DEVELOPMENT OF SCHISTOSOME SPINDALIS
(MONTGOMERY 1906) IN THE MOLLUSCAN HOST AS
WELL AS WHITE MICE WITH PARTICULAR REFERENCE
TO THE HISTOPATHOLOGY OF THE VARIOUS
ORGANS.

A DISSERTATION
submitted in partial fulfilment of the requirements
for the degree of
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INTRODUCTION

Although parasitic worms with a few exceptions do not produce spectacular and explosive symptoms like viral and bacterial diseases, the loss, however, is incidious and constant, and, is at least as great.

For many years, schistosomiasis was considered as a disease of man and animals in several parts of the world, and it is only during recent years, that it has been recognised as one of the most important parasitic diseases.

There has been a steady interest, a constant accumulation of knowledge on many aspects of schistosomiasis now.

While human schistosomiasis is hardly a problem in India, schistosomiasis in domestic animals is of considerable significance.

Quite a large number of schistosomes are reported from cattle animals in India, namely S.indicum found in equines, camel, buffalo, sheep, and goat; and rarely buffalo; S.spindale in cattle, buffalo, sheep and goat; S.incognito in pig, dog and sheep; Orientobilharzia turkestanicum in cattle, buffalo, sheep, goat, equines and camels; O.dattai in cattle, buffalo, sheep and goat; S.nasalis confined to cattle. Except the latter, which causes nasal schistosomiasis (snoring disease), all other schistosomes mentioned above causes viscero-intestinal schistosomiasis. S.nasalis S.nasale inhabit the veins of the nasal mucosa while the others are found in the portal and mesenteric veins of the respective hosts.

Inspite of large number of schistosomes occurring in domestic animals in this country, work, on the various aspects of biology, development, pathogenicity of these, save that of S.nasale and S.indicum in the natural as well as experimental hosts has not been fully worked out.
It is therefore proposed to study the development of S. spindalis in the invertebrate as well as vertebrate hosts. As far as the writer is aware not many studies were made on the development of S. spindalis in mice except that of Buckley (1938) who recovered S. spindalis from mice exposed to cercariae responsible for paddy itch in Malaya.

**REVIEW OF LITERATURE**

Liston and Soparker (21) elucidated the life cycle of S. spindalis incriminating Indoplanorbis exustus and rarely Limnoea acuminate as the intermediate hosts. Bhalerao (1943) added L. luteola to the list of the intermediate hosts.

Abdel-Malek (4) described the migration of sporocyst of *Schistosoma mansoni* through the snails tissue (Biomphalaria boissyi). Most of the miracidia has settled close to the site of entry in the head, foot, organ, the mantle collar, or the pseudo-branch and the mother sporocysts were formed. Their daughter sporocysts were motile and moved at random through the tissue or were carried by the blood to various organs. The digestive gland and ovotestis provided the most favourable environment for subsequent development of daughter sporocysts and this accounts for the fact that the most were found there.

Sinha and Srivastava (29) have also described the migration of sporocysts of *S. incognitum* through the snail tissue (Lymanea luteola var. australis) and the damage produced in the snail. They noted that the earlier stages of the daughter sporocysts did not produce any apparent damage in the snail.
The digestive gland and the gonads appear to be riddled with innumerable sporocysts and cercariae, liberated in the tissues. The liver become friable and appears to be of slate or greenish brown color, instead of healthy yellow color. The shell becomes thin and brittle. The presence of so many sporocysts produced pressure atrophy. A large percentage of the liver tissue became replaced by these sporocysts. The sporocysts probably absorbed nutrition which shortened the life of the snail. Infected snails were found to perish easily under unfavourable conditions in the laboratory.

In Vertebrates:

*Schistosoma bovis* was the first species of schistosome discovered in the portal venous system of cattle in Egypt, by Sonsine in 1876.

The earliest record of the occurrence of *Schistosomoses* in India was made by Cobbold in 1882, and later Montgomery (22) found *S. spindale* in the mesentric vessels of two plain cattle (*Bos indicus*) at Mukteswar, India, and their spindle shaped ova.

Vryburg (1907) reported *S. spindale* as occurring in Sumatra.

Liston and Soparker (21) worked out the life cycle of *S. spindalis* by artificially infecting kid with the furcocercous cercaria liberated by the snail, *Indoplanorbis exustus*, and by infecting snails with the miracidia hatched out of the spindle shaped eggs obtained from goat. The kid developed severe anaemia, diarrhoeæ, paraplegia with oedematous swelling under the jaw and round the neck and died a fortnight later. Postmortem showed numerous worms in all stages of development in the liver and the larger portal veins were packed with worms, the females contained the characteristic spindle shaped ova.
Soparker (27) published a detailed description of the cercaria of *S. spindalis*.

Fairley and Mackie (13) studied the histopathology of *S. spindalis* in goats and proved that it affected exclusively the portal system and its ova were voided only with the faeces.

Fairley (10) and Fairley et al (14) tried to infect monkeys and guinea pigs artificially with the cercaria of *S. spindalis* obtained from *Indoplanorbis exustus* and as a result of this they discovered that monkeys possessed some natural immunity by virtue of which the development of cercaria did not proceed beyond the Schistosome stage in them. In the case of guinea pigs they found that only male worms attained full development, while no female worms were demonstrable at autopsy. They suggested that in these animals some host factor antagonistic to the development of female Schistosome underlined the phenomena of exclusive male survival rather than initial unisexual infestation. Two water buffaloes were also successfully infected with *S. spindalis* by Fairley and Jasudasan (10) and these buffaloes developed bilharzial pseudo-tubercles in the liver within three months.

Fairley and Mackie (13) and Rao (2) confirmed the observation of Liston and Soparker and reported that only male worms developed in the guinea pig. However Dutt (7) found that the female of *S. spindalis* also develop in the guinea pig.

Fairley and Jasudasan (11) published two papers dealing with the seasonal infection of the snails, *Indoplanorbis exustus*, with the cercaria of *S. spindalis* while the records of the experiments depicted in the other, point to the conclusion that among ruminants alimentary infection with Schistosome is more
important, since the contents of the mu rumen give an alkaline or neutral reaction. The schistosome cercaria cannot survive in the normal acidity of the gastric contents in animals other than ruminants.

Fairley and Mackie (13) dealt with the pathological changes with the infection of *S. spindalis* brought about in the host tissues they state that the lesions occurring prior to the deposition of ova included verminous phlebitis, involving the branches of mesentric and portal veins, and toxic changes in the liver and kidneys, pseudotubercles and periportal cellular infiltrations, in the liver.

The infection of the calves was carried out by Fairley and Mackie (1932) by three methods:— (1) Drenching with cercaria (2) Infection through the nasal mucous membrane and (3) infection through the nasal mucous membrane and by drenching. The same methods was adopted by Rao (2) in the calves.

No lesions were seen in nasal cavities in both of the calves. The calf which was infected through the nasal mucous membrane passed blood-stained faeces on the 40th day and from 46th day onwards its faeces showed a fairly large number of typical ova of *S. spindalis*.

The calf which was drenched showed ova of *S. spindalis* in the faeces in small number from 70th day onwards. The portal and mesentric veins were having worms, both male and female. Sections of liver, large and small intestines revealed a very large number of pseudotubercles and verminous phlebitis. It was also seen that many of the ova in the liver and the intestines had "actino bodies" on them. Lungs did not reveal any schistosomes ef or
their own in the blood vessels.

Fairley and Mackie (13) found that the sexes of all the worms collected by them from eleven experimental goats, showed that the male worms were more than twice as numerous as the females.

**The habitat of S. spindalis.**

Montgomery (22), Liston and Soparker (27), Fairley and Mackie (13) and Rao (2) have found that *S. spindalis* inhabits the portal and mesentric veins.

**Morphological characters.**

**Size:** It has been admitted that the average size of schistosomes may vary with the age of the parasite, mode of preservation, time of collection after the death of the host etc., but still the question remains whether or not there is difference in size between the different species.

**Nature of the cuticle.**

Bhalerao (1932) found variation in the development of tubercles on the cuticle of the schistosomes. Fairley and Mackie (13) in their studies on *S. spindalis* did not say any thing about the nature of the cuticle, but from their excellent photographs of sections of these parasites in various organs it seems that the cuticle of the male is non-tuberculate. Rao (1) has confirmed that it is non-tuberculate.

**Number of testicular glands.**

The number of testicular glands present in *S. spindalis* is 6 to 7 according to Montgomery (22). Bhalerao (1932) however, noted in his description of this parasite, that the number of testes varied from 3 to 6 and remarked that Montgomery might have failed to notice the range of variation to be 3 to 7 which was in conformity with the above findings.
According to Bhalerao (1932) the common caecum of S.spindale measures 4.19 to 6.93 mm. Montgomery (22) said that the common caecum of S.spindale was 6 mm.

Kalapesi and Purohit (17) have made observations on histopathology of morbid tissues from a case of natural infection with Schistosoma spindalis.

Kulkarni et al (16) recorded in Bombay State unusual outbreaks of Schistosomiasis in bovines due to Schistosoma spindale associated with heavy mortality.

Cattle found most susceptible where as buffaloes were found practically refractory to the infection. Sheep and goats were also reported to have succumbed to their infection.

Susceptibility of the animal to the disease was increased on account of prevalence of an outbreak of Foot and mouth disease prior to the onset of these outbreaks. Innumerable parasites were detected in the heart, aorta, vena cava as well as in the lungs, liver, spleen, in addition to their normal sites, portal and mesentric veins.

Eggs of the S.spindalis were detected in the tissues of the liver, brain, kidney, spleen and lungs.

Eggs were also seen in the urine and bile. Few eggs were also detected in the discharge mixed with blood from the nostrils.

They stated that "Generalised schistosomiasis due to S.spindalis in the form of outbreaks as a disease could not be recognised in India or abroad so far, but the number of deaths recorded in bovines of the two villages brought about by S.spindale infestation in-bovines-of-the-two-villages-brought-about-by seems to prove that generalised schistosomiasis is definitely a disease entity! They believed that the toxins discharged by the parasites were responsible for the lesions and consequently death of animals."
Meleny et al. (25) have infected mice, hamsters, rats, guinea pigs and rabbits with *S. mansoni*, *S. japonicum* and *S. haematobium* (bisexually), and recorded the histopathological changes in the liver, intestine, spleen and bladder. The most typical lesions were in the liver where they developed into characteristic pseudo-tubercles and eventually resulted in scarring. Living worms did not cause a tissue reaction but dead worms gave rise to intense perivascular reaction. The worms disappeared and scar formation resulted in the liver, and there was periporal infiltration by leucocytes especially in the early stages of infection. It was an allergic reaction which did not contribute significantly to fibrosis. Areas of coagulative necrosis in the liver parenchyma might be due to infarcts. In the mucosa and submucosa of the intestine and bladder, fertile eggs caused little tissue reaction while in the muscularis and serosa, lesions similar to those in the liver were seen. In the spleen, areas of focal necrosis were seen in early *S. mansoni* infections and increase of fibrous tissue in late infections in some mice. The early periporal cellular infiltration of the liver was an allergic phenomenon due to the presence of worms in the portal mesenteric veins but did not contribute ultimately to liver cirrhosis.

Kagan and Meranze (15) have studied the histopathology of the liver in mice experimentally infected with *Schistosomatium douthitti*. Female and bisexual infections caused essentially similar lesions which were associated with eggs and the formation of granulomata. Male infections produced inflammatory reactions, mainly with mononuclear cells, around large veins and in some animals there were intense accumulation of pigment.

Mice which received a challenging infection 60 days after the
initial exposure showed no enhanced cellular response to it. Mice which were cured of the infection by 12 intraperitoneal injections of 4 mg of tri (n-dodecyl-mercaptan) s-antimonious acid in peanut oil showed a return to normal histology of the liver in about 270 days.

Kagan and Meranze (16) also dealt with the histopathology of the spleen, intestine, lungs, bone marrow and lymph nodes in the mice infected with Schistosomatum douthitti. In early infections the intestinal muscularis mucosa confined the eggs to the submucosa. No ulceration of the villi was detected. In males infections of the liver and lungs showed accumulation of mononuclear cells around the veins and the spleen was markedly congested. There were typical pseudotubercles around the eggs and adults in the lungs. No marked changes with could be detected in the immune host when challenged with cercariae. More male than female worms were recovered.

Methods of infecting the mice.

Watson and Abdel Azim (30) showed comparative efficiency of various methods of infecting mice with S. mansoni. The methods are as follows:

<table>
<thead>
<tr>
<th>Method</th>
<th>Percentage of success</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Partial immersion method</td>
<td>(44.75%)</td>
</tr>
<tr>
<td>(2) Vaseline ring method</td>
<td>(8.75%)</td>
</tr>
<tr>
<td>(3) Tube method</td>
<td>(41.5%)</td>
</tr>
<tr>
<td>(4) Cover slip method</td>
<td>(38.7%)</td>
</tr>
<tr>
<td>(5) Oral method</td>
<td>(35.7%)</td>
</tr>
<tr>
<td>(6) Subcutaneous injection</td>
<td>(17.6%)</td>
</tr>
<tr>
<td>(7) Intra peritoneal method</td>
<td>(11.0%)</td>
</tr>
</tbody>
</table>

and (8) Gastric injection method (Nil)

Mice were employed as the species most commonly used in experimental bilharziasis. Partial immersion of the animals in
a known volume and depth of water containing a known number of cercariae was the most satisfactory method.

De Mellon and Paterson (8) experimented colonies of white mice injected with S. mansonii, the normal increment in weight and the weaning rate decreased, while the mortality rate increased, but the size of the litter and their weight at birth and weaning were unaffected.

**MATERIAL AND METHODS**

Furcocercous cercaria obtained from the mollusc (Planorbis) collected from a stagnant pond near Borivili a suburb of Bombay, were used for in the investigation.

Each of the snails that were brought to the laboratory, after they were cleaned of the adherent dirt and debris were placed in test tubes containing clean tap water, and were kept over night. Some of the infected snails discharged mature furcocercous cercaria with in a few minutes. The test tubes containing the snails were examined by naked eye or with a hand lens for the presence of cercaria next morning.

The mice used in these experiments were laboratory bred and free from any other infection.

**Method of infection:**

The mouse was kept in a plastic tube measuring 10 cms in height and 3 cms in diameter, with a round hole at the bottom through which the tail was made to project into a small test tube containing the cercarial suspension. The mouth of the plastic tube was closed by wire net, to prevent the mouse from wriggling out, and was fixed to a stand as shown. (Plate No.1 Fig.1)

The mice was kept in this position, with the tail in the cercarial suspension for half an hour to ensure the penetration of the tail by the cercaria.
Several estimations of the number of cercariae in a measured volume of water were made by counting them under the microscope and the average total cercarial number was calculated. About 500 cercaria were given in this manner to each mice.

Of the various techniques for infecting mice with Schistosome cercaria that have been devised by various authors, each technique has certain advantages and disadvantages depending on the experimental design. The method used here is the one that was in general use in the School of Hygiene and Public Health, Baltimore, U.S.A.

A total of 4 white mice that were infected in this manner were necropsied and studied. Observations were made on two mice exposed without counting the cercaria and the other two mice with about 500 cercaria of *S. spindale*.

One mice was exposed to the cercaria discharged from one single snail and after necropsy several worms belonging to both sexes were recovered.

The mice were killed by chloroform inhalation, fastened to an autopsy board and were opened by ventral incision. Before killing, the general appearance of the peritoneal cavity and the abdominal organs such as the liver, spleen, kidney, pancreas, intestines, mesentric veins were examined for the Schistosomes. Respiratory system lungs, trachea, bronchii etc., were dissected out in a petidish containing normal saline and examined for parasites, urinary system-kidney, ureters and bladder were dissected out and kept in the normal saline, to allow the adult schistosomes to come out.

A small piece of liver tissue was pressed between two slides and examined under microscope for the worms and eggs of *S. spindale*.

The worms collected in normal saline were cleaned and fixed in appropriate fluids and preserved for subsequent study.
Small pieces of liver, lung, spleen, intestines, kidney, pancreas were cut and fixed in 10% formaline and preserved for histopathological study. Section of 6 microns were cut, stained with Harri's Haematoxylene and Eosin, and mounted in canada balsem, for further study.

Live infected snails were desected by removing the shell and fixed in 10% formalin or hot Bouin's fluid for studying the developmental stages of furcocercous cercaria.

To study morphological characters of cercaria heat method was used. A large drop containing several cercaria was placed on a glass slide and gently heated by passing it over the bunsen flame two to three times taking care not to allow the drop to boil and examined at intervals of a few seconds under a low power microscope until most of the cercaria were found to be motionless. A coverslip was placed gently on the drop of water containing cercaria without least pressure on the cercaria.

Acetic Alum Carmine (Lee's Vade Mecum) or by Borax carmine were used for staining the cercaria and both found to be satisfactory. The cercaria were also stained in the living condition with vital stains, such as methylene blue, janus green etc., particularly for staining the flame cell pattern.

Beside the mice, one rabbit and two buffaleo calves were also infected.
OBSERVATIONS AND RESULTS

SNAIL HOST

Out of 306 snails (*Planorbis exustus*) collected 24 were found to discharge furcocercous cercaria and 41 snails with other type of cercaria, (*Viz.*, Xiphido, Echino and Pigmented cercaria).

From 108 snails of Lymnea sp., 7 were found to emit pigmented cercaria, almost all the snails of lymnea died with in two days.

The following table shows the detailed collection and different types of cercaria discharged by them.

<table>
<thead>
<tr>
<th>S. No. of No. of snails collected</th>
<th>Date of collection</th>
<th>No. of snails found infected with different type of cercaria</th>
<th>Furcocercous</th>
<th>Xiphido</th>
<th>Echino</th>
<th>Pigmented</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40 16.7.63</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>-</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>130 6.10.63</td>
<td>5</td>
<td>6</td>
<td>9</td>
<td>6</td>
<td>3.8%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>136 22.10.63</td>
<td>15</td>
<td>4</td>
<td>14</td>
<td>2</td>
<td>11%</td>
<td></td>
</tr>
</tbody>
</table>

In several instances where an infected snail containing mature cercaria was placed in a test tube and fresh tap water was added the snail was observed to discharged cercaria into water with in few minutes. The life of the infected mollusc was found to be much shorter than that of nonaffected one. Signs of approaching death, in infected snails is often is seen by the red discolouration of the water with the blood of the mollusc.

Movements of the cercaria.

The cercaria can be seen with naked eye or with a hand lens. They will be seen to hang in water with the head down and the tail turned upwards. In this position the cercaria do not remain stationary, but gradually sink when suddenly they begin to swim
actively and in its action rise to certain height in the water again. This movement is in a more or less straight line either perpendicularly upwards or at an angle, but is never irregular.

If the cercaria adhere to the surface of the test tube it will move by fixing itself to the surface by means of its ventral sucker, the furcae of the tail come together and both the tail and the body then undergo rapid vibration, after a second or two the vibrations cease, the furcae open out, the portion of the body in front of the body ventral sucker is extended and the oral sucker takes hold as far as possible. The hold of the ventral sucker is thus pulled farwards and takes a fresh hold, the hold of the oral sucker is then let off and the body and the tail rapidly vibrates, thus the cercaria makes rapid progress. During this slow progress the cercaria bends its body and appears to be in the act of licking or biting at the ventral sucker which is projecting sideways every time it makes a forward move. After some time the cercaria sheds its tail which keeps on vibrating for some time, and the body moves in a slow creeping sort of movement and eventually it dies. If the cercaria meets any obstruction in its movement it will try to penetrate (vigorously) in all directions holding the object firmly by means of its ventral sucker. These movements appear to have a correlation with penetration into the skin of the host.

In a majority of instances the molluscs were infected with a single species, but in a few snails showed echinostome cercaria as well as furcocercous cercaria.

For the study of the flame cells and their ducts artificial light was found most suitable. Most of the furcocercous cercaria were found to be active inside the water for 6 to 8 hours after their emergence from the (snail) host.
The infected organ of the snail showed sometimes a yellowish brown and at others a dark blategre colour. Most of the cercaria were found to develop in the digestive gland (i.e. Liver).

The cercaria were found to develop in a small cylindrical sporocysts. The sporocyst looks under low power as brown or light spots containing 6 to 8 cercaria. The wall is very thin, elastic and allows considerable stretching by the movements of the contained cercaria, the sporocysts are firmly attached to the tissue or the digestive gland, they are not motile, and it is difficult to tease out separately. The mature cercaria were found moving in the sporocyst. No radia formation was noticed.

When the shell is opened most of the mature cercaria were found to adhere to the sides of the shell. So the cercaria when mature escape from the sporocyst by their active movements, they make their way through the tissues of the molluscan host and come to lie between its shell and the mantle cavity. From here they escape into the water from time to time and swim about actively in it.

Histopathology of the snail

An enormous number of sporocysts containing cercaria were found in the interglandular tissue of the snails liver. Almost all the tissue of the liver were replaced by those sporocysts. Some of the sporocysts were found at the ovotestis with the cercaria inside.

Some of the mature cercaria were found at the foot and at the mantle cavity, probably the discharged cercaria might have penetrated back.

Other organs of the snail were normal without any developmental stages of the cercaria.
The behaviour of the cercaria of S. spindalis in vertebrate hosts.

The frequent lashing of the tail of mice in cercarial suspension that were observed were probably due to the active penetration of cercaria. All the cercaria were trying to attack the tail without going sideways. When the mice were let out after infection they were biting their tails, probably due to the irritation caused by the cercaria.

The following table gives the detailed particulars of the experiments.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Species of cercaria</th>
<th>No. of cercaria</th>
<th>Date of infection</th>
<th>Method of infection</th>
<th>Date of killing</th>
<th>No. of days to develop</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mouse</td>
<td>without count</td>
<td>17.7.63</td>
<td>Tail penetration</td>
<td>23.9.63</td>
<td>69</td>
<td>positive</td>
</tr>
<tr>
<td>2.</td>
<td>-do-</td>
<td>500</td>
<td>18.7.63</td>
<td>-do-</td>
<td>28.10.63</td>
<td>103</td>
<td>-do-</td>
</tr>
<tr>
<td>4.</td>
<td>-do-</td>
<td>without count</td>
<td>23.10.63</td>
<td>-do-</td>
<td>16.12.63</td>
<td>54</td>
<td>-do- spleen was enlarged.</td>
</tr>
<tr>
<td>5.</td>
<td>Rabbit</td>
<td>1000</td>
<td>24.10.63</td>
<td>ear penetration</td>
<td>13.1.64</td>
<td>82</td>
<td>positive</td>
</tr>
<tr>
<td>6.</td>
<td>B. calf</td>
<td>5000</td>
<td>25.10.63</td>
<td>cutaneous</td>
<td>30.1.64</td>
<td>98</td>
<td>negative</td>
</tr>
<tr>
<td>7.</td>
<td>B. calf</td>
<td>5000</td>
<td>25.10.63</td>
<td>-do-</td>
<td>30.1.64</td>
<td>98</td>
<td>negative</td>
</tr>
</tbody>
</table>

All the mice and the rabbit were found quite healthy till they were killed. It is found that the mice do not succumb easily to heavy exposure of cercaria.

Schistosomes were demonstrated at autopsy in every instance, and the number recovered varies from 50 to 200 in individual animals. Only mice were heavily infected than others.

Number of worms in all stages of development were found in
the liver, spleen and portal veins. The mesentric veins contained several paired forms. The portal veins were found to be choked with masses of worms. Most of the females worms contained characteristic spindle shaped ova in the uterus, and numerous eggs were found in the liver, and the walls of the intestines. the worms were examined and were identified as Schistosoma spindalis. (Montgomery 1906)

In the mice, infected with S. spindalis, ova, are not commonly demonstrable within the uterus of the female worms and also in the visera till the end of 60 days onwards, and this is the period during which the female worm normally attains maturity. In every case, pieces of the entero-colon were compressed between two glass slides and examined microscopically for ova. 4 to 5% caustic soda digests liver, the small intestine were also digested and the sediment were examined for the characteristic spindle shaped ova.

When a bit of infected liver was chopped and placed in a cavity block containing water, miracidia were found emerging from the eggs with in 10 to 15 mts time. The male schistosomes were small white bodies about a quarter of an inch in length. Usually lying coiled in the mesentric veins. The females appeared dark hair like structure upto twice the length of the male.

The movements of the living worms were found to be very interesting when they were placed in a petridish containing normal saline, especially the male worms were found extending their bodies double their size. When they were in motion, the anterior and posterior portions of the body showed lashing movements, particularly the ventral sucker which projected from the body prominently on the ventral surface (just like a Loud speaker)
as if trying to gulp something. Some of the females which were
lodged in the Gynacophoric canal of the male were also found
moving like snakes.

The worm survived for several hours at room temperature
(about 24 hours) and for 3 days at 4 degrees C when transferred
to normal saline solution.

In the rabbit only few immature worms were recovered from
the mesentric vein.

In the buffalo calves about the cutaneous method was adopted
and on postmortem after about 98 days, no worms could be recovered
even after sternous search.

Recovery of adult worms is difficult from rabbit and cattle
than from the mice.

Morbid changes

Liver and spleen were invariably enlarged. In one mouse
the spleen was very much enlarged to about 10 times than normal
size. (Plate No.1 Fig.3).

Numerous, minute, greyish-white, chalky-white nodules
resembling milliary tubercles could be seen immediately beneath
the transparent capsule of liver. If an individule nodule was
removed with the point of a knife and a caustic potash prepar-
ation of the crushed material be examined under the microscope
one or two spindke shaped eggs could be seen.

When the mesentery was exposed to the light without cutting,
worms were seen coiled in the veins.

No other macroscopic changes could be noticed.
Histopathological changes of the various organs of mice.

Liver:
Hepatic cells showed fatty changes and increased granularity. Some of the nuclei also showed degenerative changes, (pyknosis etc. and in advanced cases complete vacuolisation of liver cells was seen. Scattered throughout the parenchyma were areas of granulomatous inflammation, many of which contain degenerating schistosome ova cut in various planes. The cellular infiltration in these areas is principally composed of neutrophils, lymphocytes and plasma cells and a few multinuclear giant cells are also seen.

Hepatic cells surrounding these regions showed increased acidophilic staining indicating the onset of necrosis. In some places there is complete replacement by fibrous connective tissue. Kupffer cells contain brownish black pigment (Bilharzial pigment). The degree of pigmentation varied from moderate to heavy in different animals.

The portal sheath shows heavy mononuclear infiltration. The portal veins contained a number of parasites both immature and adults. In one animal, a number of immature schistosomes were found in the portal vein.

The division of parenchyma into artificial lobes characteristic of cirrhotic changes are seen at some places.

Lung:

Acute congestion and heavy perivascular and peribronchial infiltration by mononuclear cells was evident. Some of these arterioles were thickened and contained spindle shaped eggs of S. spindalis. Some degree of oedema was seen.

A number of branches of pulmonary veins were found to contain immature schistosomes. Bilharzial pigment seen. Fibrosis of some of the areas were also noted.
Lymph node: Showed lymphoid hyperplasia, but no parasite ova were seen.

Pancreas: Parenchyma was unaffected, but there was perivascular round cell infiltration. Blood vessels contained parasites cut in different planes. (Plate No. VI Fig. 1)

Intestines: Mild enteritis as suggested by areas of infiltration with inflammatory cells. (Plate No. VII Fig. 4)

Thyroid: No pathological changes.

Spleen: Showed lymphoid hyperplasia. (Plate No. VI Fig. 1)

Kidney: Showed congestion.

Heart: Showed cloudy swelling. (Plate No. VII Fig. 5)

Spleen: Worms were found in the portal of the spleen vein, none could be seen in the histopathological sections. There was however an indication of a tissue reaction in the form of lymphoid hyperplasia.

Intestine: As pointed earlier quite a large number of characteristic eggs were recovered in the small intestines, but on histopathological examination, none could be seen. There were however areas of cellular infiltration and sign of enteritis, indicative of host reaction to eggs. (Plate No. VII Fig. 4)

DISCUSSION

The incidence of Schistosome spindale infection is closely linked up with the distribution of the snail vector, Indoplanorbis exustus which is found to occur throughout the year in the stagnating water ingested with vegetation like water Hyacinth, Ipomoea etc., in Bombay.
Soparker (21) found that the incidence of infection in the snails is highest during the autumn month and lowest during late winter and early spring. (Rate of infection in June 0.58 and October 2.0 in the year 1917 and 3.9 in June, 1.3 in October 1918), but Fairley and Jasudasan (11) showed the incidence of snail infection (Planorbis exustus) with S. spindalis cercaria to be in cool months (i.e. from November to March).

The cercaria were found to be active inside the water for 6 to 8 hours after emergence from the (snail) host.

Cercaria and sporocysts developed in the interacous connective tissues displacing glandular tissue in certain areas of the snail liver. These observations are in agreement with the findings of Soparker (27) and Fairley and Jasudasan (11).

These histopathological changes in the snail tissue by the development stages of the cercaria showed only in the liver and ovotestis. Almost all the liver tissue was replaced by the sporocyst causing enormous damage to the tissue. It indicates how the life of the infected mollusc was found to be much shorter than the noninfected one.

Some of the mature cercaria were found at the feet and mental cavity, probably the discharged cercaria might have penetrated back into the tissue. (Plate No. III Fig. 3)

Staining the cercaria on a slide requires more time than when they are stained in mass in test tube, besides considerable wastage of cercaria takes place during the change over from one fluid to the other. The procedure though laborious could be used whenever the material to be examined is limited.

Borax carmine and Acetic Alum Carmine have given good results for the staining of cercaria.
The method adopted by Fairley and Jasudasan (10), Fairley and Mackie (13) and Rao (2), for infecting the cercaria of *Schistosoma spindalis* in larger animals were not used here. As the animals used in this experiment were mostly mice where safety and skill were essential when exposing the smaller animals to cercariae. It is also essential to limit the cercaria to host skin, and also to count the number of cercariae on host skin, and also to count the number of cercaria which did not penetrate.

The method used in this present investigation is relatively simple, safe and easy to operate. The reaction of the animal, and the cercarial movements could be watched without difficult.

Watson’s methods for infecting smaller animals were found to be laborious, time consuming and the results were also indifferent.

Discussion in Vertebrates.

During recent years many different affections in cattle by *Schistosome spindalis* have been recorded in literature. Intestinal bilharziasis is a form of schistosome affecting the cattle in many parts of the world. The present observations are based on 4 mice exposed in the usual manner to bilharzial cercariae applied to the tail for a period of half an hour. The total number of pooled cercariae utilised was about 500 in two mice and in other two, without counting as detailed in the table. (Page 16)

Except mice different laboratory animals Viz., guinea pigs, goats, monkeys and buffalo calves were used by different workers, like Liston and Soparker (21), Fairley and Mackie (13), Rao (1) and Dutt (7). Mice were extensively used by various workers by infecting with *S. mansoni*, *S. haematobium* and *S. japonicum*. In all these experiments the method of infection being by cutaneous, drenching and nasal mucous membrane methods.
Buckely (1938) was the only worker to date to have recovered *S. spindalis* working on mice after exposing them to the dermatitis producing cercaria in paddy fields of Malaya.

**Habitat of *S. spindalis***

The results essentially confirmed the findings obtained in other experimental studies in schistosomiasis (Montgomery 22), Liston and So Parker (21), Fairley and Mackie (13) and Rao (1), that the habitat of *S. spindalis* was the portal and mesentric veins. Fairley and Mackie found in goats, infected heavily with cercariae of *S. spindalis*, an over flow distribution of schistosome in atypical situation in the early phases of the disease, and later a biological equilibrium became established between the worms and the definitive hosts, when the worms limited to themselves to their ordinary habitat, viz., the portal and mesentric veins. They also say that the distribution of *S. spindalis*, within the blood vascular system, corresponds to *S. mansoni* and *S. japonicum* than to *S. haematobium*. This supports the view that the *S. spindalis* resembles other schistosome, in its behaviour in selecting its habitat.

**Morpholog of *S. spindalis***

It has been admitted that the average size of schistosome may vary with the age of the parasite, mode of preservation, time of collection after the death of the host etc., but it was found that there was difference in size between the different species. The different species of schistosomes developing in the portal system of man and animals differ in their average size, even though they all have equal scope for development there. Regarding the cuticle of *S. spindalis*, the present observations agree with Rao’s (1) and Kalapasi and Purohit (17) findings, in that it is smooth. Therefore it is evident that the tuberculate
and nontuberculate nature of the cuticle is of value when considered along with other morphological features. On examination of stained male worms, it was found that the range of variation of testis was 3 to 7 which was in conformity with the range of variation observed by other authors, (Montgomery 1909, Dhulerao 1933 and Rao).

The distribution of Schistosomes in the blood vascular system.

At autopsy on the infected mice it was seen that the common habitat of the worm was in the mesentric and the portal veins, and thereafter occurred in pancreatic, splenic and pulmonary veins. Another interesting feature of these autopsies was the relatively greater frequency of the male compared with the female worms. Montgomery (22), as well as Liston and Soparker (21) have also remarked on their unequal distribution of the sexes and the whole phenomenon requires more detailed study.

The time of appearance of eggs in the viscera.

It was observed that ova begin to appear in the uterus of the female worms after 60 days onwards, and this is the period during which the female worm normally attains maturity, therefore the prepatent period is about 2 months, and the deposition of ova in the tissues takes place thereafter development. It was recorded by Fairley and Mackie (13) in goats, that the ova in the tissues were observed at the end of 6th week, and that by the 7th week a wide distribution in the various viscera including the liver, pancreas, stomach, small and large intestine and lungs. The findings of Rao (1), in one buffalo calf, which was infected through the nasal mucous membrane showed plenty of ova in its dysenteric motions on the 46th day, and the other buffalo calf which was infected by drenching showed a few ova on the 76th day.
**Prenatal infection.**

It was observed that one mouse which was pregnant gave birth to three healthy male mice 15 days after infection, but on autopsy of the mice it was found that the offspring of the pregnant mice remained free from bilharzial infection proving there by that prenatal infection was not possible.

**Rabbit.**

In the rabbit few immature worms were recovered from the mesentric vein, probably the site of infection may also play a part in their quick and delayed development as the animal was infected through the ear.

**Buffalo calves.**

The infection was not taken by the buffalleo calves. The failure may be due to less number of cermaria used and the method of infection, namely the cutaneous route, which was quite different from the method used by Rao (1), where he gave 60,000 by cutaneous, nasal and drenching methods.

**Pathological changes in the viscera.**

The pathological picture in *S. spindale* depend upon the duration of the disease, the intensity of the infection and the particular tissue involved. In 4 mice which had been artificially infected for a period varying from 54 to 103 days living worms and ova were invariably demonstrated. Liver changes were frequent with small whitish nodules scattered through out the substance (Bilhargial pseudotubercles). Ova deposition in both the large and small intestine were noticed. These observations confirm with those of Fairley and Makie (13) in goats. In one mouse the spleen was very much enlarges, about 10 times more than normal size and many worms were found in the portal veins.
Microscopical changes in the tissues.

The histopathological of the liver, spleen, lungs, pancreas, intestines, kidney and heart were studied.

From the histopathological findings it appears that cellular infiltration is a more common feature in the animals. The order of organ susceptibility thus therefore appears to be liver, spleen, mesentery, pancreas, lungs. This confirms the observations made by Fairley and Mackie (13) and also Rao (1).

Liver:

In histological examination, the hepatic cells showed fatty changes and increased granularity. Some of the nuclei also showed degenerative changes (Pyknosis etc). In advanced cases complete vacuolisation of liver cells was seen. Scattered throughout the parenchyma were the areas of granulomatous infiltration, many of which contained degenerating schistosome ova, cat in various planes. Some ova, however, were typical with a terminal spine and embryonal cellular contents (Plate No. IV, Fig. 3). The layer of "actino body" as described by Rao (1) was not present around any of the ova. The cellular infiltration in those areas (around the ova) was principally composed of neutrophils, lymphocytes and plasma cells and few multinuclear giant cells. Eosinophil element was conspicuously absent in the cellular infiltration around ova.

Hepatic cells surrounding these regions showed increased acidophilic staining indicating the onset of necrosis. In some places there was complete replacement of fibrous connective tissue.

Many of the kupffer's cells in the dilated sinusoids were loaded with golden brown and black pigment (Bilharzial pigment).
The degree of pigmentation varied from moderate to heavy, in different animals.

It was stated by Meleny and Faust (1924) that the pigment "is obviously orginated from digested haemoglobin which is excreted into the portal blood and later become localised in reticuloendothelial cells, especially in the liver".

The portal sheath showed heavy mononuclear infiltration. The portal veins contained a number of parasites both immature and adults. The division of parenchyma into artificial lobules characteristic of cirrhotic changes were seen at some places.

All the above observations in liver, were in agreement with the findings made by Kalapesi and Purohit (17) from a natural infection with _S. spindale_ in bovine. Most of the changes observed, were also similar to the findings of Fairley and Mackie (13) in goats and Rao (2) in buffalo calves.

In one mice, a number of parasites were found in the portal vein, but no eggs or tubercles were found to have developed indicating an early stage of infection.

**Lungs:**

Acute congestion and heavy perivascular and peribronchial infiltration by mononuclear cells was evident. Some of the arterioles were thickened and contained the parasite ova. Some degree of oedema was seen. A number of branches of pulmonary vein contained parasitic larvae. Bilharzial pigment was seen. Fibrosis of some of the areas were seen. Fairley and Mackie (13) in their experimental infection of goats noticed that in the lungs, Schistosomes and the ova were occasionally located in embolic foci inside the vessels as well as in alveolar walls which infiltrated with leucocytes. But Rao's (1) findings showed, no worms of eggs except small quantity of bilharzial pigment.
As per Kalapesi and Purohit (17) there were schistosomine parasites in the blood vessels, surrounded by foreign body giant cells, large monocytes, fibroblasts and an outer capsule of delicate fibrils and cellular fibrous tissue.

**Pancreas:** Microscopically it showed only perivascular round cell infiltration and blood vessels containing parasites.

**Intestines:** Mild enteritis suggestive of infiltration of inflammatory cells were present. No pathological changes as noted by Kalapesi and Purohit (17) were observed.

**Spleen:** Showed lymphoid hyperplasia.

**Kidney:** Showed congestion.

**Heart:** Showed cloudy swelling.

**Thyroid:** Showed no pathological changes.

**Lymph node:** Showed lymphoid hyperplasia. No parasites were seen.

**The Relationship of S. Spindale to Human Schistosomiasis.**

Viewed from the standpoint of comparative histopathology of mice, *S. spindale* is more closely allied to *S. mansoni*, *S. japonicum* and *S. haematobium*. All three are intestinal types of schistosomiasis and the habitat of the adult worm is dominantly the portal and mesentric veins with maximal ova deposition in the entero-colon and liver.

The histopathological changes recorded by Heleny et al (24) & 25 in mice, hamsters, rats, guinea pigs and rabbits were as identical with the present observations, but they have not mentioned the changes in lungs and pancreas.

The observations add to the evidence that the pathological changes in the liver are sometimes associated with the presence of either male or female schistosome worms or both, and the deposition of eggs.
The inconsistency of the pathological changes in mice, their wide variation in intensity, and their frequent lack of direct contact with worms, make it difficult to be certain of their origin. However the general trend of increase in severity, in proportion to duration of infection up to 2 to 3 months, strongly favours the view that they are caused by the toxic products of the worms.

Note:—The name Schistosome spindale was used in certain places for Schistosome spindalis, since it is a synonym used by certain authors.
SUMMARY AND CONCLUSIONS

The extent of literature on the subject has been briefly reviewed.

Out of 306 snails (*Indoplanorbis exustus*) collected, 24 snails were found to discharge furcocercous cercariae of *S. spindalis*; the percentage of infection being 7.84%.

The digestive gland or liver is the main site of selection for sporocytic and cercarial development and to a less extent ovotestis.

Histopathological sections reveal the glandular tissue of the liver as was being displaced by the cercariae and sporocysts. There was not much damage in other tissues.

Technique for exposure of mice, rabbit and buffaleo calves to *Schistosoma spindalis* have been described.

The frequency of distribution of schistosomes in the blood vascular system of experimentally infected mice was determined.

Fetal infection by the cercariae was not possible.

Observation on the sex ratio showed that males were more than twice as numerous as female schistosome.

Ova appeared in the uterus of the female worm after 60 days and were deposited in the tissues with in a week of development, that is 67 days after the exposure to the infestation. The number of ova observed being 8.

Morbid changes mainly noticed was in the liver, spleen, pancreas and lung.

An easy method of infecting laboratory animals such as mice with a view to maintaining the *Schistosoma spindalis* in the laboratory has been described.
Fig. No. 1) Furcocercous cercaria of Schistosome spindalis.

Fig. No. 2) Method of infection in mouse.

Fig. No. 3) Liver and Spleen of the experimentaly infected mouse. (Left side)

Note the great enlargement of the organs as compared to the normal. (Right side)

Fig. No. 4) Schistosome spindalis: Anterior end showing oral and ventral suckers... testis.
Fig. No. 1) Cercariae (S. spindalis) in interglandular tissue of snail liver. (Planorbis exustus)

Fig. No. 2) Cercariae of (S. spindalis) in the evetestis of snail. (Planorbis exustus)

Fig. No. 3) Cercaria found at the foot and mantle cavity of the snail tissue. (Repenetration)
PLATE NO. 111

Fig. No. 1) Schistosomes in the periportal veins of the liver.

Fig. No. 2) Schistosomes in the periportal veins and pigment deposit in kupffer cells.

Fig. No. 3) Section of liver showing pigment deposition.
Fig. No. 1) Section of liver showing a tubercle composed of an area of necrosis surrounded by mononuclear cells.

Fig. No. 2) Section of liver showing cytoplasmic vacuolization of liver cells. Fatty changes are also seen at the peripheral region.

Fig. No. 3) Liver showing a granulomatous reaction, typical Schistosoma ovum is seen at centre and is surrounded by the inflammatory cells. Surrounding parenchyma showing degenerative changes.
Fig. No. 1) Section of liver showing cellular infiltration and local around spine of ova.

Fig. No. 2) Section of liver showing round cell infiltration in the portal sheath.

Fig. No. 3) Section of the lung showing immature forms of parasite. Heavy infiltration of mononuclear cells seen around it.
Fig. No. 1) Section of pancreas showing the parasites in the vein. Round cell infiltration around the vein is also seen on the right side.

Fig. No. 2) Section of spleen showing a diffuse hyperplasia of lymphoid cells.

Fig. No. 3) Same section has been enlarged. (spleen)
Fig. No. 1) Kidney section showing cloudy swelling of the cells of convoluted tubules.

Fig. No. 2) Same section of the kidney has been enlarged.

Fig. No. 30) Section of the heart showing swollen muscle fibers.

Fig. No. 4) Section of Intestines showing lymphocytic infiltration in the mucosa, characteristic of mild enteritis.