the anterior section of the miracidium. Both of the penetration glands and the primitive gut open into a small space just below the terminal opening of the apical papilla. Directly posterior to the primitive gut are the paired eye spots. A pair of flame cells occurs lateromedially in the middle (3rd) portion of the miracidium. From each flame cell runs a convoluted excretory duct which courses anteriorly and medially for a short distance then briefly laterally after which it meanders posteriorly and reaches the fifth

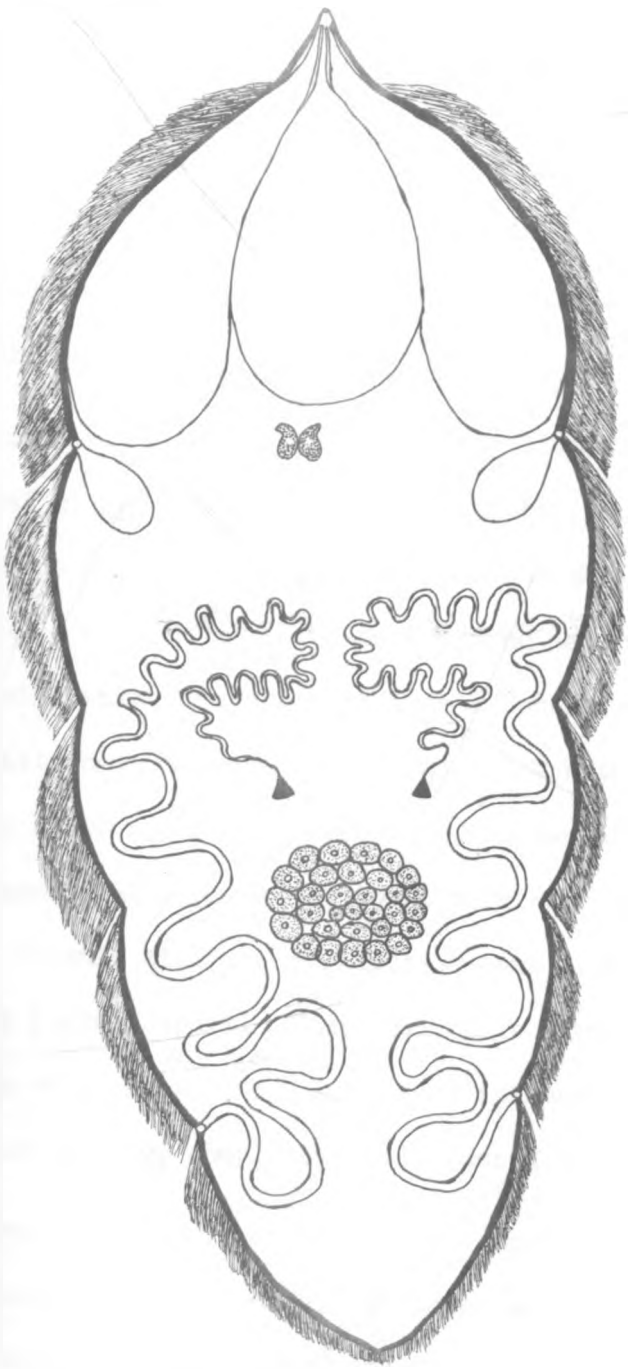
row...

row of plates. The duct then runs antero-laterally and finally opens laterally at the junction of the 4th and fifth row of plates. Directly behind the flame cells a cluster of germinal cells occupy the region marked by the posterior portion of the 3rd and anterior portion of the 4th row of plates of the miracidium. The cells have prominent nuclei and the protoplasm is surrounded by a definite cell membrane.

The miracidia, when examined in a petri dish are seen swimming rapidly from one edge of the vessel to another, remaining very active until they encounter a proper snail host. On encountering the proper snail host, the miracidium swims round the snail, sometimes many times, before it finally adheres to and penetrates the host. Most miracidia penetrate snail tissue around the mantle, although a few were seen attempting to penetrate tissues of the foot.

Miracidia are attracted to spots covered by mucous material left at the bottom of the vessel by crawling snails. Many miracidia were seen congregating arround such spots. A fast-swiming miracidium would swim past, then swim in circles arround, and finally stop at the spot.

Figure 16. The miracidium.



4.2.3 Pre-redial phase "Sporocyst" (Fig. 17 and 18)

The miracidium attaches itself to the snail and punctures the tissue using its finely pointed apical papilla. Next the miracidium loses its ciliated coat and finally penetrates the snail tissue.

Thirty six hours later, the miracidium transforms into a spherical object, 75.9 microns in diameter, surrounded by a transparent membrane. Immediately inside this membrane is a thick layer of somatic cells surrounding a cluster of germinal cells which are arranged into a sphere. All these germinal cells have a prominent nucleus each with a definite membrane surrounding the protoplasm. Between the two cell layers occurs an empty space completely surrounding the germinal cells. At this time the eye spots become detached and are situated further apart. Flame cells are displaced from the position they occupied in the miracidium and the excretory ducts seem to have completely disappeared. See figure 17.

Three days after penetrating the snail tissue, the miracidium transforms even more; it is no longer the irregular sphere observed at thirty six hours, but an elongate structure 96.6 microns long. The organism is, however, still surrounded

by.....

by a transparent membrane with somatic cells surrounding the germinal cells. The space between the two cell layers still exists although it is reduced in size. Eye spots have moved even further apart as have the flame cells, while the excretory ducts continue to be unobserved or absent. Germinal cells which occured as a single spherical mass at thirty six hours have now increased in number and occur as three overlapping masses. The larger mass is at one end followed by a smaller one and the very smallest at the opposite end. This arrangement results in an elongate structure wide at one end and tapering to a relatively narrower opposite end. As germinal cells increase in number, somatic cells tend to decrease. See figure 18.

Figure 17. The pre-redial phase 36 hours after the miracidium has penetrated the snail tissue.

" 18. The Pre-redial phase 3 days after miracidium has penetrated the snail tissue.

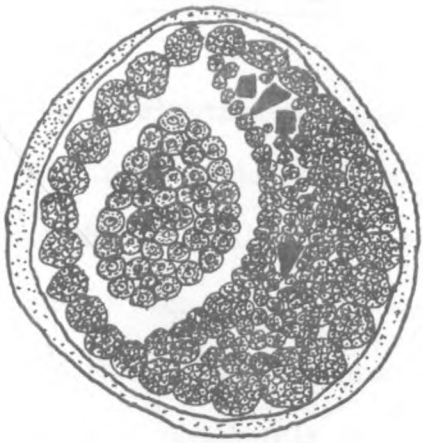


FIG. 17

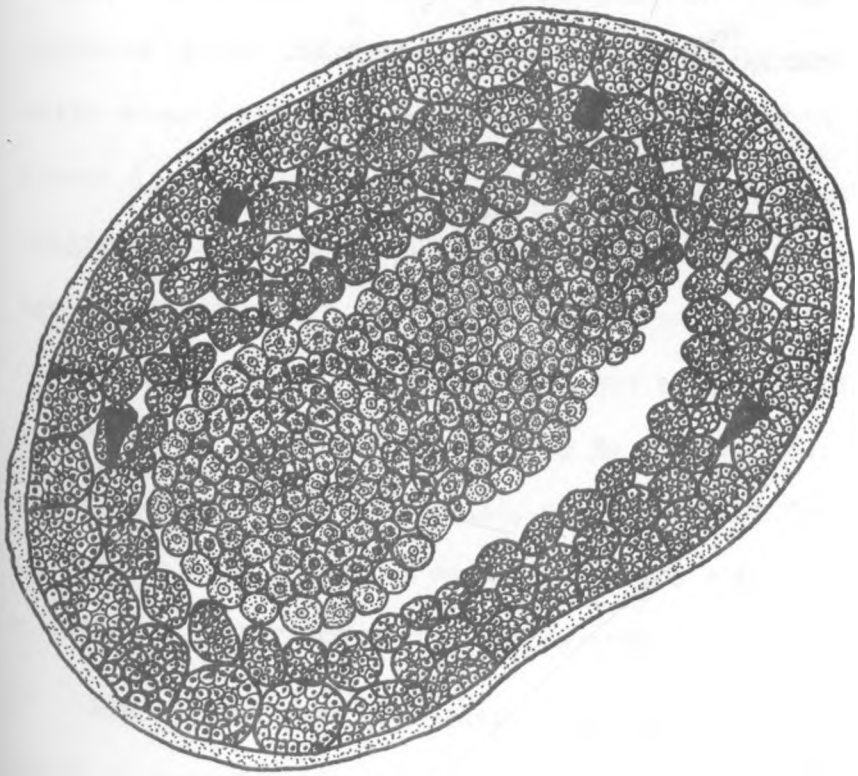


FIG. 18

4.2.4 Mother redia (Figs. 19-26)

Four days after penetrating the snail tissue, the miracidium has developed into the definite organism measuring 161 by 50.6 microns shown in figure 19, which is interpreted to be the mother redia. Some of the internal structures of this organism can be traced back to those in figure 18. Somatic cells have almost completely disappeared and the transparent membrane which is still present directly surrounds the germinal cells which completely fill the space in the organism except the anterior one third. The germinal cells still occur as three overlapping masses, however, the structure formed is wide in the middle and tapers to narrow ends. The original pair of flame cells and eye spots of the miracidium are still present in this organism. In addition, the organism has developed a syncytial primitive gut which is wide at the anterior end and tapers posteriorly to end just below the enterior edge of germinal cells, a non-muscular but cellular pharynx, a buccal opening connected to the primitive gut, and a definite collar around the narrow enterior end. See figure 19.

Eight days after penetration, the organism increases in width to measures 161 by 69 microns and more distinct morphological features become apparent. The transparent membrane seen

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in figure 19 has developed into a thick wall surrounding the organism. The pharynx becomes very slightly muscular and the gut becomes more distinct. The original pair of flame cells and eye spots of the miracidium are still present in this organism and in addition five pairs of relatively small flame cells can be seen arranged on the sides of the mother redia immediately surrounding the anterior daughter embryo. The overlapping masses of germinal cells as seen in figure 19 separate into three distinct cellular masses or daughter embryos. The anterior-most daughter embryo measures 92 by 46 microns and is the largest of the three. It is surrounded by a thin wall within which germinal cells are arranged in three overlapping spherical masses. The anterior spherical mass is large followed by a small one and finally the smallest is situated at the opposite end forming an elongate structure wide at the anterior and tapering to a narrow posterior end. An empty space occurs between the germinal cells and the wall on the postero-lateral sides of the daughter embryo. The other two of the three daughter embryos are separate spherical masses of germinal cells which have no surrounding membranes. See figure 20.

Ten days after penetration the parent organism has developed into a completely recognizable redia as seen in figure 21, a muscular pharynx, a buccal opening which leads into a distinct gut containing food particles, and a pair of appendages on the posterior end. The original pair of eye spots and flame cells are still present in addition to the newly developed five pairs of small flame cells which were seen in figure 20. The newly developed flame cells are located between the anterior extremety of the gut and the appendages of the mother redia.

The three daughter embryos are arranged in a line; the anterior one is made of three overlapping spherical masses of germinal cells (forming an elongate structure wide at the anterior end and tapering to a narrow posterior end) surrounded by a wall. An empty space occurs between this wall and the elongated structure. The second daughter embryo is equally elongate and tapering, but unlike the first one, it has no wall surrounding it. The third daughter embryo is relatively small, although it is also made up of three overlapping spherical masses of germinal cells with no wall surrounding it. This is represented in figure 21.

Snails which were exposed individually to five and ten miracidia respectively were dissected ten days post-infection. In all, 135 snails individually exposed to 5 miracidia each and 150 snails individually exposed to 10 miracidia each were dissected and examined for the first generation rediae. The rediae found in these snails were counted and recorded. These records are summarised in tables VII and VIII below:-

Table VII: REDIAE RECOVERED FROM SNAILS THAT WERE INDIVIDUALLY EXPOSED TO FIVE MIRACIDIA EACH.

No. of Snails examined	No. of Rediae Recovered	Recovery Rate %
25	5	100
37	4	80
42	3	60
7	2	40
24	0	0

Table VIII: REDIAE RECOVERED FROM SNAILS THAT WERE INDIVIDUALLY EXPOSED TO 10 MIRACIDIA EACH.

No. of snails examined	No. of Rediae Recovered	Recovery Rate %
27	10	100
39	8	80
28	7	70
9	4	40
47	0	0

These results indicate that only one mother redia could have developed from each miracidium.

Twelve days post penetration of the snail tissue by the miracidium, the mother redia increases in size to measure 826.5 by 130.5 microns. At this stage the original eye spots and pair of flame cells of the miracidium disappear, and only the newly developed five pairs of small flame cells which are evenly spread between the collar and appendages of the mother redia remain. The daughter embryos which were seen as three separate groups of germinal cells have increased to five. The first 4 are elongate tapering structures made of overlapping spherical masses of germinal cells, however, the fifth is a small single sphere of similar cells. The most anterior daughter embryo is surrounded by a thick wall, the second is surrounded by a thin wall and the third by a membrane. All of them have an empty space between the germinal cells and the wall or membrane on the posterolateral positions. The fourth and fifth embryos have neither a wall nor a membrane. The anterior largest and most advanced daughter embryo measures 103.5 by 80.5 microns. See figure 22.

Fourteen days post penetration, the mother redia increases in size and her gut elongate posteriorly to end near the appendages. The daughter embryos have increased in number to nine and each of them is a compact grouping of germinal cells surrounded by a membrane, the empty space seen in figure 16 having disappeared. See figure 23.

Sixteen days after the miracidium has penetrated the snail tissue, the mother redia which has by now developed a sixth pair of flame cells has began migration towards the snail's liver. At this time the daughter embryos are still nine in number, however, they have undergone further development and occur as definite single units of germinal sacs scattered throughout the mother's hollow body. The three most advanced daughter organisms, two of them placed anterior and one posterior to the mother's appendages begin to show poorly formed pharynges. The other six are scattered throughout their mother's body. For the first time since the miracidium penetrated the snail tissue, there is clear indication that a second generation of rediae are developing. See figure 24.

Nineteen days post penetration the daughter organisms which have well developed pharynges but no guts nor buccal openings are present, and at 21 days these daughter organisms begin to look like future daughter rediae. See figure 25 and 26 illustrating stages of development at 19 and 21 days respectively.

Figure 19. Mother redia at 4 days post penetration of the snail tissue by the miracidium.

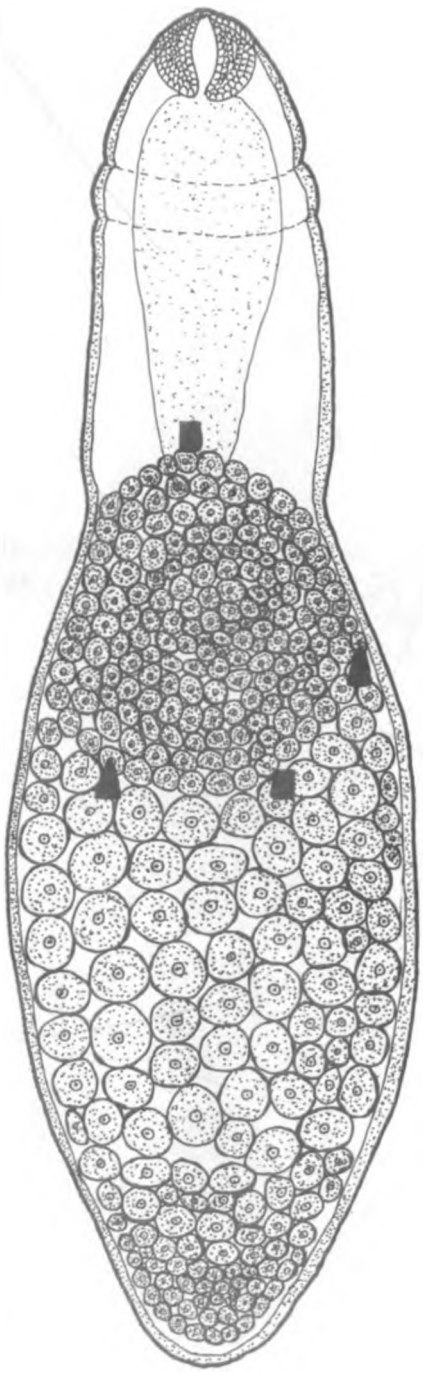


Figure 20. Mother redia at 8 days post penetration of the snail tissue by the miracidium.

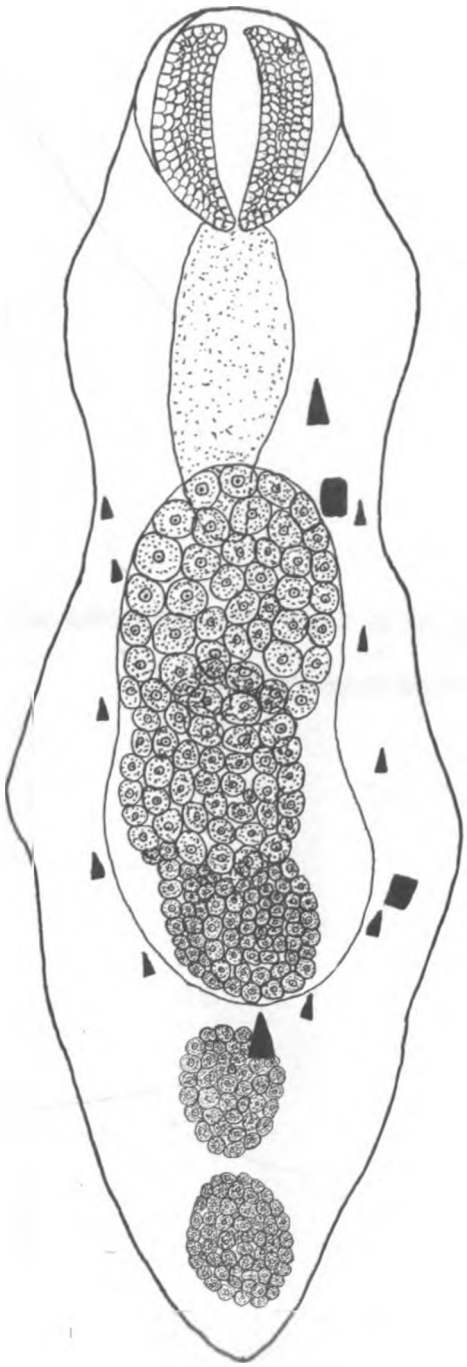


Figure 21. Mother redia at 10 days post penetration of the snail tissue by the miracidium.

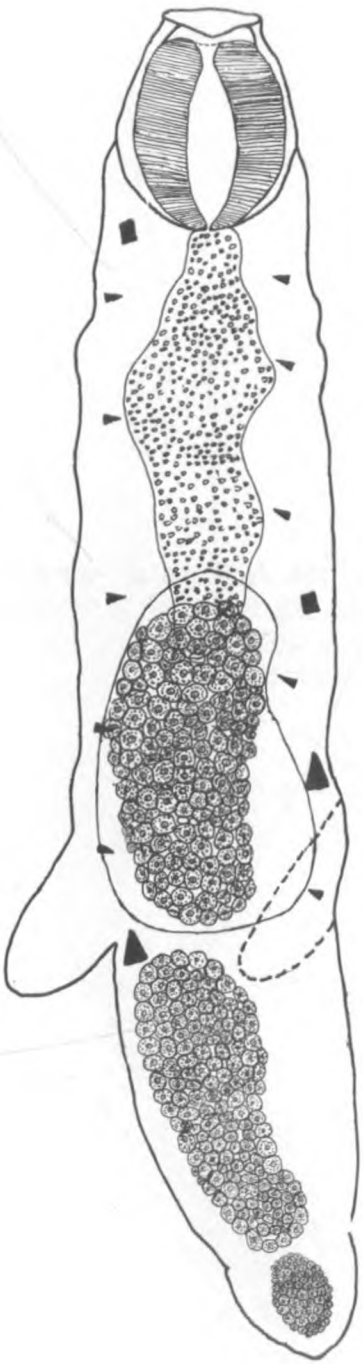


Figure 22. The mother redia 12 days post penetration of the snail tissue by the miracidium.

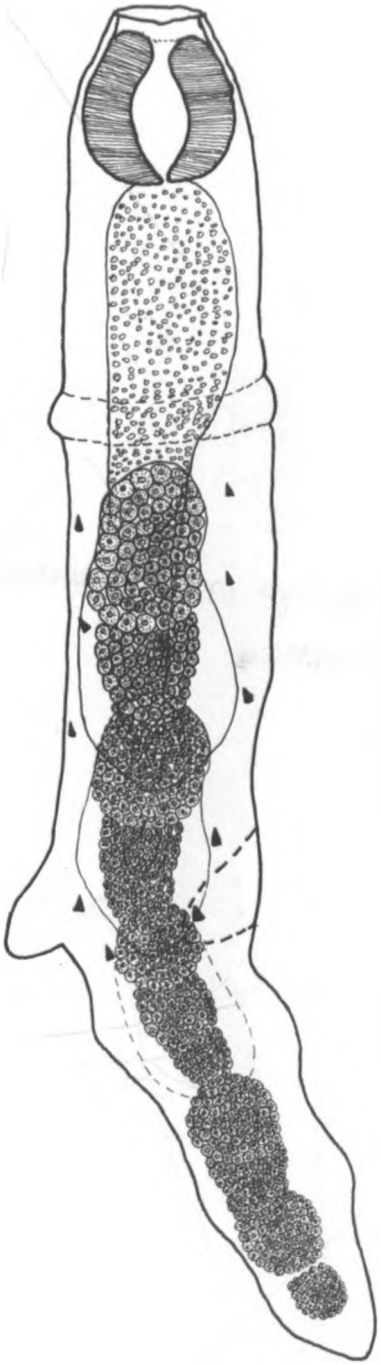


Figure 23. Mother redia at 14 days post penetration of the snail tissue by the miracidium.

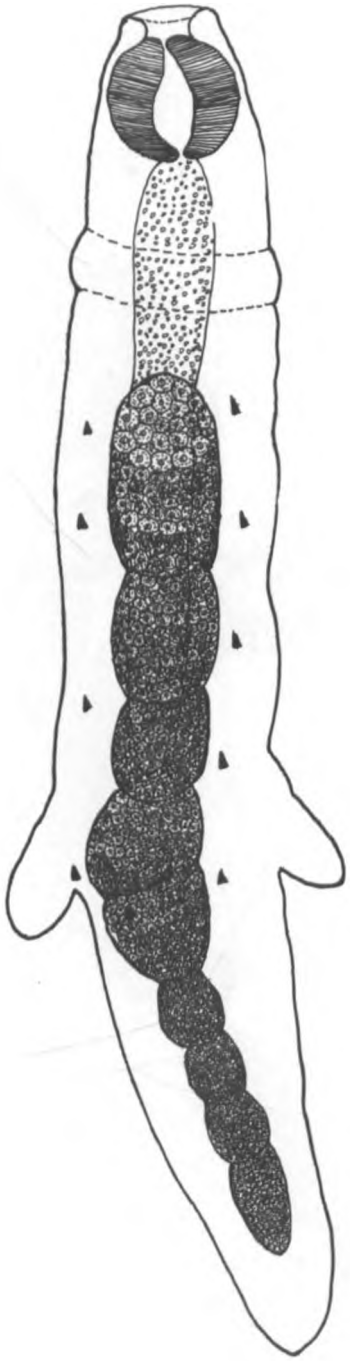


Figure 24. Mother redia at 16 days post penetration of the snail tissue by the miracidium.

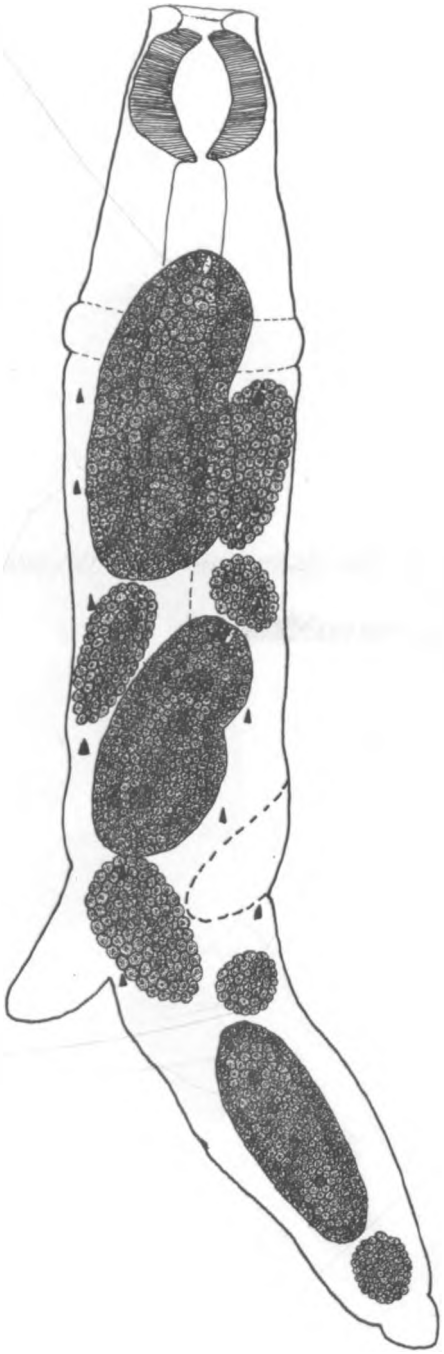


Figure 25. Mother redia at 19 days post penetration of the snail tissue by the miracidium.

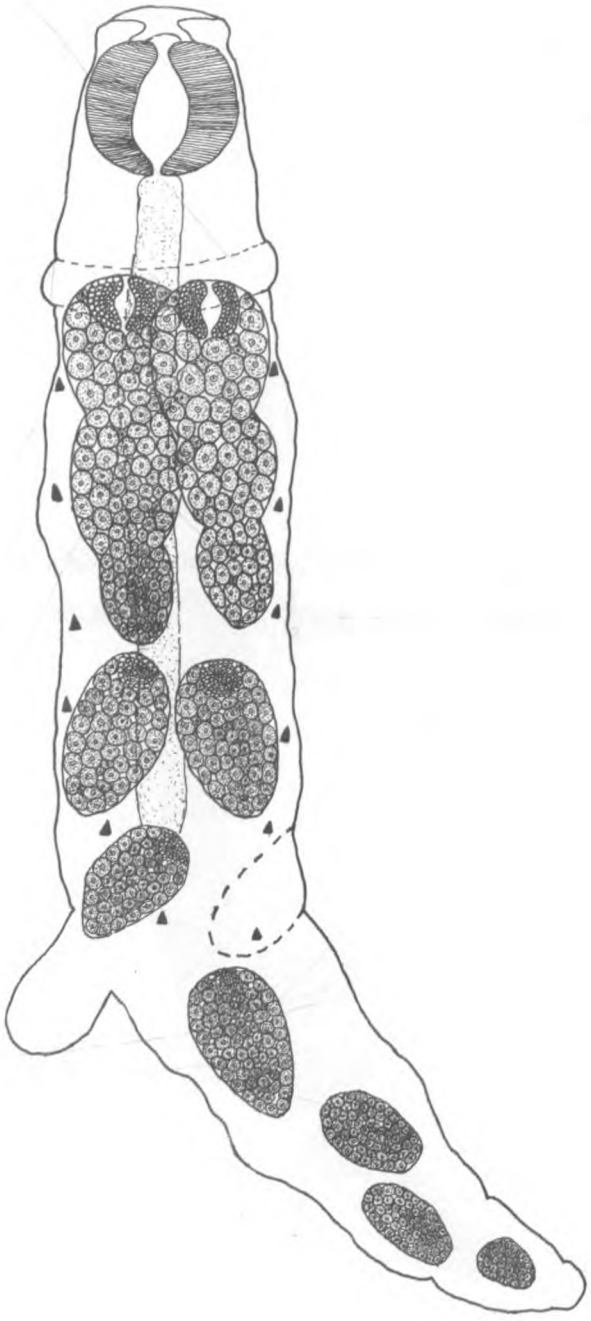
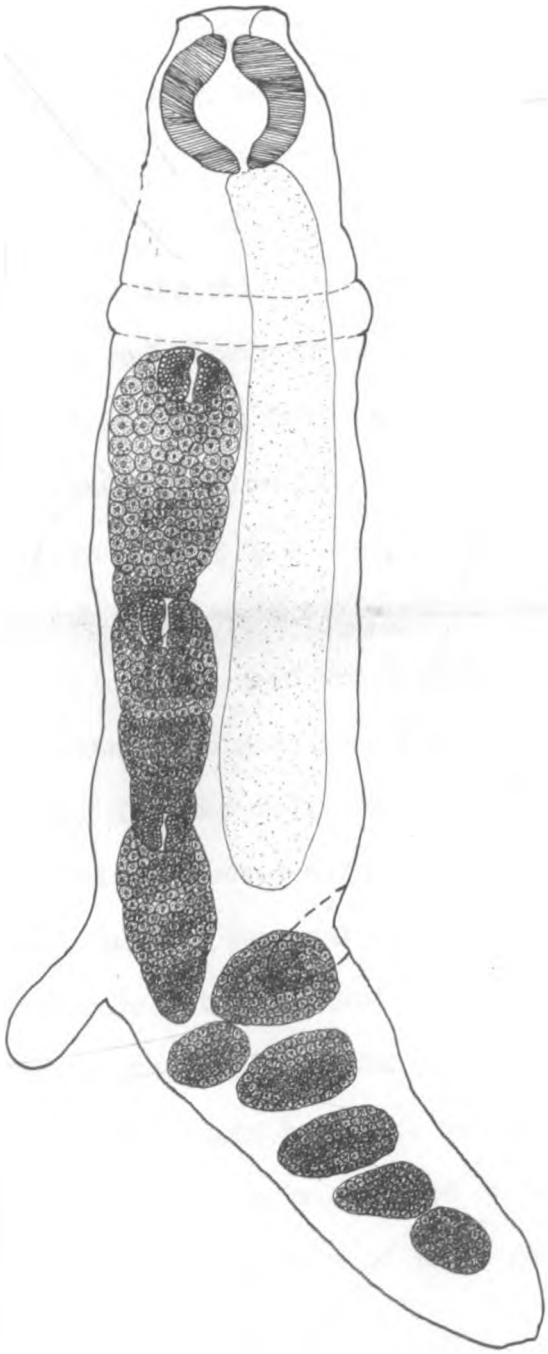


Figure 26. Mother redia 21 days post penetration of the snail tissue by the miracidium.



4.2.5 Daughter redia (Figs. 27-28)

Twenty three days post penetration, the mother redia has reached maturity, and its gut contains very few food particles. Daughter rediae, each one having a fully developed muscular pharynx, a buccal opening which leads into a gut containing many food particles and a pair of appendages appear in the mother redia's hollow body. The daughter rediae are scattered throughout the mother's hollow body in addition to grand daughter germinal sacs which develop into daughter rediae enventually. At this time occurs grand daughter germinal sacs scattered throughout the hollow bodies of the daughter rediae which are still contained within the mother redia. See figure 27(a) and 27(b) illustrating a mother redia containing daughter rediae and a daughter redia dissected out of the mother redia and containing grand daughter germinal sacs respectively.

Twenty five days after penetration of the snail tissue by the miracidium, the daughter rediae which are filled with grand-daughter germinal sacs begin to escape out of the mother redia through the birth pore which is situated on the collar. The daughter rediae contain many more of these germinal sacs than the mother redia had. See figure 28.

Figure 27(a) Mature mother redia.

" 27(b) Young daughter redia dissected out of mother redia's hollow body 23 days post penetration of the snail tissues by the miracidium.

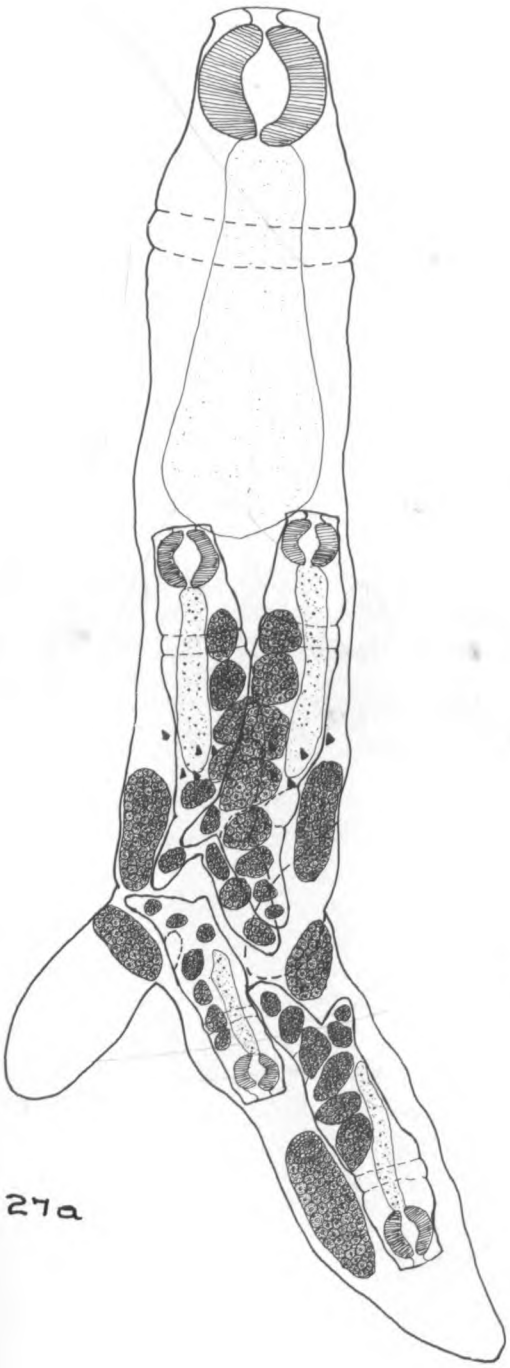


FIG. 27a

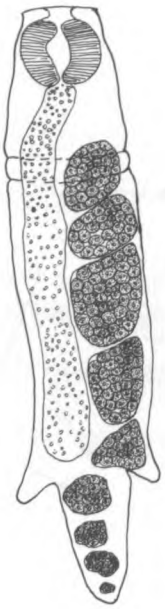
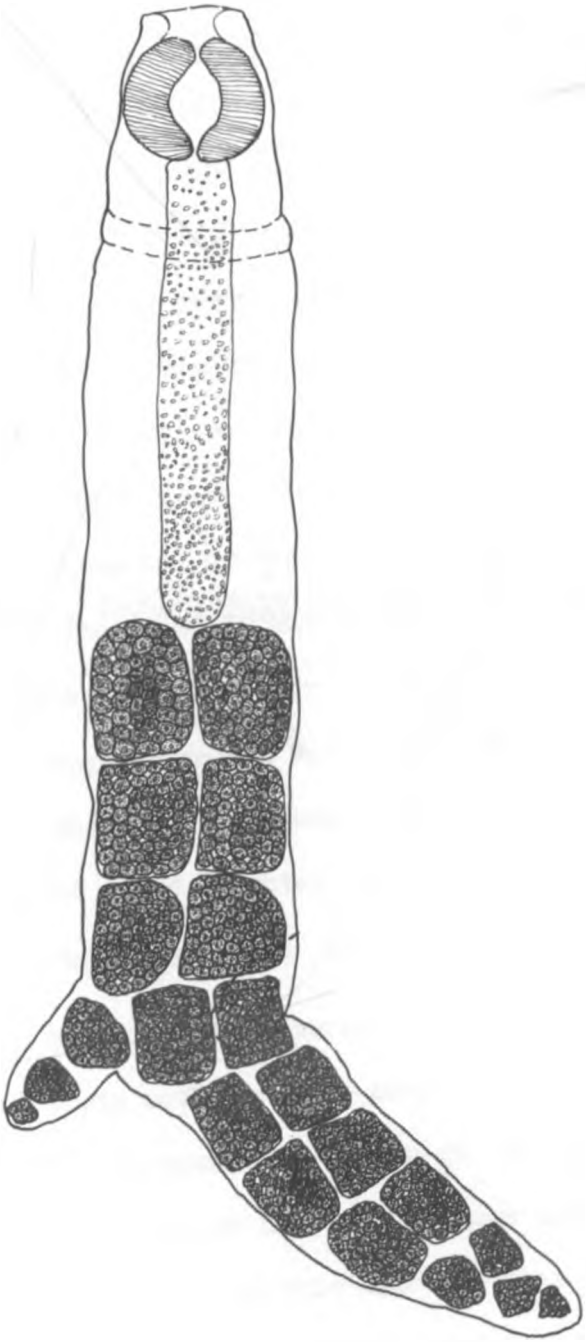


FIG. 27b.

Figure 28. Daughter redia after escaping from the mother's hollow body 25 days post penetration of the snail tissue by the miracidium.



4.2.6 Grand daughter redia (Figs. 29, 31 and 32)

Thirty one days post penetration of the snail tissue by the miracidium, the daughter redia's hollow body is filled with fully developed grand daughter rediae which have a muscular pharynx, a buccal opening that leads into a gut filled with many food particles, a collar, and a pair of appendages each. In addition, there are developing grand daughter rediae which have only their pharynges formed, and many grand daughter sacs. All the three categories of grand daughter organisms are scattered throughout the daughter redia's hollow body. The grand daughter redia's hollow body is filled with many great grand daughter germinal sacs. See figure 29. Some of the daughter rediae were still contained within the mother redia when they (daughter rediae) already contained fully developed grand daughter rediae whose bodies were filled with grand daughter germinal sacs.

Thirty two days post penetration of snail tissue by the miracidium, empty mother rediae completely devoid of any daughter rediae or germinal cells begin to appear indicating that all daughter rediae have matured and escaped into the snail's liver tissue. At this time all that remains of the mother redia is an empty bag with a muscular pharynx, buccal opening, an empty gut and a pair of appendages. These empty

bags...

bags are found in the snail's liver tissue into which the mother redia migrates before her daughters begin to escape. Figure 30 illustrates an empty mother redia.

Forty days post penetration of the snail tissue by the miracidium the grand daughter redia develops even further and its contents of great grand daughter germinal sacs which almost completely fills her hollow body increase both in size and number. Figure 31 illustrates this stage of development.

In addition to the great grand daughter germinal sacs some of which have concretions occuring in some spaces between adjacent individual germinal cells, poorly developed cercariae begin to appear in the grand daughter redia's hollow body 44 days post penetration of the snail tissue by the miracidium. Each of these developing cercariae has a short thick tail, poourly formed oral sucker and acetabulum, and concretions that are restricted to the cercaria's body. The undifferentiated great grand daughter germinal sacs with concretions in them eventually develop into cercariae while those without develop into great grand daughter (fourth generation) rediae. Figure 32 illustrates this stage of development.

Of all grand daughter rediae examined at any time, only one of them was found to be abnormal - a case of "Siamese twins" in rediae! It had two buccal openings, two pharynges, two collars, and two guts with food particles in both of them; a single body which was joined a short distance below the collar and a single appendage. This abnormal grand daughter redia, however, had a normal developing cercaria and normal great grand daughter germinal sacs. This monster is illustrated in figure 33.

Figure 29. Mature daughter redia containing developed grand daughter redia.

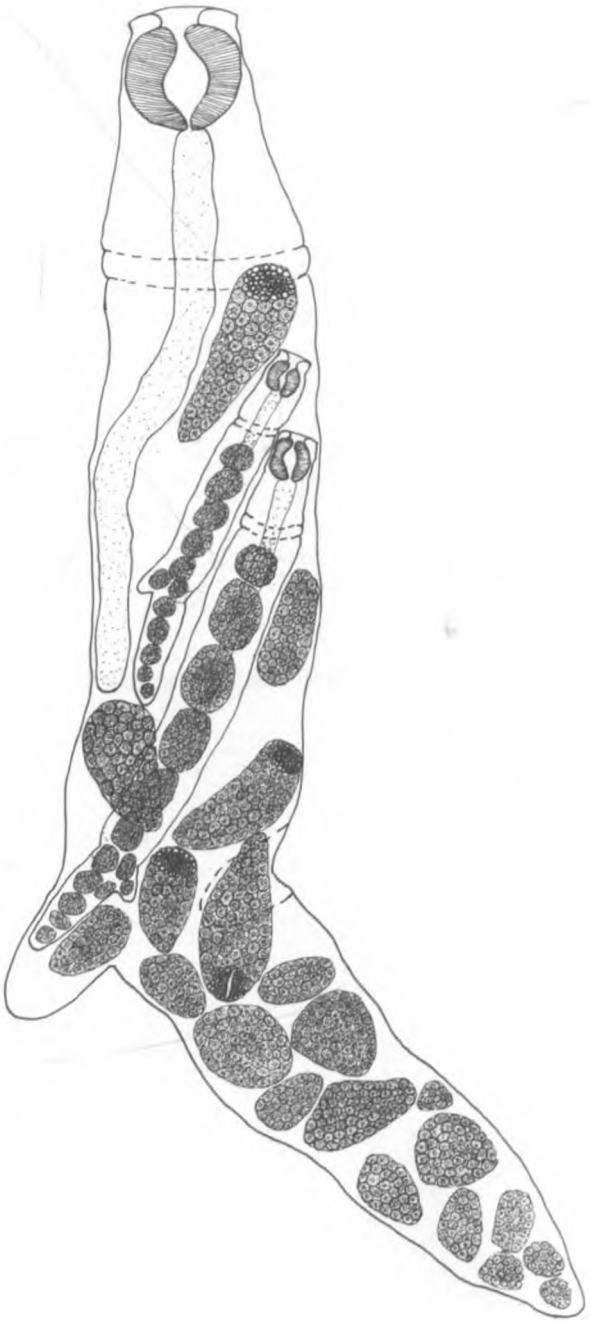


Figure 30. Empty mother redia.

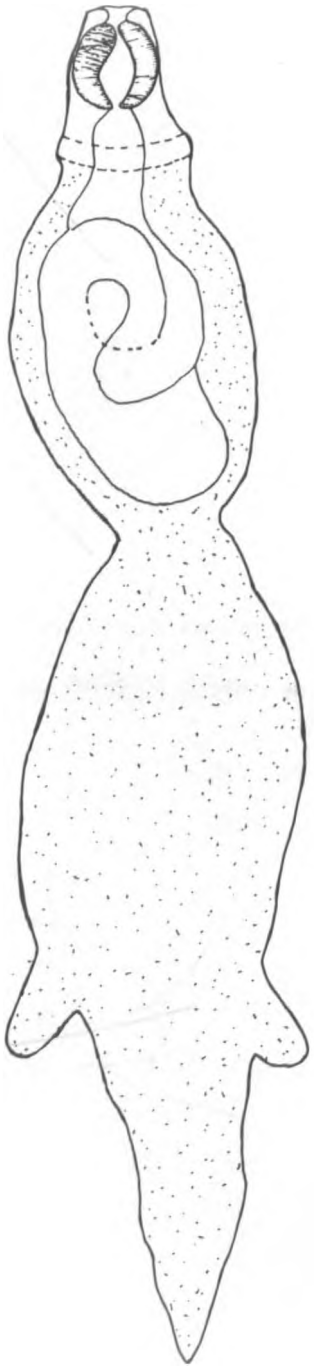


Figure 31. Mature daughter redia 40 days post penetration of the snail tissue by the miracidium.

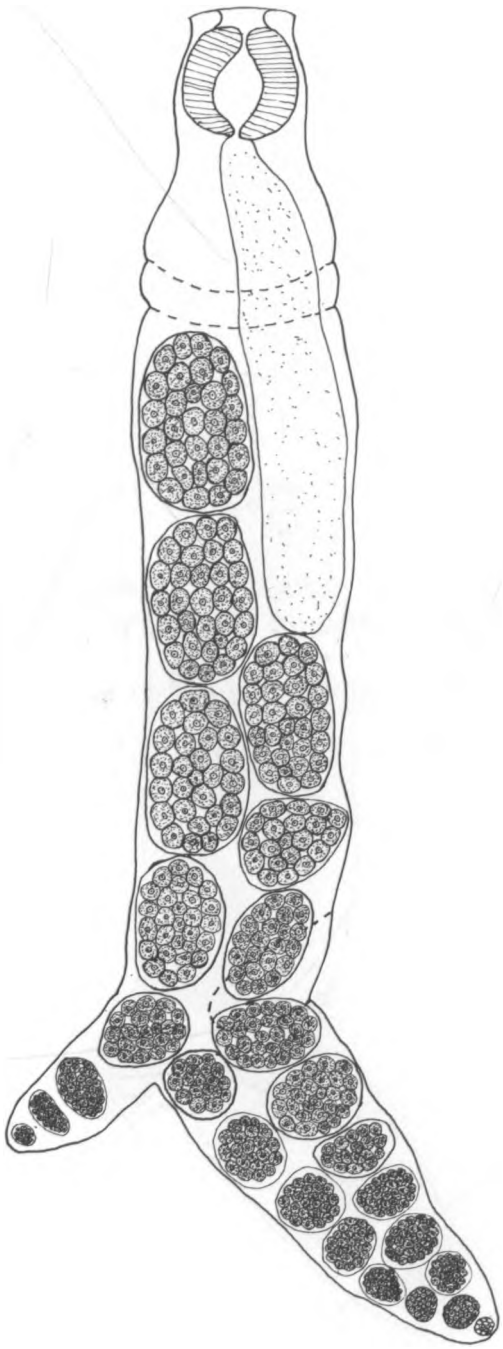


Figure 32. Mature grand daughter redia.

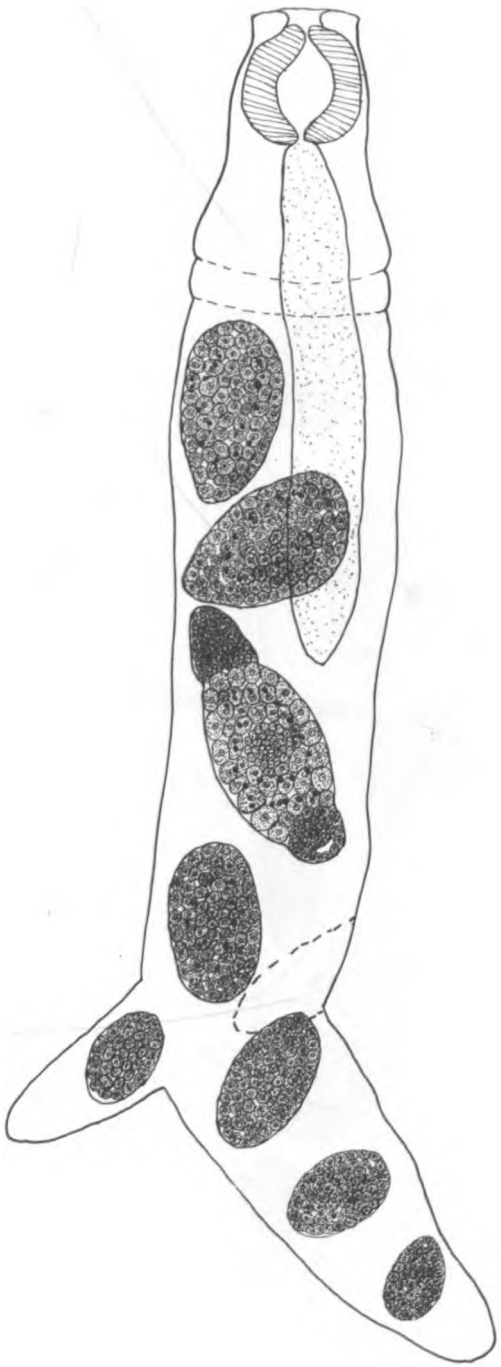
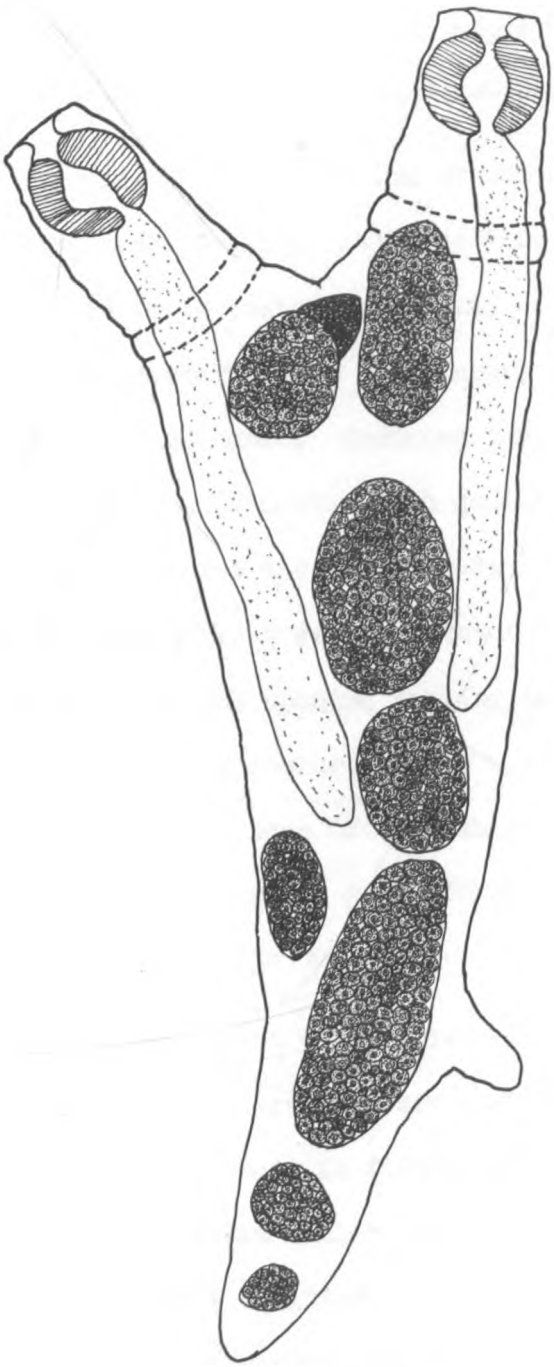


Figure 33. Abnormal grand daughter redia.



4.2.7 Great grand daughter (4th generation) redia (Figs. 34-37)

Fifty one days post penetration of snail tissue by the miracidium, the grand daughter redia contains fully formed cercariae with definite tails, acetabula, oral suckers, and pharynges. These cercariae are by no means mature, however, they are easily recognizable. Occuring together with cercariae, are great grand daughter (4th generation) germinal sacs scattered throughout the hollow body of the grand daughter redia, and a great grand daughter (4th generation) redia. This has a buccal opening leading into a gut filled with food particles, a muscular pharynx a collar and a pair of appendages. Germinal sacs of the 5th generation are scattered throughout the great grand daughter redia's hollow body. Figure 34 illustrate this stage of development.

Seventy five days post penetration of the snail tissue by the miracidium, most cercariae are mature; the grand daughter redia's hollow body is filled with cercariae and a great grand daughter redia which also contains developing cercariae. A few great daughter (4th generation) undifferentiated germinal sacs are still present at this time. See figure 35.

Eighty days post penetration of the snail tissue by the miracidium, easily recognisable cercariae are evident in the great grand daughter (4th generation) redia's hollow body; in addition, there are undifferentiated fifth generation germinal sacs which are destined to become cercariae eventually. Figure 36 illustrates this stage of development.

Eighty five days post penetration of the snail tissue by the miracidium, the great grand daughter (4th generation) redia's hollow body is completely filled with mature cercariae and some undifferentiated 5th generation germinal sacs which eventually develop into cercariae. Figure 37 illustrates this stage of development. A careful examination of the great grand daughter (4th generation) rediae failed to reveal a fifth generation redia; all 4th generation rediae had only cercariae in them. This is therefore a strong indication that only four redial generations are present in the life cycle of Fasciola gigantica.

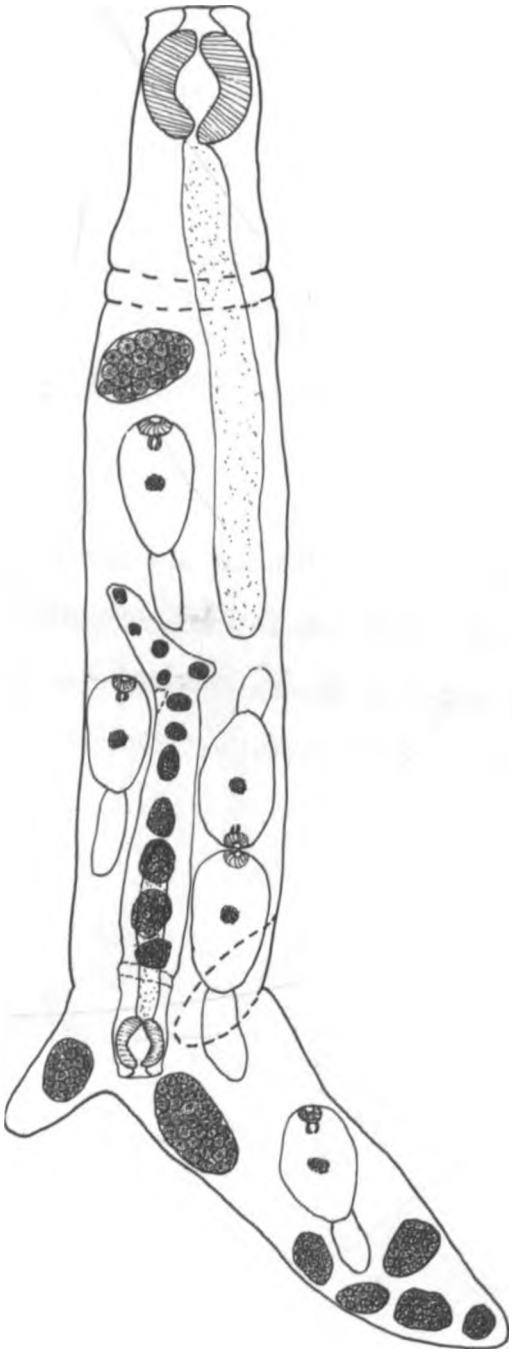


Figure 35. Grand gaughter redia containing 1st generation cercariae and a great grand daughter redia in whose hollow body are developing 2nd generation cercariae.

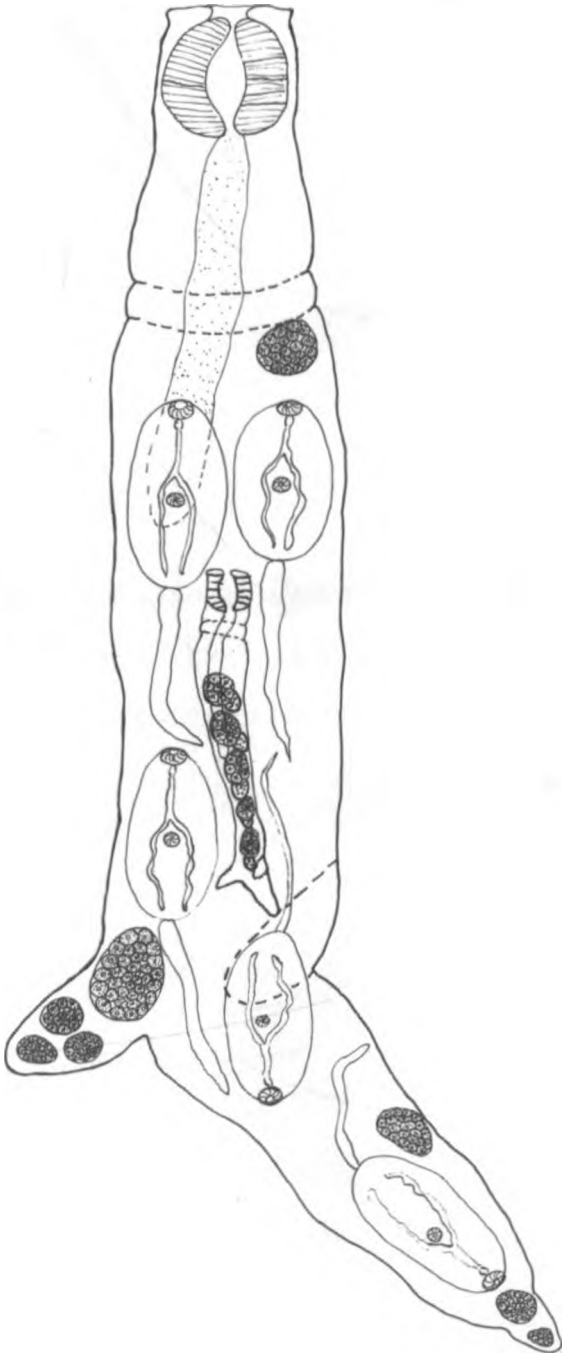


Figure 36. Mature great grand daughter redia.

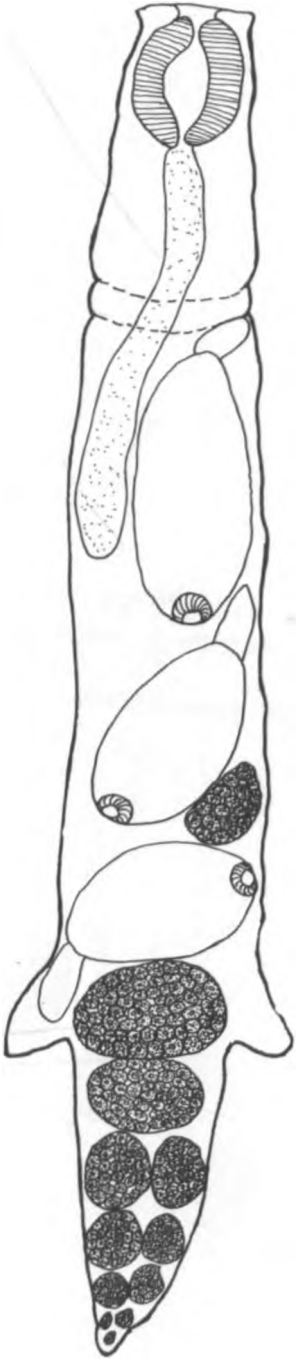
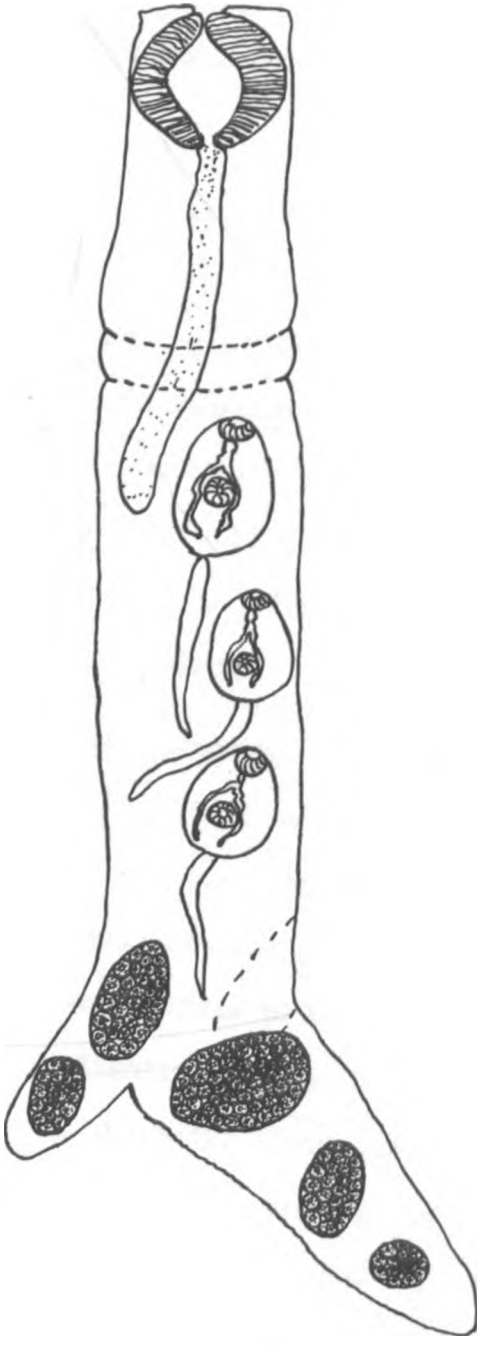


Figure 37. Mature great grand daughter redia containing mature cercariae of the 2nd generation.



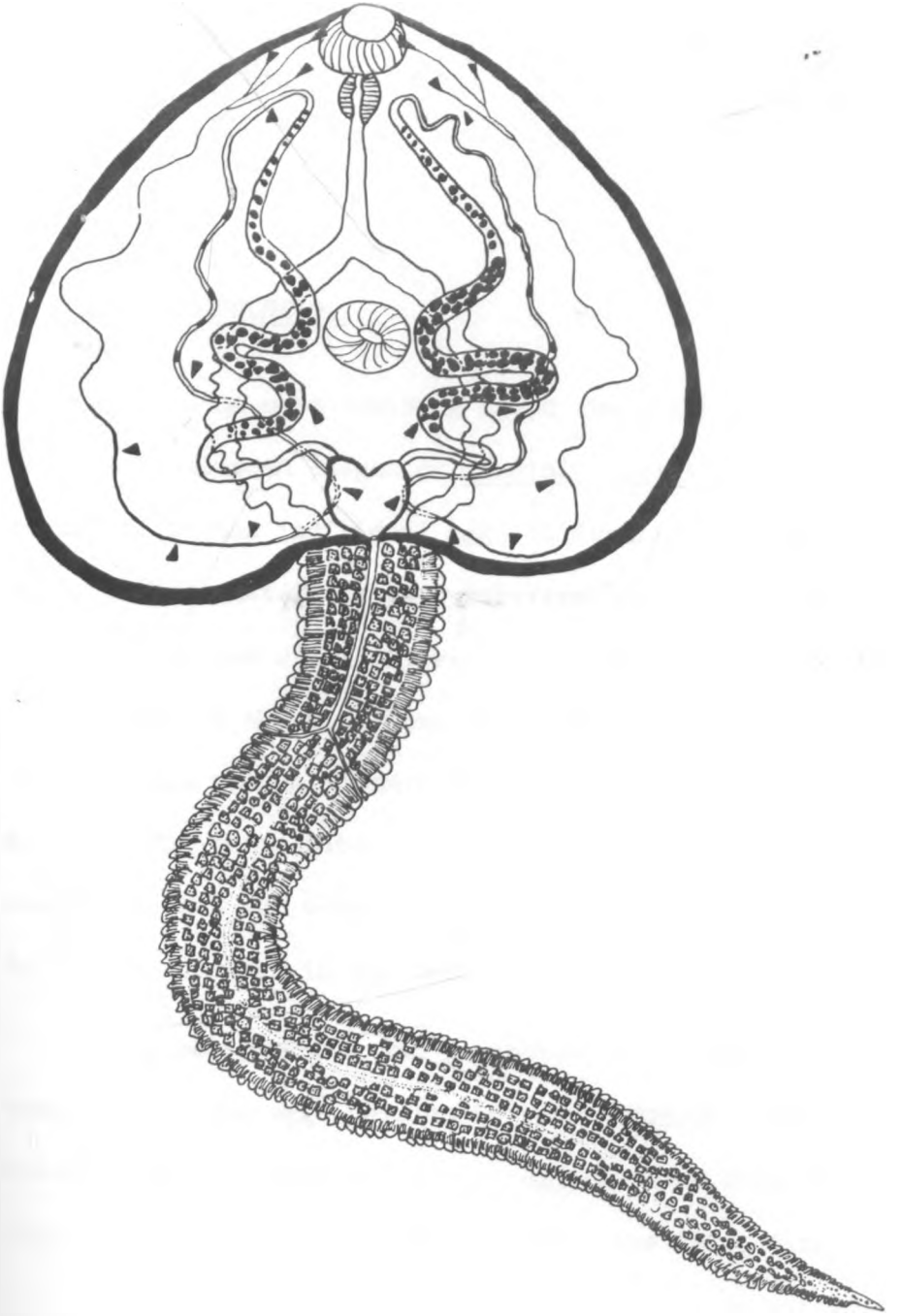
4.2.8 Cercaria (Fig. 38)

The first mature cercariae escape from the grand daughter (3rd generation) rediae 53 days post penetration of the snail tissue by the miracidium. These constitute the first generation of cercariae. A second generation of cercariae develops in the great grand daughter (4th generation) rediae which develop at the same time with the first generation of cercariae in the grand daughter (3rd generation) rediae. Cercariae, whether of the first or second generation are identical in anatomy, size and behaviour.

The cercaria appears as a whitish organism with a short, broad body measuring 200.1 by 287.1 microns and a long tail measuring 669.9 microns. Upon release in water, the cercaria swims very actively and finally rests on any solid surface, loses its tail and encysts to become a metacercaria. The caece are very slightly convoluted and almost reach the posterior end of the body. Each excretory duct which is highly convolted has two descending limbs and one ascending limb which has six ciliary patches. The first descending limb leading towards the thick-walled excretory bladder is the thickest of the three limbs, and is full of crystalline concretions. The second descending limb is the thinnest of the three and terminates in flame cells.

The ascending limb which is the middle portion of the whole excretory duct has five ciliary patches. The excretory products thus collected by flame cells are conducted through the ducts into the excretory bladder from where they are passed through the excretory tube running through the tail and finally voided through the two lateral excretory tubes. Because of numerous cystogenous material in the cercaria's body, it was not possible to see many of the very fine tubles joining each flame cell to the excretory ducts.

Figure 38. Cercaria.



4.3 DISCUSSION

One of the most striking facts about the development of the intramolluscan stages of Fasciola gigantica is the time required for the individual eggs to develop. One would normally expect that fluke eggs recovered from bile have all reached the same stage of development and would therefore go on to hatch at the same time when incubated under same conditions. Eggs remain dormant while in the bile or alimentary canal of the vertebrate host. Dinnik and Dinnik (1959) have suggested that egg development at this time is arrested due to oxygen shortage in the environment.

After being washed and incubated in tap water at room temperature, the eggs develop at very different rates; the first miracidia hatch out of the eggs 17 days following the start of incubation while 111 days after the first miracidia

appeared.....

appeared very actively swimming miracidia are still hatching. Dinnik and Dinnik (1959) working at a constant temperature of 26°C observed hatching between 17 and 90 days after incubation was first started. Thomas (1883) observed this phenomenon of delayed hatching in Fasciola hepatica. The miracidia hatching later in the hatching period are just as capable of penetrating and developing in the snail as those hatching earlier. This phenomenon of delayed hatching may perhaps be a case of adaptation by the parasite to the environment for the success of the species. That is, when miracidia are hatched at different times, some have a chance to escape adverse conditions that may be existing in the environment at that particualr time. Dinnik and Dinnik (1959) have suggested that the prolongation of the hatching period increases the chances for the miracidia to encounter a snail host; at the same time, the possibility of an over-infestation of snails with too many miracidia might be avoided. This view sounds reasonable, especially when it is realised that all miracidia hatching out of the eggs at anytime during the hatching period are capable of penetrating and developing in the snail host. One cannot, however, help questioning the idea of avoiding over-infestation when one realises that during the earlier part especially the first two weeks of the hatching period very many

miracidia...

miracidia hatch while very few indeed hatch during the latter part of the hatching period. It would therefore seem that chances of snails being over-infested are much greater during the earlier part of the hatching period and almost none in the latter part. The author, however, is in full agreement with Dinnik and Dinnik's view that chances of encountering a snail host by the miracidium are enhanced by the prolongation of the hatching period.

The sporocyst as a phase developing from the miracidium and giving rise to several mother rediae has been reported in the life cycle of Fasciola gigantica by all workers and is therefore an accepted "fact". Thomas (1882) has gone even further and reported that on rare occassions two sporocyst generations are formed by the constriction and finally division of the mother resulting in two daughter sporocysts in Fasciola hepatica. There exists, however, other contrary views on the existence of the sporocyst in the life cycle of other trematodes. Najarian (1963) has reported that Johnson (1920) believed that the miracidium of Echinostoma revolutum, a trematode in the same order as Fasciola gigantica metamorphoses directly into a redia. In his discussion of the life cycle of Fasciolopsis buski, Faust (1939) states, ".....On entering the snail and reaching the lymph spaces, the miracidium becomes

transformed...

transformed into a sporocyst which is atypical, in that it possesses a functional rhabdocele gut like a redia but lacks a pharynx." Bennet and Humes (1939) observed the development of the redia in the miracidium and reported the absence of the sporocyst in the life cycle of Stichorchis subtriquetrus. Bennet and Humes (1939) quote Wesenberg-Lund (1934) as having stated that the omission of the sporocyst stage in a trematode life history was reported by Szidat (1932) in the monostome Tracheophilus sisowi Skrjabin. Van der Woude (1954) has traced the germinal propagatory cell from which the germinal cell of the first generation redia were derived back to the developing miracidium in the life cycle of Megalodiscus temperatus. These findings by the authors mentioned above is in agreement with this writer's observation of the redia developing directly from the miracidium of Fasciola gigantica. What Faust (1939) considers to be "atypical sporocyst" with a gut but lacking a pharynx is almost the description of what the writer observed 4 days following penetration of the snail by the miracidium. The only difference is that the present writer observed a poorly formed pharynx. It may be possible that Faust overlooked this poorly formed pharynx in his specimens.

Dinnik and Dinnik (1963) have stated "...The largest of the six embryo balls in the sporocyst developed into the redia which ruptured the wall and escaped from the sporocyst. The remaining embryo continued their development into rediae, either in the ruptured body of the sporocyst or lying outside it in surrounding tissue of the snails." This writer disagrees with Dinnik and Dinnik on the possibility of embryo balls flowing out of their ruptured "sporocyst" and maturing in the snail tissue. Faust (1920) and Agersborg (1924) have shown that infected snail tissue especially liver tissue secrete substances that are both enzymic and absorptive. Agersborg who worked with Lymnaea obrussa among other snails maintained that when penetrated by the miracidium, the snail host reacts by producing a secretion from the cells of all tissues which is visible as osmium-staining granules in the intercellular spaces. It is therefore evident that the snail tissue is a hostile environment to the parasite, and the latter is only able to resist this environment through its protective wall. It would seem very difficult for the young embryo balls which have not yet developed this protective wall, and having prematurely flown out of their ruptured brood chamber to develop into rediae in this hostile environment of the snail tissue. Alicata (1938) and Dinnik and Dinnik (1963) observed

mother.....

mother rediae having escaped from the sporocyst 5 days and 6 to 8 days respectively following penetration of the snail by the miracidium. This writer demonstrated that as early as 4 days following penetration of the snail by a miracidium, the mother redia can be recognized even though some of its structures are not fully formed. It is therefore possible that what these three authors thought to be mother rediae developing out of the sporocyst were in fact mother rediae developing directly from the miracidium. The diagram of the sporocyst drawn by Thomas (1883) shows a structure with a pharynx and a gut which he calls the mother redia enclosed within a larger structure with a pair of eye spots. The way the "mother redia" is positioned in the "sporocyst" is such that what Thomas considers as the mother redia could be the pharynx and developing gut of the whole structure, the mother redia. This diagram very much resembles what the writer observed and later confirmed to be the mother redia. This stage is illustrated by figure 20 which shows a developing gut, a non-muscular pharynx and a pair of eye spots.

The two prominent features i.e. the eye spots and a pair of flame cells which were observed in the miracidium have been used as "tags" to trace the transformation of the miracidium into the mother redia. These structures were observed in

stages....

stages illustrated in figures 17, 18, 19, 20, and 21 and yet figure 20 and 21 illustrate fully formed rediae. This means that these eye spots and flame cells are the ones seen in the miracidium. If mother rediae developed as individuals inside the sporocyst then it would be very unlikely for them to have these eye spots and flame cells that were observed in the miracidium. The logical thing would be for the sporocyst to have acquired these eye spots and flame cells. Since fully formed rediae still had these original structures i.e. eye sports and flame cells seen in the miracidium, it is only reasonable to conclude that the mother rediae developed directly from miracidia. If it is accepted that more than one mother redia will develop from one sporocyst, it would be logical to expect more mother rediae than the number of miracidia to which the individual snail was exposed. An attempt to demonstrate this fact failed. On the contrary the maximum number of mother rediae ever recovered from any individual snail was equal to the maximum number of miracidia to which the snail was exposed; these results are shown in tables VII and VIII.

Based on these observations, it is the firm conclusion of the present writer that the sporocyst stage does not exist in the life cycle of Fasciola gigantica.

Varied views exist as to how many redial generations there are in the life cycle of Fasciola gigantica. Thomas (1882) and Leuckart (1882) observed two redial generations in the life cycle of Fasciola hepatica and reported that each of the two redial generations is capable of producing cercariae depending on temperature conditions of the environment. Poter (1920) observed two redial generations; she stated ".....The first generation redia which develops from the sporocyst produce cercariae and towards the end of its life, gives rise to a second generation of rediae." Alicata (1938) observed two redial generations in addition to the sporocyst with cercariae developing in rediae of the second generation only. Dinnik and Dinnik (1964) observed three redial generations in addition to the sporocyst with cercariae developing in all rediae of all these generations. If, as it is the writer's contention, what has been hitherto regarded as a sporocyst is in fact a first generation redia, then what Thomas, Leuckart, Porter and Alicata observed could be regarded as three redial generations, instead of a sporocyst and two redial generations, while Dinnik and Dinniks' observation would be regarded as four redial generation, instead of a sporocyst and three redial generations. This last observation is in agreement with the writer's observation of four

redial...

redial generations. Alicata's observation of cercariae being produced by what this writer considered third redial generation agrees with his (writer's) observation of cercariae being produced for the first time by rediae of the third generation. Porter's observation of the second redial generation being produced by the first redial generation, the latter having been producing cercariae does not agree with the present writer's observation; however, there are some aspects that may have some relevance. This writer observed rediae of the third generation producing both first generation cercariae and very few 4th generation rediae. It is possible that Porter missed by accident one generation in between and in fact what she observed are what this writer considers to be the third generation rediae with first generation cercariae and 4th generation rediae developing in them. Dinnik and Dinnik (1963) observed alternation of generations of rediae and cercariae in first generation of rediae; they further reported that this phenomenon is practised by all subsequent redial generations, this is contrary to observations by Thomas (1882) and Alicata (1938). Several attempts were made to try and demonstrate this phenomenon but it was never observed.

It is therefore concluded that there are four redial generations in the life cycle of Fasciola gigantica and that

the...

the earliest redial generation ever to produce cercariae is the third. In this third generation of rediae, the first generation of cercariae are produced together with the 4th redial generation which in turn produce a second generation of cercariae.

Two cercarial generations have been observed, the first generation developing in the third generation rediae while the second develops in the 4th redial generation. The cercaria has a complicated excretory system which begins with flame cells and courses into the excretory bladder. Wright (1927) observed seven pairs of flame cells in Fasciola hepatica, while Kuntz (1957) observed eight pairs in F. gigantica. The present writer, however, observed 10 pairs of flame cells in Fasciola gigantica. A lot of time and effort was spent studying the excretory system of many live cercariae of F. gigantica; however, owing to the vast amount of cystogenous material in the body it is possible and in fact probable that there may be more flame cells than were actually observed. While examining living cercariae for flame cells, five ciliary patches were observed in the ascending limb of each excretory duct. Kuntz (1957) observed five ciliary patches in the ascending limb of the excretory duct of Fasciola gigantica. Very probably these ciliary patches which flicker in the duct of a live cercaria help in the movement of excretory material along the excretory channels.

LITERATURE CITED

Agersborg, H.P.K. 1924. Studies on the effect of parasitism upon the tissues I. With special reference to certain gastropod molluscs. Quart. J. Micr. Sci., 68:361-401.

Alicata, J.E. 1938. Observations on the life history of Fasciola gigantica, the common liver fluke of cattle in Hawaii, and the intermediate host Fossaria ollula. Hawaii Agric. Expt. Stat. Bull. No. 80:1-22.

Alicata, J.E. 1941. Studies on control of the liver fluke of cattle in the Hawaiian Islands. Amer. J. Vet. Res., 2:152-164.

Alicata, J.E. 1953. The snails, Pseudosuccinea columella (Say) new intermediate hosts for the liver fluke Fasciola gigantica Cobbold. J. Parasit., 39:673-674.

Alicata, J.E. and Swanson, L.E. 1937. Fascicla gigantica, a liver fluke of cattle in Hawaii, and the snail, Fossaria ollula, its important intermediate host. J. Parasit., 23:106-107.

Barlow, C. 1937. The value of canal clearance in the control of schistosomiasis in Egypt. Amer. J. Hyg., 25:327-348.

Bennet, H.J. and Humes, A.G. 1939. Studies on the pre-cercarial development of Stichorchis subtriquetrus (Trematoda:Paramphistomidae). J. Parasit., 25:223-231.

Berg, C.O. 1953. Sciomyzid larvea (Diptera) that feed on snails. J. Parasit., 39:630-636.

Bhalerao, G.D. 1933. Preliminary note on the life history of the common liver fluke Fasciola gigantica in India. Ind. J. Vet. Sci. and Anim. Husb., 3:120-121.

Bitakaramire, P.K. 1968. Bovine Fascioliasis in Kenya. Bull. epizoot. Dis. Afr., 16:107-113.

Boray, J.C. 1963. The ecology of Fasciola hepatica with particular reference to its intermediate host in Australia. Proc. 17th Wld. Vet. Congr., Hanover., 1:709-715.

Boray, J.C., Happich, F.A. and Andrews, J.C., 1969. The epidemiology of fascioliasis in two representative endemic regions of Australia. Aust. Vet., 54:549-533.

Chenin, E. 1956. The control of Australobis glabratus populations by the leech Helobdella fusca under laboratory conditions. Amer. J. Trop. Med. Hyg., 5:308-314.

Chenin, E. 1957. Studies on the control of schistosome-bearing snails. VII. Streptomysin-induced inhibition of growth and reproduction in the schistosome-bearing snail, Australorbis glabratus. Amer. J. Hyg., 66:321-330.

Chenin, E., Michelson, E.H., and Augustine, D.L. 1956. Studies on the Biological control of schistosome-bearing snails. 1. The control of Australorbis glabratus populations by the snail, Marisa cornuarietis under laboratory conditions. Amer. J. Trop. Med. Hyg., 5:297-307.

Coyle, T.J. 1956. Liver fluke in Uganda. Bull. epizoot. Dis. Afr., 4:47-55.

Coyle, T.J. 1958. Experiments in the diagnosis and treatment of fascioliasis in Uganda cattle. Bull. epizoot. Dis. Afr., 6:255-272.

Dawes, B. 1959. Penetration of the liver fluke Fasciola hepatica into the snail, Lymnaea truncatula. Nature, 184:134-135.

Dawes, B. 1960. Penetration of Fasciola gigantica Cobbold 1856 into snail hosts. Nature, 185:51-53.

Dias, E. and Dawood, M.M. 1955. Preliminary trials on the biological snail control with Bacillus pinottii in Egypt. Mem. Inst. Oswaldo Cruz, 53:13-29.

Dinnik, J.A. 1958. Identification of liver fluke and stomach fluke eggs recovered from feces of infected animals. Bull. epizoot. Dis. Afr., 6:135-138.

Dinnik, J.A. and Dinnik, N.N. 1959. Effect of the seasonal variations of temperature in the development of F. gigantica eggs in Kenya highlands. Bull. epizoot. Dis. Afr., 7:357-369.

Dinnik, J.A. and Dinnik, N.N. 1963. The effect of seasonal variations of temperature on the development of F. gigantica in the snail host in Kenya highlands. Bull. epizoot. Dis. Afr., 11:197-207.

Dinnik, J.A. and Dinnik, N.N. 1964. The influence of temperature on the succession of redial and cercarial generations of F. gigantica in a snail host. Parasit., 54:59-65.

Dollfus, R.P. 1912. Une metacercaire margaritigene parasite de Donax vittatus Da Costa. Mem. Soc. Zool. Fr. 25:85-14.

Faust, E.C. 1920. Pathological changes in the gastropod liver produced by fluke infection. Johns Hopkins Hosp. Bull. No. 31:79-84.

Faust, E.C. 1939. Human Helminthology. 2nd Edition pp. 181-182. Lea and Febiger, Philadelphia. Pa. U.S.A.

Ferguson, F.F., Oliver-Gonzales, J. and Palmer, J.R. 1968. Potential for biological control of Australobis glabratus, the intermediate host of Puerto Rican schistosomiasis. Amer. J. Trop. Med. Hyg., 7:491-503.

Froyd, G. 1959. The incidence of liver fluke and gastro-intestinal parasites of cattle in Kenya. Bull epizoot. Dis. Afr. 7:179-182.

Gordon, H.M. 1955. Some aspects of fascioliasis. Vet. J., 31:182-189.

Kendall, S.B. and Parfitt, J.W. 1953. The life history of Fasciola gigantica Cobbold 1856. Nature, 171:1164-1165.

Kuntz, R.E. 1957. Development of the cercariae of Fasciola gigantica Cobbold 1855, with emphasis on the excretory system. Trans. Amer. Micro. Soc., 76:269-274.

Leuckart, R. 1882. Zur Entwicklungsgeschichte des leberegels. Zool. Anz., 5:524-528.

Leuckart, R. 1886. Die Parasiten des Menschen und die von inhen herrunrenden Krankheiten. Leipzig u. Heidelberg. (Original article not seen.)

Looss, A. 1896. Recherches sur la Faune parasitaire de l'Egypte. Mem. Inst. Egypt., III:1-252.

MacDonald, M. 1965. Treasure of Kenya. Collins, St. James' Place, London.

Malek, E.A. 1956. Factors conditioning the habitat of bilharziasis hosts of the family Planorbidae. Bull. Wld. Hlth. Org., 18:785-818.

Manipol, F.S. 1938. The molluscan hosts of Fasciola gigantica in the Philippines. Univ. Philippines, Nat. and Appl. Sci. Bull., 5:335-262.

Michelson, E.H. 1957. Studies on the biological control of Schistosome-bearing snails. Predators and parasites of fresh water Mollusca: A review of literature. Parasit., , 47:413-426.

Mitchel, J.R. 1968. Durch Fleisch ubertragene wurm zoonosen in Ostafrika. Die Fleischwirtscharf No. 8.

Monnig, H.O. 1934. Veterinary Helminthology and Entomology. The Williams and Wilkins Co. Baltimore, Md. U.S.A.

Mozley, A. 1960. Consequenses of disturbance. H.K. Lewis and Co. Ltd., London.

Nagar, H.E. 1958. Control of Schistosomiasis in the Gezira, Sudan. J. Trop. Med. Hyg. 61:231-235.

Najarian, H.H. 1955. The life history of Echinoparyphium flexum (Linton 1892) (Dietz 1910) (Trematoda: Echinostomidae). Science, 117 No. 3047:564-565.

Ogambo-Ongoma, A.H. 1969. The incidence of Fasciola hepatica Linnaeus, 1758 in Kenya cattle. Bull. epizoot. Dis. Afr., 17:429-431.

Ogambo-Ongoma, A.H. 1970. Fascioliasis Survey in Uganda. (part of unpublished thesis.)

Ogambo-Ongoma, A.H. 1970. Snail population Dynamics. (Part of unpublished thesis).

Oliver-Gonzales, J. 1946. The possible role of the guppy Lebistes reticulatus, on the biological control of Schistosoma mansoni. Science, 104 (2712) : 605.

Oliver-Gonzalez, J. and Ferguson, F.F. 1959. The probable biological control of Schistosoma mansoni in Puerto Rican Watershed. Amer. J. Trop. Med. Hyg., 8:56-59.

Ollerenshaw, C.B. 1959. Ecology of liver fluke (Fasciola hepatic). Vet. Res. 71:957-963.

Pantelouris, E.M. 1965. The Common Liver Fluke. Pergamon Press, Oxford.

Pennak, R.W. 1963. Fresh-water Invertebrates of the United States. The Ronald Press Company, New York, U.S.A.

Porter, A. 1920. The life history of the African sheep and cattle fluke F. gigantica. S. Afr. J. Sci., 17: 26-130.

Radke, M.G. Ritchie, L.S. and Ferguson, F.F. 1961. Demonstrated control of Australorbis glabratus by Marisa cornuarietis under field conditions in Puerto Rico. Amer. J. Trop. Med. Hyg., 10:370-373.

Reinhard, E.G. 1957. Landmarks in Parasitology. I. The discovery of the life cycle of the liver fluke. Expt. Parasit., 6:208-232.

Schmid, F. 1929. Beitrag zur Kenntnis der Trematodenlarven aus der Leberegelschnecke. Sitzungsberichter der Gesellschaft Naturforschender Freunde, pp. 256-267. (Original article not seen).

Taylor, E.L. 1964. Fascioliasis and the liver fluke. F.A.O. Agric. studies Bull. No. 64:1-234.

Thomas, A.P. 1882. The rot in sheep or the life history of the liver fluke. Nature, 26:606-608.

Thomas, A.P. 1883. The life history of the liver fluke (Fasciola hepatica). Quart. J. micr. Sci., 23:99-133.

Uganda Meat Pakcers Ltd., Annual Returns 1954-1969.

Wager, V.A. 1936. Possibility of eradicating bilharzia by extensive planting of tree Balanites. S. Afr. Med. J., 10:10-11.

Wright, C.A. 1957. Guide to Mclluscan Anatomy for Parasitologists in Africa. British Museum (Natural History), London.

Wright, W.R. 1927. Studies on larval trematodes of North Wales. 1. Observations on the redia, cercaria and cyst of Fasciola hepatica. Ann. Trop. Med. and Parasit., 21:47-56.

Van der Woude, A. 1954. Germ Cell Cycle of Megalodiscus temperatus (Stafford, 1905). Harwood, 1932 (Paramphistomidae: Trematoda). The Amer. Mid. Nat., 51:172-202.

Yamaguti, S. 1958. Systema Helminthum. I. The Digenetic Trematodes of Vertebrates. Part I and II. Inter. Sci. Publ. Co. NY.

A P P E N D I X I

1. Kigezi District.

Kabale, Kisoro, Cyanika, Rubaya, Maziba, Kanungu, Rukungiri, Bugangari and Ikumba.

2. Ankole District.

Kikagati, Nsongezi, Rubongota, Chiture, Kagunza, Rwentobo, Rubaare, Ntungamo, Gayaza, Mbarara, Kinoni, Sanga, Kabwohe, Kayonta, Bushenyi, Mitoma, Kyamahungu, Rutoma, Rubirizi, Katunguru, Ibanda, Kiruha, Busheshe and Kazo.

3. Toro District.

Ntoroko, Kijura, Katoke, Kyenjojo, Butiti, Fort Portal, Bundibugyo, Kakabara, Kyegegwa, Mubuku, Kasese, Muhokya, Nyabirongo, Dwera, Nyakatonzi, Kamwenge and Bihanga.

4. Bunyoro District.

Matunda, Katulikire, Kiryandongo, Kigumba, Masindi Port, Kibangya, Butiaba, Biso, Kinyara, Bikonzi, Bwijanga, Bulindi, Kitoba, Hoima, Munteme, Kitoma, Bulumagi, Muhororo, Kibalo and kakumiro.

5. West Nile District.

Panyimu, Parombo, Goli, Paidha, War, Nebbi, Ojollo, Pakwach, Ragem, Utrutru, Uleppi, Arua, Terego, Ovujo, Maracha, Omugo, Koboko, Londonga, Aringa, Yumbe, Lori and Rumogi.

Appendix I Contnd.

6. Madi District.

Moyo, Metuli, Laropi, Laufori, Nyeru, Pachara, and Adjumani.

7. Acholi District.

Te Okot, Lolim, Aparanga, Amaka, Alero, Alelelele, Keyo, Lamogi, Gulu, Laminabili, Parabong, Pabo, Patiko, Pawe, Atiak, Palabek, Padibe, Madi Opei, Kitgum, Naam-Okora, Aswa, Atanga, Pajule, Opit, Paranga, Patongo and Adilang.

8. Karamoja District.

Loyoro, Kotido, Morulem, Nakiloro, Moroto, Kangole Lorengedwat, Loro, Nabilatuk, Amudat and Moruit.

9. Lango District.

Minakulu, Atura, Ibuje, Akokoro, Namasale, Amolatar, Nabyeso, Kachung, Aduku, Bar, Aloi, Lira, Aboke, Anyeko, Otwala and Agur.

10. Teso District.

Orungo, Amuria, Katukwi, Tiriri, Wera, Magoro, Kaberamaido, Kalaki, Arapai, Soroti, Gweri, Bugondo, Kadunguru, Serere, Ngora, Kumi, Bukedea, and Kachumbala.

Appendix I Contnd.

11. Bugisu District.

Siroko, Budadiri, Buwalasi, Mbale, Bududa, Busisu, Mayenze, Buliru, and Bubulo.

12. Sebei District.

Binyin, Kaburoron, Kapchorwa, Sipi, Kyosweri, and Bukwa.

13. Bukedi District.

Agulo, Gogonyo, Pallisa, Kamuge, Kibuku, Budaka, Iki Iki, Naboa, Busolwe, Budumba, Nagongera, Molo, Tororo, Sukulu, Busia and Majanji.

14. Busoga District.

Kidera, Buyende, Kagulu, Nawaikake, Kamuli, Mbulamuti, Naigobira, Nsinze, Busembatia, Luzinga, Busesa, Iganga, Bugiri, Kakira, Magamaga, Ikulwe, Jinja, Kityerera and Lugala.

15. East Mengo District.

Nakitoma, Lwampanga, Nabiswera, Nakasongola, Galiraya, Kakoge, Bale, Luwero, Kiziba, Kayunga, Kapeka, Bowa, Wobulenzi, Bombo, Kikakala, Nakifuma, Nagalama, Nagojje, Mukono, Lugazi, Seta, Buikwe, Ngogwe and Kibanga.

Appendix I Contnd.

16. West Mengo District.

Lukona, Matuga, Kasangati, Kakiri, Kawanda, Kawempe, Kampala, Nsangi, Kajansi, Kisubi, Nkumba, Entebbe, Kasanje, Mitala Maria, Kabasanda, Mpigi, Kamengo, Kiriri, Kanoni, Kabulasoke, Maddu and Port Bell.

17. Mubende District.

Bukwisi, Ntwetwe, Kiboga, Nabingora, Mubende, Kiwumola, Kasanda, Bukonera, Kikandwa, Busunju, Sekanyonyi and Mityana.

18. Masaka District.

Ntusi, Sembabule, Lyantonde, Lukaya, Kalungu, Kawoko, Makoko, Masaka, Kaboyo, Mbirizi, Kiziba, Kalisizo, Kyotera, Kakuto and Mutukula.

A P P E N D I X II

1st	day	2nd	day	3rd	day	4th	day	5th	day
Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails
1	20	15	0	20	1	8	0	41	6
62	100	30	3	27	2	49	3	26	0
28	103	40	56	43	3	58	16	17	0
30	48	28	28	8	0	16	0	62	21
51	93	52	50	2	0	61	23	37	3
49	139	26	0	37	5	4	0	43	10
16	32	32	0	12	0	29	3	31	0
22	91	42	2	32	2	19	4	29	5
20	12	58	108	15	0	36	7	52	11
23	88	57	67	38	3	23	0	38	0
8	100	44	21	18	2	31	4	15	8
45	39	54	61	21	1	25	5	40	2
26	17	22	3	19	2	62	26	1	1
39	141	48	40	13	0	11	0	16	2
14	13	37	10	16	0	42	6	25	0
5	7	21	14	49	6	28	2	53	10
53	208	6	3	54	8	33	1	12	0
19	18	39	22	1	0	14	0	54	7
3	30	50	13	61	9	34	2	49	6
31	70	4	0	56	4	9	0	55	18
TOTAL	1369	TOTAL	501	TOTAL	48	TOTAL	102	TOTAL	110

Appendix I Contnd.

6th day		7th day		8th day		9th day		10th day	
Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails
34	8	39	1	19	4	50	1	49	6
1	0	43	10	27	1	56	3	8	0
57	6	5	0	53	0	17	0	3	0
64	10	8	0	50	4	53	5	15	0
31	4	16	0	5	2	48	2	31	0
12	0	49	8	47	14	41	4	39	4
37	0	15	4	33	0	5	0	41	3
49	3	13	0	25	3	42	3	10	0
29	7	25	8	54	18	28	1	36	3
53	4	56	7	45	0	19	0	22	0
42	0	1	0	7	5	51	3	33	2
58	9	49	6	28	0	60	6	44	1
25	3	38	5	58	9	59	4	9	0
38	1	27	3	30	0	14	0	46	2
47	5	33	6	14	0	30	0	4	0
51	2	57	10	56	3	36	1	42	3
50	1	8	0	16	1	9	0	53	7
16	3	42	4	40	0	31	1	29	0
39	0	53	9	12	0	49	3	45	3
9	1	10	3	48	6	61	6	38	4
TOTAL	67	TOTAL	84	TOTAL	70	TOTAL	43	TOTAL	38

Appendix IX Contnd.

. 11th day		12th day		13th day		14th day		15th day	
Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails
8	0	24	0	42	1	44	4	39	0
27	3	29	0	5	0	31	0	47	0
46	3	12	0	1	0	52	3	3	0
39	1	6	0	8	0	25	0	53	4
43	4	16	0	32	2	38	4	62	1
53	2	19	0	3	0	32	0	36	0
3	0	39	2	21	0	37	0	55	1
13	0	1	0	28	0	1	0	18	0
25	7	61	3	62	8	7	0	19	0
31	0	38	0	17	0	50	2	33	0
45	4	15	0	10	0	10	0	40	0
50	3	52	2	18	0	6	0	14	0
48	6	47	2	4	0	56	6	46	1
62	10	59	1	55	1	16	0	10	0
7	0	20	0	12	0	47	1	16	0
58	9	43	2	59	2	29	0	25	0
23	0	13	0	38	0	46	2	43	0
1	2	9	0	58	3	25	0	41	1
10	0	30	0	14	0	35	1	21	0
19	1	60	2	12	0	21	0	7	0
TOTAL	55	TOTAL	14	TOTAL	17	TOTAL	23	TOTAL	8

Appendix II Contnd.

16th day		17th day		18th day		19th day		20th day	
Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails
35	0	17	0	3	0	38	2	63	7
52	0	35	0	49	5	30	0	18	0
4	0	38	0	18	0	8	0	12	0
54	4	9	0	60	7	21	0	16	0
19	0	61	4	9	0	27	0	25	0
9	0	10	0	6	0	34	0	4	0
12	0	41	0	61	4	24	0	47	10
14	0	15	0	25	0	10	0	40	1
51	3	44	0	54	1	9	0	35	1
2	0	33	0	52	0	49	2	9	0
30	0	27	0	16	0	33	0	19	0
33	0	37	0	64	10	45	3	53	8
3	0	14	0	62	3	19	0	34	0
16	0	52	0	29	0	25	0	46	2
17	0	12	0	35	0	54	5	36	3
31	1	25	0	44	1	28	0	7	0
56	0	11	0	8	0	15	0	14	0
64	2	2	0	17	0	20	0	41	3
15	0	54	5	38	0	31	0	55	4
39	0	21	0	31	0	12	0	24	0
TOTAL	10	TOTAL	9	TOTAL	31	TOTAL	12	TOTAL	39

Appendix II Contnd.

21st day		22nd day		23rd day		24th day		25th day	
Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails
3	0	8	0	43	2	6	0	13	7
15	0	4	0	3	0	20	2	7	0
54	10	47	2	56	15	9	1	3	0
12	0	61	20	45	0	41	0	57	13
17	3	52	13	4	0	44	6	35	0
52	6	38	2	52	0	59	11	39	9
11	0	22	0	12	0	2	0	22	0
27	2	12	0	34	0	27	3	29	27
9	0	36	0	39	0	7	2	51	0
50	7	21	0	58	13	17	0	12	8
40	3	49	14	5	0	31	7	31	0
25	0	39	0	25	0	28	0	54	4
24	0	11	0	63	17	58	20	34	0
1	0	12	0	42	0	22	3	21	8
7	0	9	0	22	0	46	2	59	0
13	2	55	11	28	0	16	0	8	0
30	0	16	0	50	3	62	18	17	0
25	0	6	0	37	0	54	4	25	0
37	5	33	0	62	20	11	0	47	10
8	1	32	8	16	8	8	3	2	0
TOTAL	39	TOTAL	70	TOTAL	78	TOTAL	82	TOTAL	86

Appendix II Contnd.

26th day		27th day		28th day		29th day		30th day	
Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails
11	0	36	8	19	2	3	2	9	6
29	11	32	1	50	11	11	1	56	20
28	0	42	7	32	4	58	7	27	11
58	7	15	1	39	2	49	6	43	5
1	0	30	1	41	7	60	20	50	17
14	6	27	2	28	4	19	3	16	5
22	0	64	19	15	5	47	8	11	4
33	0	26	3	2	0	20	3	37	12
21	0	5	2	1	0	62	25	25	4
4	0	40	10	59	14	45	7	12	6
50	17	12	3	61	15	43	2	49	8
43	0	51	11	40	3	40	10	28	6
9	0	[illegible]	0	21	1	52	14	52	9
44	6	7	2	64	21	13	7	21	7
17	0	11	6	9	1	57	18	15	10
34	5	8	2	46	13	9	8	57	14
25	4	1	0	22	0	22	9	8	11
23	0	3	0	14	4	1	2	36	13
54	21	55	14	5	2	17	6	18	10
37	0	46	6	25	0	63	25	54	12
TOTAL	77	TOTAL	98	TOTAL	109	TOTAL	183	TOTAL	190

Appendix II Contnd.

31st day		32nd day		33rd day		34th day		35th day	
Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails
27	6	46	14	16	5	54	28	50	8
44	12	3	3	49	17	61	33	39	3
24	7	52	11	48	19	47	13	19	9
31	9	16	6	15	4	17	15	3	1
4	2	12	10	62	19	26	14	58	3
15	7	40	9	10	13	25	15	23	1
3	4	9	10	38	16	28	11	49	4
49	6	33	12	12	6	15	17	37	8
26	9	45	16	3	4	14	12	6	0
57	4	55	17	1	2	22	13	7	0
7	8	31	9	37	14	38	18	34	3
40	3	44	14	40	12	1	12	13	2
16	11	25	9	27	11	31	19	55	2
51	12	42	13	29	16	20	11	59	4
47	10	4	3	11	4	12	18	51	3
29	3	21	11	7	14	8	8	43	6
60	13	24	10	4	3	49	25	44	3
25	7	28	10	61	20	41	14	9	0.
9	6	17	8	25	14	43	13	52	3
62	19	47	15	53	15	55	23	15	4
TOTAL	158	TOTAL	210	TOTAL	228	TOTAL	332	TOTAL	67

Appendix II Contnd.

36th day		37th day		38th day		39th day		40th day	
Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails
27	16	10	6	38	16	61	28	3[illegible]	17
43	15	14	7	50	14	10	11	21	19
38	5	25	10	16	16	52	24	64	18
54	14	39	13	23	20	9	15	16	2
29	6	34	4	11	14	49	18	52	21
52	13	7	11	7	12	56	13	11	16
18	7	17	5	45	17	5	14	41	22
41	10	21	9	14	14	36	12	50	12
1	4	16	4	47	15	12	22	34	16
30	5	4	6	57	19	34	13	1	8
59	19	57	14	5	10	32	11	62	19
13	4	23	7	26	16	21	4	5	2
2	6	12	4	3	6	16	13	28	21
16	17	18	8	1	5	29	16	55	14
28	5	48	9	52	20	39	11	63	35
45	9	56	4	18	7	14	14	12	1
32	13	40	3	19	16	40	13	58	16
24	11	63	20	42	14	59	10	49	10
46	7	60	17	62	31	48	14	37	7
11	18	14	6	39	18	27	21	50	11
TOTAL	204	TOTAL	167	TOTAL	300	TOTAL	297	TOTAL	317

Appendix II Contnd.

41st day		42nd day		43rd day		44th day		45th day	
Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails
53	32	15	16	62	41	41	30	61	31
63	40	52	18	17	15	2	11	31	13
30	15	59	28	58	29	40	29	16	11
12	12	7	9	33	22	26	28	36	20
55	27	28	16	3	17	42	33	33	17
16	13	20	11	57	31	15	18	15	12
27	17	33	17	2	14	57	38	47	18
7	10	30	8	42	18	19	23	59	28
50	21	6	15	28	22	6	22	29	14
22	6	4	10	52	28	30	27	19	15
52	24	12	17	63	47	31	25	51	27
56	31	13	5	19	20	12	24	10	11
18	16	45	19	6	14	37	31	38	15
60	32	29	13	29	17	43	35	35	12
1	8	64	35	7	15	51	28	7	9
32	14	11	9	47	18	21	26	13	17
14	15	47	31	56	25	29	30	6	8
4	7	49	28	54	16	59	40	55	26
26	10	34	16	46	19	23	20	27	14
61	29	27	15	51	20	25	21	45	19
TOTAL	379	TOTAL	336	TOTAL	448	TOTAL	539	TOTAL	337

Appendix II Contnd.

46th day		47th day		48th day		49th day		50th day	
Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails
27	21	9	8	38	16	26	14	35	20
6	10	22	13	59	30	59	19	44	14
17	15	16	11	51	22	5	8	60	29
56	20	64	36	50	17	18	16	10	9
19	14	31	26	23	11	14	15	20	23
22	17	4	6	55	18	22	14	12	8
16	12	35	20	40	6	64	30	45	10
12	13	50	27	18	14	13	13	53	21
1	5	25	15	49	21	12	10	14	10
9	11	15	11	44	15	52	15	30	15
50	19	19	16	15	13	55	17	31	12
2	10	1	4	62	33	4	13	29	14
3	16	13	18	17	12	9	11	40	18
54	23	38	17	2	9	42	14	38	12
55	25	59	31	14	15	3	10	23	16
38	21	7	13	1	6	23	12	18	11
64	30	26	18	20	13	25	13	34	13
43	9	6	14	45	20	48	15	50	23
40	23	24	16	16	10	30	11	48	15
32	16	2	8	37	16	37	10	6	8
TOTAL	330	TOTAL	328	TOTAL	317	TOTAL	280	TOTAL	301

Appendix II Contnd.

51st day		52nd day		53rd day		54th day		55th day	
Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails
24	18	61	29	23	15	12	15	12	23
18	16	14	21	31	14	18	14	26	19
31	14	30	15	56	12	19	11	38	37
17	11	21	16	53	20	61	30	40	14
27	17	27	14	24	13	51	23	55	41
10	9	59	17	52	18	7	16	11	15
30	10	52	23	16	10	24	19	8	18
51	27	11	14	38	15	55	31	53	24
23	14	3	13	59	30	43	22	44	21
21	16	34	17	57	21	6	10	20	20
12	13	64	32	32	10	47	19	63	43
38	16	15	11	39	14	8	14	17	25
57	24	33	18	45	9	29	20	13	15
58	20	42	13	62	28	56	34	2	17
60	31	44	19	27	12	53	28	7	15
39	7	57	16	22	15	35	18	23	20
61	28	25	12	4	13	44	16	36	25
54	16	9	15	58	15	10	9	64	54
43	10	26	10	5	10	26	19	24	26
47	11	29	11	2	8	37	15	37	28
TOTAL	328	TOTAL	336	TOTAL	302	TOTAL	383	TOTAL	500

Appendix II Contnd.

56th day		57th day		58th day		59th day		60th day	
Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails
34	26	3	19	1	13	62	50	29	21
56	52	4	15	51	53	36	35	56	41
50	48	58	49	42	31	24	20	38	32
33	36	55	31	43	28	7	17	7	23
6	18	25	30	40	35	29	21	19	21
9	27	8	24	3	18	45	28	1	18
18	30	59	61	37	39	25	32	37	26
7	16	51	28	19	31	61	44	27	40
48	40	29	32	50	44	48	33	9	23
47	35	12	28	11	17	26	28	63	61
42	28	19	30	52	56	16	23	25	27
25	24	1	14	10	24	2	22	31	29
35	27	50	37	29	26	50	41	53	29
45	42	42	35	38	40	51	39	13	14
38	29	47	37	12	23	17	14	42	36
55	49	6	21	30	27	42	26	40	30
23	17	49	34	24	17	56	37	39	24
19	16	45	29	41	23	31	20	60	64
10	19	40	24	58	43	10	15	3	17
8	20	41	26	31	12	14	21	33	25
TOTAL	599	TOTAL	604	TOTAL	600	TOTAL	566	TOTAL	601

Appendix II Contnd.

61st day		62nd day		63rd day		64th day		65th day	
Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails
14	21	24	28	36	35	39	43	7	46
2	17	52	58	49	66	40	36	9	48
35	41	59	61	24	30	9	40	57	70
22	27	36	26	39	34	59	60	47	43
30	21	19	29	40	43	48	42	4	41
36	34	16	27	51	65	36	38	22	36
24	28	6	20	8	30	52	61	39	55
19	32	35	29	7	36	38	47	42	48
31	37	8	25	38	45	13	48	2	32
62	48	44	32	54	59	63	76	31	50
6	36	40	38	37	38	53	48	41	58
44	33	42	35	26	39	31	42	40	53
4	21	17	32	16	32	19	44	16	49
18	35	23	32	56	68	10	42	61	81
22	26	13	28	44	42	49	61	26	48
32	24	21	24	2	29	26	48	37	38
13	20	24	32	23	35	29	41	50	61
55	41	14	30	6	36	43	44	15	40
60	55	37	29	48	38	25	36	29	52
49	40	33	29	61	69	34	38	5	38
TOTAL	637	TOTAL	644	TOTAL	869	TOTAL	935	TOTAL	987

Appendix II Contnd.

66th day		67th day		68th day		69th day		70th day	
Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails	Block No.	No. Snails
33	43	37	89	23	74	15	86	43	88
36	48	13	78	29	68	44	93	44	97
51	91	36	84	16	70	52	91	25	64
24	40	31	57	3	51	16	79	40	73
9	39	1	40	62	131	61	142	21	78
44	65	55	89	43	86	47	89	51	95
49	78	12	70	12	62	8	62	14	87
37	81	52	78	22	75	27	87	64	161
29	79	28	61	28	79	22	77	15	59
19	43	21	63	15	71	11	68	53	86
11	54	58	95	6	65	3	71	24	71
45	82	10	61	39	63	43	106	49	91
62	95	11	58	47	78	28	86	30	81
24	58	23	66	31	82	51	93	4	66
36	78	38	74	50	89	38	97	8	72
41	59	54	96	7	57	7	67	10	75
40	68	48	86	57	96	30	88	59	102
25	76	19	59	11	63	48	72	27	95
14	63	7	67	18	68	1	49	62	132
6	66	8	56	30	74	64	54	12	68
TOTAL	1306	TOTAL	1427	TOTAL	1502	TOTAL	1657	TOTAL	1741

Appendix II Contnd.

71st day		72nd day	
Block No.	No. Snails	Block No.	No. Snails
57	106	37	128
8	83	54	82
9	74	25	81
11	68	57	98
60	102	63	135
3	71	28	99
22	97	23	70
2	66	18	69
16	70	52	87
43	91	10	73
4	98	38	103
1	53	56	138
62	165	42	76
7	66	60	171
40	95	4	71
42	101	59	147
27	93	2	69
64	183	48	121
63	156	47	86
52	99	11	92
TOTAL	1937	TOTAL	1996