OBSERVATIONS ON ORNITHOBILHARZIA TURKESTANICUM AND SCHISTOSOMA BOVIS IN IRAN

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by

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ABSTRACT

The geography and freshwater ecology of Khuzestan and those human activitiss which affect the snail ecology are described. O.turkestanicum. S.bovis and S.haematobium were the only animal and human blood flukes found in Khuzestan. The molluscan hosts of both species of Schistosoma is B.truncatus whereas O.turkestanicum is transmitted by L.gedrosiana. The infection rate of O.turkestanicum in ruminants was high and causes considerable economic loss.

The morphology of O.turkestanicum and its distinguishing charactristics from other schistosomes are discussed. The prevalence of O.turkestanicum and S.bovis infection in ruminants in Khuzestan abattoirs was studied. Infection rates of O.turkestanicum in cattle was higher than in sheep and goats. The intensity of O.turkestanicum in naturally infected cattle and sheep was determined. Sheep suffered more than cattle from the disease. The intensity of O.turkestanicum declined with increasing age in cattle but increased in sheep.

/Experimental....

Experimental studies were carried out on calves, sheep, goats and buffalo-calf infected with O.turkestanicum and S.bovis . The intensity, pathogenicity and pathology of O.turkestanicum and S.bovis in naturally and experimentally infected ruminants were compared. S.bovis caused more serious damage to ruminants than O.turkestanicum. In O.turkestanicum infections the duodenum was the most intensely involved organ and the liver was less affected and large intestine was free from infection. S.bovis was evenly distributed along the alimentary tract.

Susceptibility of different species of rodents, carnivores and birds to O.turkestanicum were tested. Only Tatera indica (a wild local rodent) was found to be susceptible to O.turkestanicum infection. Carnivores and birds were resistant to infection.

In the heterologous immunity experiments mice were immunized with different numbers of O.turkestanicum cercariae and challenged with S.bovis, S.haematobium and S.mansoni. Homologous immunity was

/ also studied....

also studied in mice with S.bovis. In these experiments mice produced a partial protection against the challenge infections.

Reciprocal heterologous immunity experiments were carried out in calves and sheep using S.bovis and O.turkestanicum . Calves showed a high degree of protection but the effect was poor in sheep. Calves immunized with repeated inoculations of S.haematobium cercariae developed some immature worms and produced considerable resistance against challenge infection with O. turkestanicum and S.bovis . Calves also developed a considerable immunity in homologous system with O.turkestanicum and S.bovis.

Studies were carried out on the distribution and ecology of Lymnaea gedrosiana the intermediate host of O.turkestanicum in Khuzestan. Detailed studies were made on the population dynamics of this snail by fortnightly surveys in different type of habitats over a period of 12 months. The results showed that peaks of snail

/ population....

population occurred in 2 seasons, spring and autumn. Transmission occurred throughout the year in canals; in spring, summer and autumn in swamps; in spring and autumn in ponds. Canals and swamps accessible to livestocks were found to be important transmission sites of O.turkestanicum.

Laboratory experiments were carried out to study the development of larval stages of O.turkestanicum in L.gedrosiana after the snails had been exposed to various number of miracidia. The results showed that the miracidial exposure dosage did not affect the cercarial prepatent period, but the life-span of infected snails was shorter and all the snails exposed to 20 miracidia shed cercariae. The snails exposed to 1 and 2 miracidia each shed fewer cercariae than those exposed to 5-20 miracidia.

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PART III: EXPERIMENTAL STUDIES ON ACQUIRED RESISTANCE TO SCHISTOSOMIASIS USING HOMOLOGOUS AND HETEROLOGOUS SYSTEMS

PART IV: FIELD AND LABORATORY OBSERVATIONS ON *LYMNAEA GEDROSIANA*, THE INTERMEDIATE HOST OF *O. TURKESTANICUM* IN KHUZESTAN. 221

GENERAL INTRODUCTION.

The present study was undertaken to assess the veterinary importance of *Ornithobilharzia turkestanicum* and *Schistosoma bovis* in livestock in Iran by determining the prevalence and intensity of infection, the pathogenicity of the parasites and the factors affecting transmission. Observations were also made on various aspects of the immune response to infection with these parasites and their inter-relationship with the schistosomes of human.

The field and laboratory studies were carried out in Iran but the development of the experimental techniques and the analysis of the data was carried out in the Department of Medical Helminthology in the London School of Hygiene and Tropical Medicine.

For convenience the thesis is divided into separate sections to cover the main topics. The general introduction which includes a geographical account of the area with particular reference to factors

likely

likely to affect transmission of schistosomiasis, followed by a section on materials and methods. This is followed by four distinct "parts". The first deals with the study of *Ornithobilharzia turkestanicum* in the definitive host; this is followed by the parallel studies on *Schistosoma bovis*. Part III deals with the observations on homologous and heterologous immunity and the interrelationship of the bovine and human schistosomes. The final section, Part IV, is an account of the studies in the field and laboratory on *Lymnaea gedrosiana* the intermediate snail host of *O.turkestanicum*.

KHUZESTAN: GENERAL DESCRIPTION OF THE ENDEMIC AREA,

Khuzestan is an area of Iran about 157,000 squire kilometres. Its southern boundary is the Persian Gulf and in the north and east it extends to the Zagros Mountain Range (Map, 1). In the west the Iran-Iraq borther forms an artificial separation between Khuzestan and Mesopotamia. In fact, Khuzestan is a prolongation eastward of the Mesopotamian plain and the two areas are indeed continuous and homogeneous in their physical geography and also to a certain extent in their human geography.

The plain is essentially alluvial. Its sub-soil contains appreciable deposits of gypsum and salts which tend to come to the surface when the ground water level is near the surface. Brown desert soils predominate. In the higher land which dominates the plain on the north east, in calcareous formations, there are numerous and relatively large springs.

The climate of the Khuzestan plain is semi-arid(classification of Thornthwaite). The maximum temperatures are very high in the summer(over 50 °C.) and the minimal winter rates are around 0°C. Daily

temperature

temperature variations are very great. The relative humidity is usually fairly low but it varies greatly depending on the direction of the wind. It is low, particularly in summer, when the winds blow mainly from the north and the north-east. It rises when the winds blows from the south, which happens more frequently in winter.

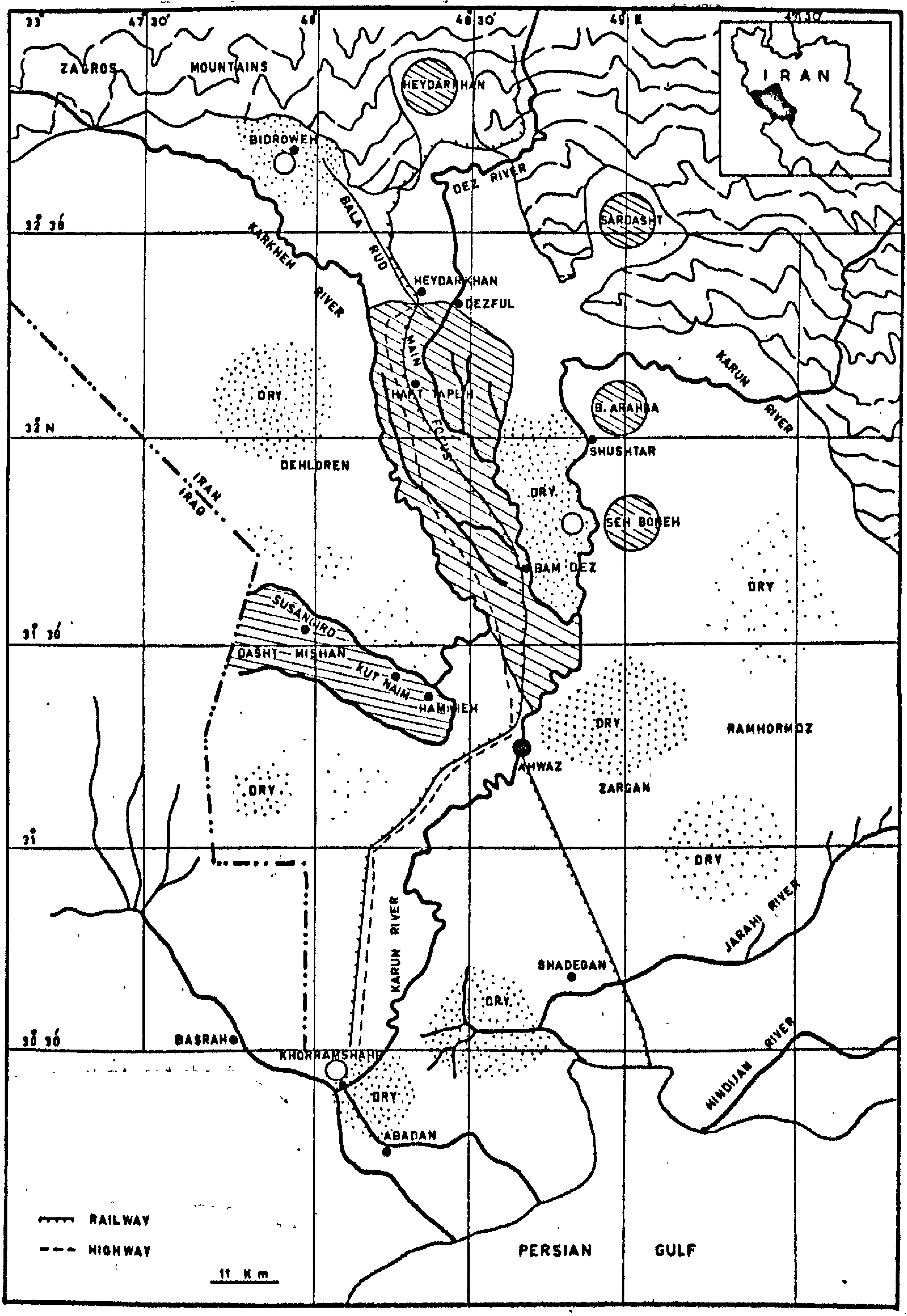
Rain is limited to the period from November to April with an average of 100 to 200 mm. but there is considerable variation in rainfall not only from place to place but from year to year. Much of this rain falls are in the form of violent downpours. Monthly temperature and rainfalls records are shown in (Fig. 1.). The nebulosity is low and the sun is very strong.

Owing to these climatic conditions, the major part of Khuzestan is a sub-desert steppe. But the plain is crossed by important streams which flown from the south-western slopes of the high Zagros Mountain Range. This combination of a semi-arid climate with relatively abundant surface waters makes Khuzestan a country of striking contrasts. Here, sand dunes are found not far from large swamps. A wide xerophilic animal fauna is overflown by an exceptionally abundant aquatic avifauna.

Rice

Map, 1.

Part of Khuzestan to show the main endemic foci of Lymnaea gedrosiana, Bulinus truncatus and the main river system



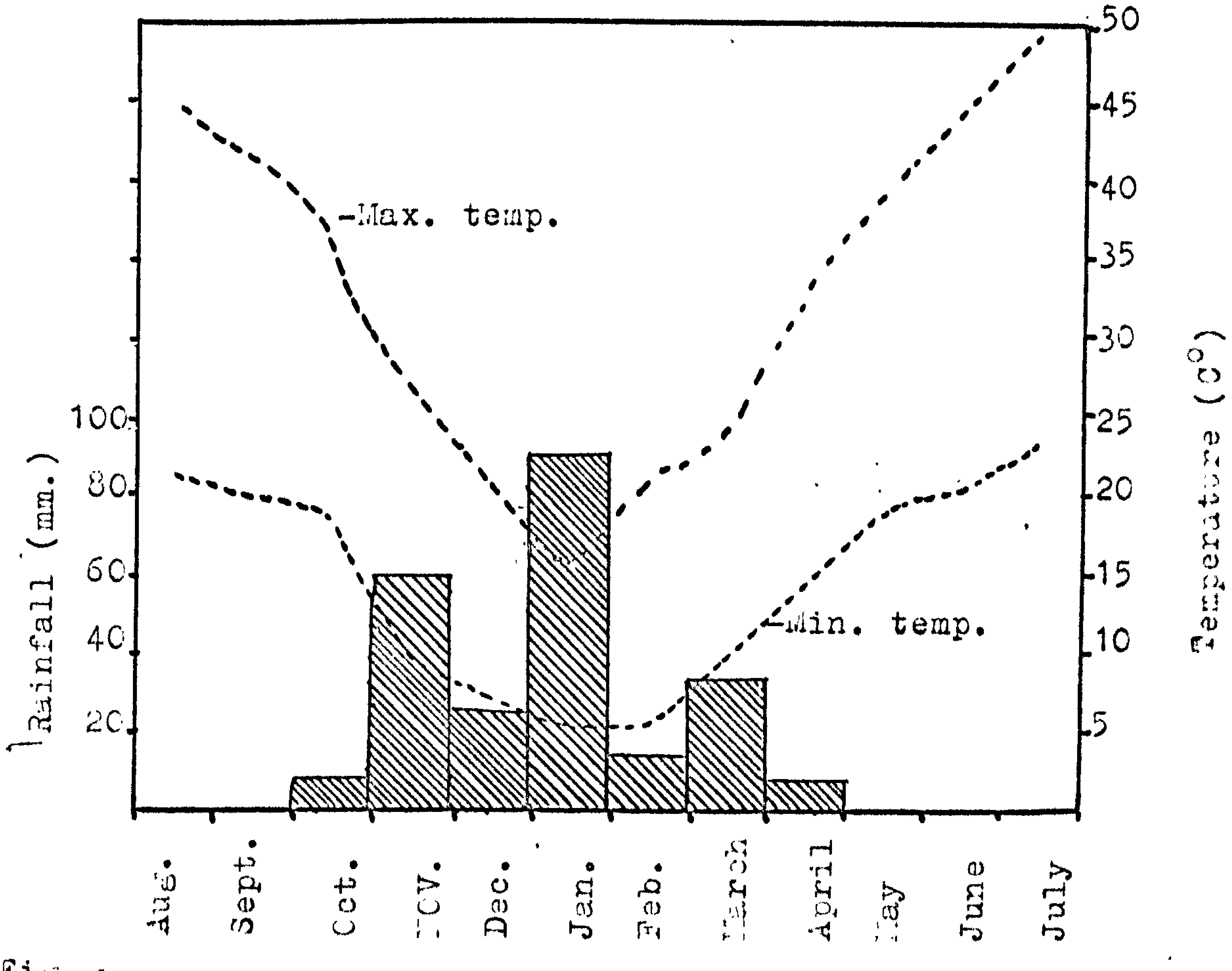


Fig. 1
Average rainfall and mean maximum and minimum temperatures at Dezful

Rice plantations alternate with cornfields. Among the herds driven back every evening to the village buffaloes walk side by side with sheep, goats and cattle. The population of Khuzestan plain is about 2½ million, mostly rural peasants practicing agricultural and cattle raising. The number of sheep, goats and cattle in the whole of Khuzestan amount to 2,700,000 head(Department of Agriculture)

Four different ethnic elements, Lur, Bakhtiari, Dezfuli and Arabs live in the Khuzestan plain: Lur in the west, Bakhtiari in the east, Dezfuli in the north and Arabs in the south. The population density is high in the irrigated areas, low or very low elsewhere. The principal crops are wheat and barley, both being produced with little irrigation, as well as rice, sesame and bedns. The present output is low, because of the archaic agricultural practices and lack of water in summer. Apart from the oil areas, the economic and social development of the country is poor.

General Ecological Aspects

Hydrography:

Rivers and large natural drains,

Five major water courses drain the south-west slopes of the Zagros Range and traverse the plain of Khuzestan (Map,1.).

Lying

Lying in order from the south-east to the north-west they are the Hindijan, the Jarahi, the Karun, its large tributary the Dez, and the Karkheh. The most important is the Karun-Dez river, not only because of the great size of its drainage basin (54,000 square kilometres) but because of its average volume of water flow (750 m^3/sec.). After it comes the Karkheh (200 m^3/sec.). Both the Hindijan and the Jarahi have an average volume of flow of about 150 m^3/sec. The flows in the summer are smaller than those of winter and spring, and the flow volùmes are very irregular from one year to another. Floods are not usual. The water is very turbid but the amount of solids carried is comparatively small. The water of the Dez is of low salinity and clear. The Karun and the Karkheh are more salty and the Hindijan and the Jarahi have a relatively high salt content.

Between the Karhkeh and the Dez there is an unusual water course, the Shahur. This springs in the north mostly from irrigation overflows. It follows a course that is probably an ancient irrigation canal, and at the lower end it becomes divided into numerous irrigation channels. Its volume of flow is small but it merits mention

/because.....

because it may play a very important role in the transmission of human and animal schistosomiasis. The other main streams in the north part of Khuzestan are the Abjirob, the Lureh and the Shureh, east of the Dez river. Downstream, these drains assume rather rapidly the aspect of a river, and before flowing into the Dez, they give rise to new irrigation network. All these rivers and streams have an irrigular and occasionally semi-torrential flow. Their course are winding and imprecise; they often spread in flood plains which sometimes reaches several kilometres in width.

Limnology:

Physico-chemical data,

In summer the water temperature remains lower than that of the air during a 24 hour period. Particularly in the daytime, the extreme atmospheric dryness results in very strong evaporation which largely compensates for the effects of solar heat and the maximum temperature observed in water remains under 37°C. seldom exceeding 35°C. while the air temperature in the shade reaches and exceeds 50°C.

/Surface.....

Surface water in Khuzestan contain a pH generally higher than 7, from 7 to 8 for running waters of rivers and canals, and from 7 to 8.5 for stagnant waters. In the stagnant waters, the pH varies in time. It is rather higher in winter with some sharp drops to 7-7.5 after heavy rainfalls.

Flora and fauna,

All stagnant or slow running waters are rapidly overrun by abundant aquatic vegetation. The vertical emerging vegetation is particularly abundant. The most representative groups are *Typha*, club-rushes, rushes and various species of couch-grass. Rushes are scarce and rather weak. *Polygonum* is often found at waters edge and is more or less immersed. By contrast, the floating vegetation is scanty. One can find *Potamegeton* and occasionally *Lemna* and *Salvinia*. Immersed macroscopic vegetation is irregular, sometimes absent but sometimes very abundant forming a thick layer. The most common groups are Zonichielles in winter, *Ranonculus* in spring, *Ceratophyllum* and characeae in summer and autumn. Various filamentous algae are also found and occasionally, *Hydrodyction*.

/The aquatic....

The aquatic fauna is abundant and includes tadpoles, as well as diptera and odonate larvae with usually only one stage of the life-cycle in water, hemiptera(hydrometres, nautonectes, naucrores, belostomes) which are plentiful and coleoptera which are fairly rare. Small crustacea are very abundant, particularly Ostracods, Choncostracs and Cladocers (Daphnia). Shrimps are only found in large permanent swamps. Hydricarines are numerous. Leeches are very scarce (Gaud et al, 1962).

Vertebrates actually living in water are represented by rather rare species of fish. Those found in small stagnant water collections seemed unable to reach their full development in such an environment, judging by the size of the specimens obseved.

On the other hand, the fauna of aquaphilic vertebrates is extremely abundant. The birds constitute its important element. They include a great number of species: pelicaniforms(cormorants and pelicans), ardeiforms(herons and storks), anseriforms(geese and ducks), larforms(terns), ralliforms(rattles and coots), coraciadiforms (kingfishers), but mostly charadriiforms(glareoles, plovers, lapwings,

/ruffs....

ruffs, snipes, sandpipes, stilt-plovers). In addition to birds, one may observe the presence of numerous turtles of the Clemmys types, and snakes of the Tropidonotus type. The amphibia, particularly frogs, are specially abundant.

The aquatic molluscan fauna is rather limited in species. The pulmonates are represented by species of: Bulinus truncatus, found in different types of habitats; Physa acuta, only found in a limited area in north of Ahwaz in canals and swamps; Lymnaea truncatula found in some swamps and drains; Lymnaea gedrosiana, the most common species occurring in the area in different types of habitats; Gyraulus intermixtus found in most habitats with other species; Planorbis planorbis is rare but sometimes found in swamps and drains. Amongs the prosobranches found are: Melanoides tuberculata in running waters and in some swamps; several species of Melanopsis are found in running waters, Melanopsis costata, Melanopsis praemrosa and Melnopsis nodesa; Viviparus bengalensis found in some large swamps; Theodoxus cintellus in some streams. Siphonommatophores (succinea) are very scarce. Pelecypodons, Lamellibranches are mostly found in

/running....

running waters and irrigation canals.

Part played by man in the ecology of the snails,

This section deals with those human activities which are more or less directly connected with the ecology of the snails. Agriculture is obviously the most important of these activities, and following five types of habitat are considered: gardens, rice fields, marketgardens, cattle breeding areas and the clay pits.

Gardens,

What they usually call gardens in this area should more properly be called orchards, as behind the high walls which surround these places, one finds mostly trees, mainly fruit-trees, but also other types of trees. The area covered by these gardens is generally larger than that of the village itself. It may be twice or four times as large as the village area or even more. In most cases, only one gate gives access to the garden. The gardens are cool places, with trees poorly cut, growing sometimes in great disarray. The irrigation canals form a network which seems more orderly devised than the paths themseves. Their terminal branches link up with one or two drains and

/end outside....

end outside through apertures made in the lower part of the walls. Excess water sometimes forms small ponds inside the gardens but more often drainage water collects in ponds and swamps outside the walls. Inside the gardens ponds and drains play a role in breeding of snails in hot seasons. Swamps and ponds outside the gardens are most favourable breeding places for snails.

Rice fields,

Almost each village is provided with its own rice field and the areas devoted to rice cultivation differs each year. Rice is sown in seed beds in June, thinned out in July-August and harvested by the end of October. During cultivation there is no drying until a little time before the harvest. The quiet, tepid and shallow water of rice-fields constitutes a favourable breeding place for snails particularly Lymnaea gedrosiana. During the summer the rice field drainage waters maintain even to a larger extent than the garden drainage waters, some breeding places which otherwise, would be dried up.

Directly related to rice culture a practice is being

/developed....

developed which seems to be favourable to the spread of bulinid snails in particular. The villagers used _Typha_ leaves for tying the bundles of rice, when planting it out. The plants grows spontaneously in swamps and on the stream banks. The villagers themselves transplant these _Typha_ leaves to nearby ponds and swamps to get sufficient amount of _Typha_ for the next season. In this way they spread the _Bulinus_ snails to the new ponds with _Typha_ roots and leaves.

Market gardens,

Around each village some vegetables are extensively cultivated for the personal use of the villagers: onions, carrots, radishes, beetroots, melons, watermelons, cucumbers, eggplants,spinach and various aromatic herbs. There are some areas, owing to the vicinity of Dezful or the highway, in which market gardening is continuously and intensively practiced, the products intended either to be sold in the town or exported to the north of Iran, particularly Teheran. In addition to the above mentioned vegetables, they also cultivated in these areas tomatoes, pimentoes, garlic, but mainly lettuce. In these gardens, intensive fertilizing(human and animal mannure) is required because of the

/villagers....

villager's desire to obtain several crops per year, for many consecutive years. Lettuce, whose cultivation alternates by rotation with that of cucumbers, particularly needs fertilizers. In these areas were found Bulinus and large population of Lymnaea in the drainage waters. On the course followed by the drains, some ponds are dug which are used for washing the vegetables before carrying them to the market. The remains of the vegetables after washing constitute an abundant food for Bulinus and Lymnaea. In the meantime excess waters from these gardens permanently supply water for swamps and ponds nearby.

Cattle breeding areas,

Livestock are abundant in the cultivated areas, consisting of about 20,000 equines (horse, donkeys, mules), 30,000 bovines (oxen and buffaloes), 15,000 goats and most important about 100,000 sheep. These animals directly affected the ecology of Bulinus and Lymnaea, because they usually drink water from the canals, causing leaks which feed stagnant waterponds. Also their excreta fill waters with organic matter which is favourable for snail breeding. Finally all these animals, particularly buffaloes, may carry bulinid snails in the mud which stick to their feet and body and may therefore afford these snails an apportunity to be disseminated rather far away from their original breeding places.

/Clay pits....

Clay pits,

Clay plays a considerable part of the life of the villagers. It is the main material for wall building and when mixed with straw, for roofs. It is used for making several utensils: bread ovens, animal mangers, nests for setting hens, vessels of various forms and capacity, particularly individual storing places for corn. In order to have clay available at any time, the peasants dig pits near the villages to which they convey either the drainage water of a nearby garden or the water from an irrigation canal. The slow sedimentation of water in these ponds allows for sorting of the clay particles which settle on the rougher grains of sands. These ponds are rapidly invaded by microscopic algae, then by aquatic vegetation. They soon become of no use as clay pits but they are not filled up. All these ponds constitute snail breeding places which are all the more dangerous as their banks may easily be used for human and animal defaecation.

S.haematobium, S.bovis and O.turkestan icum are the only mammalian schistosomes occuring in the Khuzestan endemic area. O.turkestanicum and S.bovis occurs mostly in ruminants, the prevalence of O.turkestanicum is higher than S.bovis. S.haematobium is the only human schistosomes and there is no evidence of animal infection with this parasite.

MATERIAL AND GENERAL METHODS

Schistosomes,

The four species of schistosomes used were maintained in the laboratory in the following hosts:

Ornithobilharzia turkestanicum (Skrjabin,1913) Price,1929.

An Iranian strain freshly isolated from naturally infected cattle was maintained in *Lymnaea gedrosiana* and calves.

Schistosoma bovis Sonsino,1876.

An Iranian strain freshly isolated from naturally infected cattle was maintained in *Bulinus truncatus* and calves.

Schistosoma haematobium Bilharz,1852.

An Iranian strain freshly isolated from naturally infected human urine after passage was maintained in *Bulinus truncatus*.

Schistosoma mansoni Sambon,1907.

A Puerto Rican strain originally isolated from man was maintained in *Biomphalaria glabrata* and albino mice.

/Snails ....

Snails,

Lymnaea gedrosiana. A laboratory colony was established from a batch of wild snails collected from the field. This snail is the most susceptible snail to *O. turkestanicum* in Khuzestan.

Bulinus truncatus. A colony was built up from the local wild snails in laboratory. This snail is susceptible to the both local species of schistosomes *S. bovis* and *S. haematobium* in Khuzestan.

Snail culture

The snails were reared in the laboratory in plastic tanks (14 x 8 x 8 inches) containing stored river water and weeds with a fine gravel substratum. Light was adequate and water temperature ranged from 25-27°C. The aquarium water was changed whenever necessary from a main storage container of dechlorinated river water. The snails were fed daily on dried and fresh lettuce with occasional addition of powdered barley and rice seeds. Occasionally the tanks were infested by the free-living nematodes, *Chaetogaster* spp. and *Cyprodopsis* and sometimes by a brownish growth on the sides of the tanks believed to

/be of bacterial....

be of bacterial origin and harmful for growing snails (McClelland, 1964), which necessitated clearing out the tanks.

Snail infection

Faeces from infected large animals were diluted in 0.9 % saline and sieved through a wire mesh to separate the large particles and repeatedly sedimented until the supernatant became clear, about 25 minutes being allowed for each sedimentation. A final washing with ice-cold water was made to remove traces of saline. After the final washing the sediment was poured into petri-dishes, diluted with dechlorinated fresh water at 30°C. and placed under a strong light. Miracidia hatched in a few minutes. This technique was also used for determining prepatent periods of O. turkestanicum and S. bovis in ruminants.

The miracidia were transferred by a Pasteur pipette to a watch glass. Snails 4-6$^{mm.}$ size were added and kept for over 4 hours under adequate light and temperature. Infected snails were kept in big plastic tanks at 25-27°C. until they started to shed cercariae.

/ Shedding ....

Shedding and counting of cercariae

At least 100 snails infected with 5-7 miracidia were used for the cercarial shedding. The snails were put in 250$^{ml.}$ beaker containing fresh water at 27^{o}C. and exposed to direct bright sun light to stimulate cercarial shedding. After 2 hours the cercarial suspension was separated from the snails and the number of cercariae per ml. were counted by the ninhydrin staining technique of McClelland(1961).

Exposure of rodents to cercariae

A variety of small rodents and carnivores (laboratory bred and wild caught) were exposed to cercariae of O.turkestanicum in the laboratory. Before exposure to cercariae the animals were left for about 20 minutes in warm water to stimulate the evacuation of faeces and urine which might ultimately reduce the viability of the cercariae (Azim and Watson,1948). The animals were exposed to infections with a counted number of cercariae in a glass jar for about one hour.

/ Recovery ....

Recovery of adult worms from rodents

The perfusion technique described by Smithers and Terry (1965) was used. The animals were anaesthetized by injecting a dose of 0.5$^{ml.}$ heparinized nembutal intraperitoneally. The perfusion solution was prepared by adding 15 grams of sodum citrate to one litre of 0.9 % of saline. The animal was skinned, the thoracic and abdominal cavities were opened and part of the ribs were removed to expose the heart. The animal was suspended on the vertical plate of the apparatus, the portal vein was slit open and a perfusion needle was inserted into the left ventricle of the heart. The pressure for perfusion was provided by a rotary pristaltic electric pump. The perfused worms collected on a fine wire mesh and were then transferred to a petri-dish containing saline for counting. The bowels and livers were removed and washed in saline to collect any adhering worms. The liver was then crushed between two glass plates to detect any remaining worms. After perfusion the liver and bowel were preserved in the deep-freeze for egg counts.

Tissue egg counts in rodents

The pepsin digestion method of Nelson et al(1966) was used.

/The liver...

The liver and bowel were digested separately. Each organ was minced and transferred to a polythene flask containing the digestive fluid (1 % pepsin and 0.5 % HCl in normal saline). After an incubation period of 16 hours at 37°C. the digested tissues were precipitated in conical urine flasks for 30 minutes, after which the supernatant decanted and 25 ml. of 0.5 % hydoxide sodium was added to dissolve any remaining undigested tissues. Three 0.1 ml. samples were taken by a MacDonald pipette for counting the eggs after agitating the whole sample.

Infection of large animals,

For infecting the large animals cercariae were collected from at least 100 infected snails. Different species of animals (calf, sheep, goat and buffalo-calf) aged 7-9 months were used after repeatedly examination of faeces to ensure freedom from any schistosome infections.

The large animals were infected by foot immersion technique: the right front leg of the animals were closely clipped by electric

/hair clippers....

Plate, 1

Photograph illustrating the infection technique used for exposing large animals to cercariae.

By this technique recovery rates of up to 70% for S.bovis and 48% for O.turkestanicum were achieved.

hair clippers, wahhed and cleaned with warm water. The leg up to about 3 inches above the knee was then immersed in a thick polythene bag containing the cercarial suspension. The upper part of the bag was tied firmly to the leg to prevent loss of the cercarial suspension. The animals were immobilized by tying the feet together and they were kept still for 45 minutes (see Part, I).

Recovery of adult worms from large animals

The animals were starved for 12 hours before autopsy to avoid having to deal with a full and distended rumen. The animals were killed by exsanguination and to prevent the shift of the adult worms to liver, no anaesthetic was used. Fast bleeding of the animals prevented any significant clotting of the blood in the mesenteric and portal veins.

A mid-ventral incision was then made and the abdomen was opened. The worm recovery technique was similar to that used by Cheever (1968) on human cadavers. Worms were recovered separately from the liver and mesenteric veins in the following manner: The viscera

/ were removed....

were removed en bloc from oesophagus to rectum and the rectum was ligated. The rumen, abomasum and reticulum were then removed from the intestine. The rest of the viscera including lungs, liver, small and large intestine were then transferred to the laboratory. The liver and lung were separated from the intestines and the liver and intestine were perfused separately. Before perfusion a wedge of liver tissue was removed for histological study.

A glass cannula with a nylon tube connector was inserted into the inferior vena cava above the liver for perfusion of the liver in a direction reverse to that of the blood flow, 7-10 litres of tap water were used, the liver was massaged intermittently during perfusion. The liver was then sliced to collect any remaining parasites and rinsed in tap water to remove adherent worms.

The mesenteries were also perfused. First the small intestine were removed from the mesentery (After pushing all the worms in capillary veins on the surface of the intestine into larger branches of mesenteric veins). The mesenteric veins were then perfused from the portal vein in a retrograde manner after cutting the mesenteric veins

/ near their....

near their insertions to the bowel. The free margin of the mesentery was massaged during perfusion to help expel the worms. The major branches of the mesenteric veins were then perfused individually. The colon and caecum were then removed and the veins in this area separately searched for the worms and pushed out by a needle pressure on the surface of the veins. The intestines were placed in a separate container and the mucosa was separated from the bowel wall by hand and searched for any worms remaining in the veins.

In general there were about 10 litres of fluid from liver perfusion and 20 litres from inteine. The fluids were seived through a no. 100 gauge wire mesh to separate the worms. Worms were transferred to petri-dishes, males and females were counted separately.

Tissue egg counts in large animals

Three portions of liver weighing 20 grams each were taken from each animal: from the portal tract and the peripheral areas of both the right and left lobes, were minced and digested in 10 volumes of 4.5 % potassium hydroxide in saline water for 5-6 hours at 50°C.

/Three...

Three 0.1$^{ml.}$ samples taken by MacDonald pipette were counted. The mean number of eggs per gram of liver from the 9 specimens was taken as the egg count per gram of liver. A total of 13 organs: lung, liver, spleen, rumen, reticulum, omasum, abomasum, duodenum, jejunum, ileum, caecum, colon and rectum were isolated. The contents in the digestive tract were washed out. From each part 20 grams were digested in potassum hydroxide (KoH) as described before and egg counts were performed.

Faecal egg counts in large animals

A single specimen of 10 grams of faeces was taken and diluted in 10 % formol saline and a few drops of glycerol were added to remove the eggs from adherent particles. After 30 minutes samples were seived through a mesh to separate large particles. After seiving they were sedimented for 30 minutes and repeated several times in normal saline to clear the sediments. After a final wash the samples were made up to 50$^{ml.}$ with water and 7-10 samples were taken by MacDonald pipette and the eggs were counted.

Post mortem examination of animals for natural infection

Examinations were carried out by transilluminating the mesenteric veins with a strong direct light. This technique seemed

/to be very....

to be very effective as even a single schistosome worm could be readily detected by this method. The heavily infected organs were transferred to the laboratory for the further perfusion of the adult worms and tissue egg counts as described above. Also, other organs of infected animals particularly liver were carefully examined, gross pathological changes were recorded and pieces of different tissues were taken for histological studies.

PART I

STUDIES ON ORNITHOBILHARZIA TURKESTANICUM (SKRJABIN,1913) PRICE,1929.

CHAPTER 1.

INTRODUCTION,

The genus *Ornithobilharzia* was originally described by Rudolphi (1819), and Odhner (1912) from Red Sea birds. The related blood fluke which was first found by Skrjabin (1913) in cattle in Russian Turkestan first called *Schistosoma turkestanicum*, but Price (1929) transferred *S.turkestanicum* to the genus *Ornithobilharzia* (Odhner,1912) and called it *Ornithobilharzia turkestanicum* on the basis of its morphological similarity to other species of that genus. He suggested that in view of the morphological relationship of *O.turkestanicum* to species occuring in birds it may be assumed that this parasite may be only an accidental and facultative parasite of cattle and sheep that they are normally parasites in birds of some sort. Further taxonomic modifications were proposed by Dutt and Srivastava (1955) who suggested transfering the mammalian *Ornithobilharzia*, species to a new genus called *Orientobilharzia*, while Le Roux (1958) proposed the name *Eurobilharzia*.

/*O.turkestanicum* ...

O.turkestanicum has been recorded by Popov (1926) from the cat in Kazakstan; by Yamagiwa (1931) in mongolian cattle; by MacHattie and Chadwick (1932) and MacHattie (1936) in large animals in Iraq; by Hsu (1938) from sheep from North China; by Abdussalam and Sarvar (1952) from a sheep in Pakistan at 6500 feet above sea level; by Srivastava and Trisal (1957) and Dutt et al (1964) from cattle in India; by Boev (1944), Lavrov et al(1964, 1967), Tuaev (1946), Zakhrylov (1964), and Azimov et al(1965, 1966, 1968) from various part of the southern USSR. Wi^t^enberg and Lengy (1966) reported O.turkestanicum from field rats in the South Turkey. O.turkestanicum was first reported from Khuzestan, Iran by the Bilharziasis Pilot Project (1962), and later by Sabbaghian et al (1964) and Arfaa et al(1965) from large animals.

The wide distribution of O.turkestanicum in Khuzestan and the economic importance of the parasite in ruminants necessitated the present detailed studies on different aspects of this parasite in various laboratory and domestic animals.

THE MORPHOLOGY OF O.TURKESTANICUM

Male,

Length 2-10^{mm}. oesophagus shows only one posterior

/dilatation ....

dilatation. The oral sucker is subterminal covered with spines. The testes varies in number from 50-90 and occupy the intracrural space, starting some what behind the acetabulum and continuing to about the middle of the body. In ventral view they appear as transversely-elongated pear shaped bodies arranged in two interlooking rows, with their narrow ends pointing towards the mid-line of the body. The testes do not stain deeply with aceto-carmine staining like other schistosomes. The males of O.turkestanicum can be easily distinguished from males of Schistosoma by their smaller size and the large number of testes.

Female,

Length 2-8$^{mm.}$ oesophagus is simple. The common caecum extends for more than half the length of the body and divides in the anterior half. The ovary is situated anterior to the caecal union and is a spiral, elongated and anteriorly coiled organ. The vitellaria consist of rounded follicles situated on either side of the intestinal caecum. The oviduct starts from the posterior extremity of the ovary and after a short winding course passes into the uterus which continuous anteriorly between the two intestinal caeca and contains only a single egg.

The egg usually bears a blunt spine at the one pole and a roughly nipple-shaped appendage at the other end; This appendage may bend away from the axis of the egg (Plate, 2). The eggs are of variable shape, mostly elongated and oval and about 76^{μ} x 26^{μ} size, immature eggs being smaller in size. Females of O.turkestanicum can be distinguished from females of Schistosoma by the smaller size and single typical intra-uterine egg and spiral ovary.

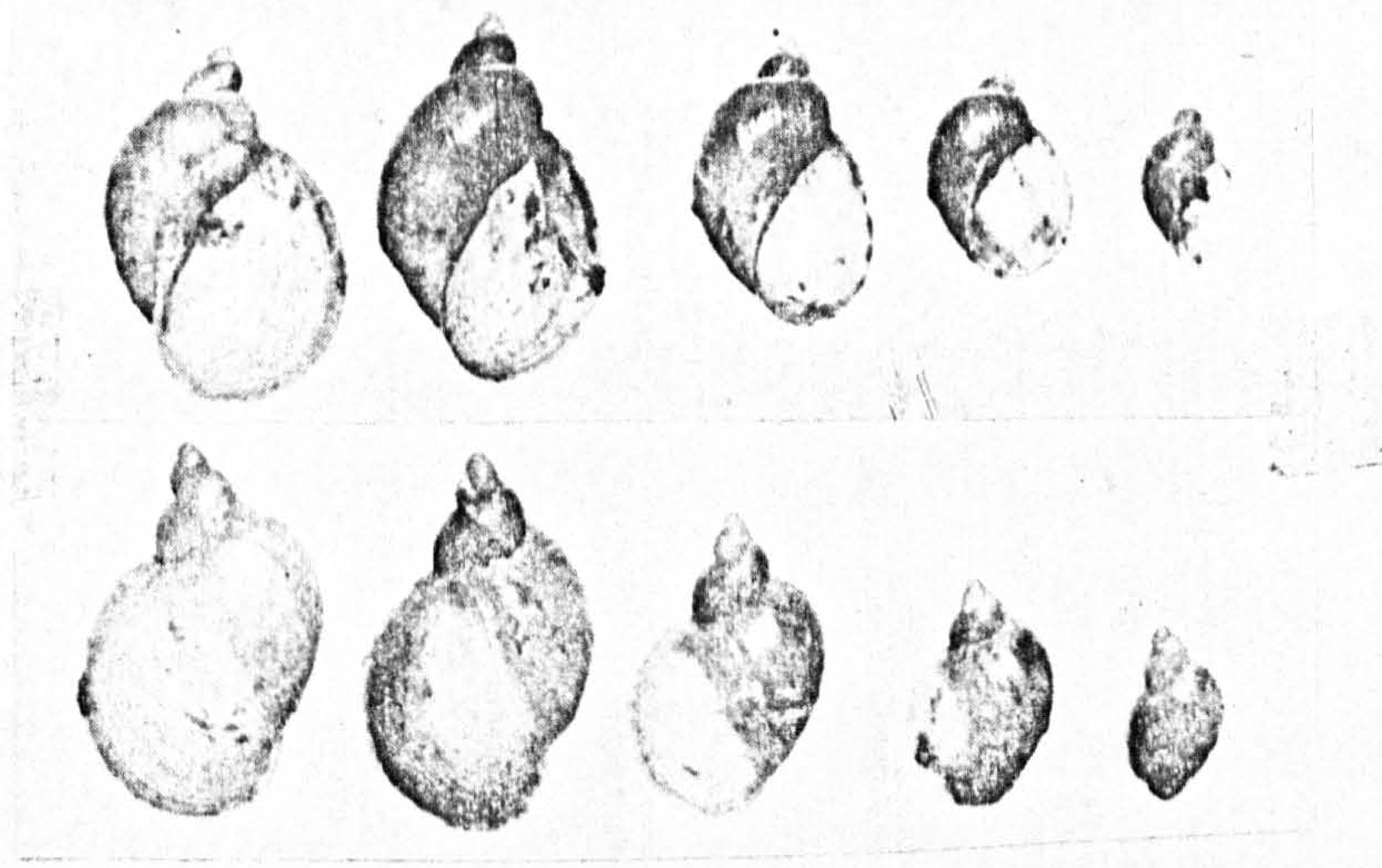
The females are usually in copula with the males and therefor it is difficult to see them, because they are completely hidden in the gynaecophoric canal. Sometimes, particularly in the small laboratory animals, two or more females are seen in the gynaecophoric canal of a single male. We agreed with MacHattie (1936) that the "tuberculate" appearance of the worms is due to contraction of the cuticule of the male worms during fixation, and we did not find

/any of the....

any of the tuberculate O.turkestanicum, refered to by Bhalerao (1932).

The miracidia of O.turkestanicum are very active and move rapidly in water. They can be distinguished from S.bovis and S.haematobium miracidia under the low power of the dissecting microscope by their smaller size and fast movement. The percentage of eggs with mature miracidia in the intestinal wall and liver of ruminants was low and most of the eggs were excreted without a fully developed miracidia. The size of immature eggs in the faeces was much smaller than mature eggs with a viable miracidia.

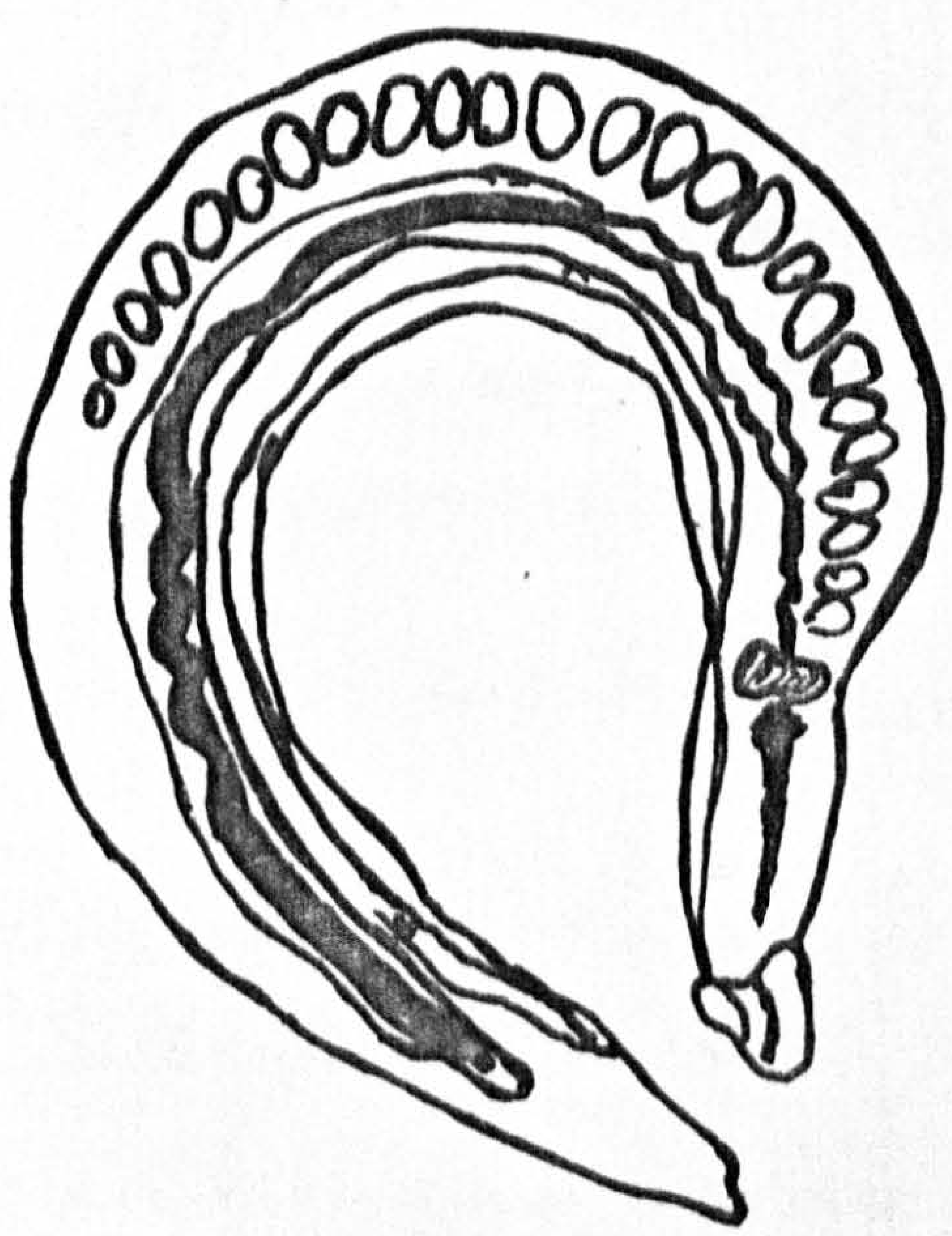
The cercariae of O.turkestanicum is apharyngeal brevifurcate distome with no eye spots, the cercariae are discharged from the snails in " puffs " emergence usually takes place at early morning and it is most rapid in direct sun-light. The cercariae did showed no predilection for the sub-surface position as shown by the S.bovis and S.haematobium cercariae, but were fairly well distributed throughout the water and after sometimes most of the cercariae were found resting on the bottom of the tube. The cercariae of O.turkestanicum contrast to ~~in spite of~~ Schistosomes cercariae (the) always progress head first.



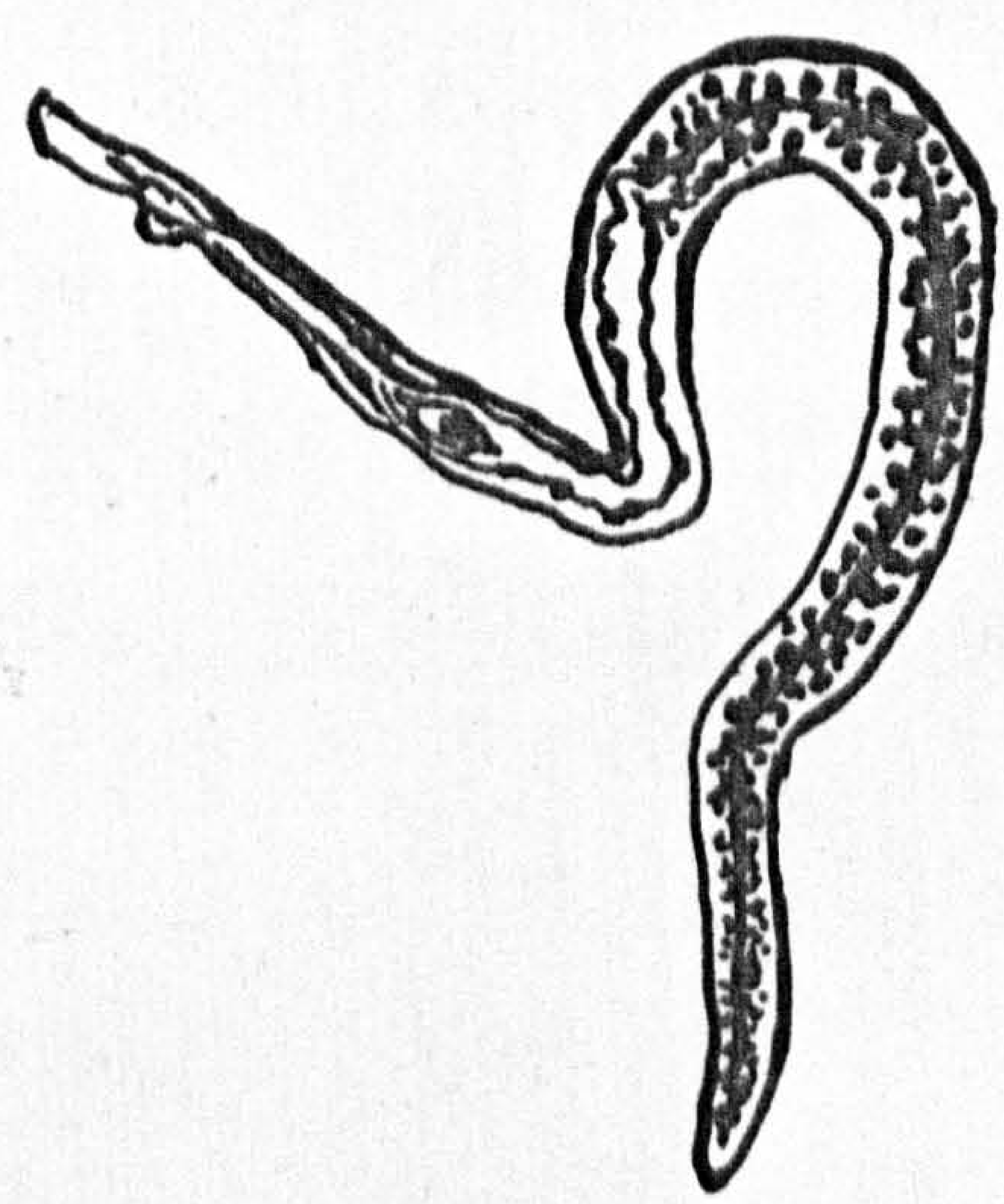
LYMNAEA GEDROSIANA

&

ORNITHOBILHARZIA TURKESTANICUM



Male and Female Worms



Free Female

CHAPTER 2

STUDIES ON THE PREVALENCE AND INTENSITY OF O.TURKESTANICUM IN DOMESTIC ANIMALS IN KHUZESTAN, IRAN.

INTRODUCTION,

O.turkestanicum is prevalent in Khuzestan, south-west of Iran. In Khuzestan, the most affected area, is in the nogthern and central parts (Dezful, Shushtar, Masjid-i-Solaiman, Ahwaz and Dasht-Mishan). In the southern part (Abadan, Khurramshahr and Shadegan)the parasite is rare because there is no animal raising practice and also no suitable breeding places for the snail intermediate host exist (See Map, 1.).

In other parts of Iran it can be assumed that there is a patchy distribution of *O.turkestanicum*. In northern Iran near the Turkestan and Caucasus areas where Russian investigators have reported a high prevalence of *O.turkestanicum*, Arfaa *et al*(1965) reported one case of *O.turkestanicum* infection in cattle slaughtered in a Teheran abattoir apparently originating from this area. Also Ale-Dawood (1963) reported infection in 4/9 sheep and 4/8 cattle

/examined....

examined in the Babolsar abattoir in the Caspian area(north of Iran). Furthermore 2/8 cows and 2/11 sheep were found infected in the Esfahan abattoir in the central part of Iran.

The Esfahan infection may have originated in Khuzestan due to the seasonal migration of Bakhtiari tribes with their herds from Esfahan area to Khuzestan in their search for winter pasture. The infections in northern Iran probably originated from southern Russia.as

MATERIAL AND METHODS,

A total of 632 cattle, 607 sheep, 89 goats and 96 buffaloes were examined in Dezful and Ahwaz abattoirs in search of schistosomes infection. Twenty one heavily infected cattle and 12 sheep in different age groups were perfused and tissue egg counts were performed. Before perfusion, samples from liver, and gut were taken for histopathological studies. The condition of the animals and their sex and place of origin were recorded. Animals (usually sheep and goats) which were from outside the Khuzestan area were not included in the data. We also examined other animals which died during this period in the area including: Four donkeys, one wild boar, 2 foxes and 3 mongooses.

/Results....

RESULTS,

Prevalence,

O.turkestanicum is prevalent among cattle and sheep in the villages in the most of the irrigated areas in Khuzestan (Table, 1.), though it is difficult to ascribe infection to precise villages as animals are watered at snail breeding places situated in different areas. Infection rates of 31.6 % and 25.0 % were found in the northen part (Dezful and Shushtar) and central part (Ahwaz and Dasht-Mishan) of Khuzestan respectively, whereas no infection were found in Abadan and Khurramshahr (southern part).

Table, 2 shows the prevalence of *O.turkestanicum* in the different type of hosts in the northern part of Khuzestan. The infection rate in cattle, sheep, goats and buffaloes was 30.3 %, 15.6 %, 6.7 % and 2.1 % respectively.

Adult worm recoveries,

A total of 21 cattle and 12 sheep heavily infected with *O.turkestanicum* were perfused and tissue egg counts were performed. The cattle were studied in 3 groups according to their age (Tables, 3, 4, 5); sheep were studied in 2 separate age group (Tables, 6, 7). The data on cattle and sheep, collected from May, 1969 to September 1970, showed that the number of worms and the number of eggs per

/gram....

gram of tissues declined considerably in the oldest cattle. In sheep however, no such reduction was observed (Fig. 2, 3).

The number of worms in the mesenteric veins of cattle varied from 2 to 7374 and in sheep varied from 2 to 712. In cattle the mean number of worms was 1188 ± 312 from the 1-3 years old age group, 1823 ± 749 from 3-5 years old age group and 764 ± 252 from the 5-7 years old age group (Tables, 3, 4, 5). In sheep the mean worm burdens was higher in the 3-5 years old (Tables, 6, 7).

Tissue egg counts,

In cattle the tissue egg counts declined with the age, but in sheep there was slightly increased with age (See Tables, 2-7).

The number of eggs per gram of tissues in cattle was highest in the 1-3 years old age group with a mean of 615 ± 298 eggs per gram in the liver and 1149 ± 3765 eggs per gram in the small intestine. In the 3-5 years old age group 166 ± 48 eggs were found in liver and 7339 ± 3455 eggs in the small intestine. In the 5-7 years old age group fewer eggs were recovered(123 ± 71 eggs

/per gram....

per gram of liver and 1444 - 628 eggs per gram of small intestine). There were no eggs in the large intestine and other organs, and the number of eggs in ileum was very low compared with the high number found in the duodenum. The pattern of egg densities relation to the age of cattle and sheep are shown in Fig. 2, 3.

DISCUSSION,

Prevalence,

Most of the cattle, sheep, goats and buffaloes slaughtered in Dezful abattoir were brought from the northern part of Khuzestan and the animals slaughtered in Ahwaz abattoir were brought mainly from the Dasht-Mishan area. In the south of Khuzestan (Abadan and Khurramshahr) the animals came mainly from outside Khuzestan from Kazerun, Behbahan and Kermanshah. Schistosomes recovered from the ruminants were mostly O.turkestanicum as indicated by the small size of the worms and shape of the eggs.

The prevalence of O.turkestanicum in cattle was higher than

/in other....

in other ruminants (cattle 30.3 %, sheep 15.6 %, goats 6.7 % and in buffaloes 2.1 %) in spite of the fact that the laboratory experiments (see chapter, 3) showed that goats are as susceptible to this parasite as sheep. The low prevalence of infection in goats may be due to their habits avoiding unnecessary contact with water, kneeling on their fore legs at the edge of the bank while drinking and not standing in it as other animals so often do. Only 2 very light infection with immature worms were found in buffaloes. In contrast to goats buffaloes spend most of the time in very close contact with infested water bodies, resting inside swamps and ponds for a long period every day. Subsequent laboratory experiments showed that buffaloes are very poor hosts for <u>O.turkestanicum</u> compared with cattle and sheep.

There are certain discrepancies between these results and those of previous workers. For example MacHattie (1936) reported that in the Marsh-Arab area of Iraq(near the Dasht-Mishan area in Khusetan) about 80 % of the animals were infected with <u>O.turkestanicum</u>. Again, Arfaa <u>et al</u> (1965) reported up to 69 % of cattle, 28 % of sheep, 35 % of buffaloes and 100 % ogf goats in the Dèzful area in Khuzestan

/ were ....

were infected with O. turkestanicum. The discrepancies may be due to their data being based on apparently ill and emaciated animals from restricted areas rather than being random samples from the general animal populations as in the present observations.

Intensity.

The worms load and tissue egg counts declined with increasing age in the cattle suggesting that they develop some degree of resistance to infection. This effect was demonstrated experimentally in calves (see Part, II). In contrast in sheep there was no such reduction, it was later shown that the immune response is less well developed in sheep.

The most striking result of the tissue egg counts was the pattern of distribution in the different organs. The duodenum had the highest egg densities; the liver showed relatively low egg counts especially in cattle where this organ was less affected than in sheep and goats. The large intestine was entirely free from eggs. This

/contrasts...

contrasts with S.*bovis*, S.*mansoni* and S.*japonicum* in definitive hosts where the large intestine is the most favourable site of egg deposition and the liver is much more affected.

The other animals examined, did not seem to be important hosts, although one of the donkeys and the wild boar had a few worms. None of the mongooses and foxes were infected.

Table, 1

Distribution of *O.turkestanicum* in Cattle
in the Khuzestan area

Locality	Total No. examined	Total No. positive	Percentage of infection
Northern part	512	162	31.6 %
Central part	120	30	25.0 %
Southern part	80	0	0

Table, 2

Prevalence of O.turkestanicum, in the ruminants in Khuzestan, Iran.

Type of animal	Total No. of animals examined	Total No. of positive	Percentage of infection
cattle	632	192	30.3%
sheep	607	95	15.6 %
goats	89	6	6.7 %
buffaloes	96	2	2.1 %

Table, 3

Recovery of adults and eggs of *O. turkestanicum*, from naturally infected Cattle 1-3 years old

No.	Sex	Worms Recovery			Tissue egg counts /gram		
		Female	Male	Total	Liver	Small intestine	Large intestine
1.	Male	57	206	263	100	35	0
2.	"	888	1135	2023	1400	8600	0
3.	"	716	1220	1936	1700	27900	0
4.	"	660	1231	1891	200	8300	0
5.	"	210	397	607	150	13250	0
6.	"	190	218	408	140	13610	0
Mean (S.E.)		454 (139)	734 (208)	1188 (312)	615 (298)	11948 (3765)	0

Table, 4

Recovery of adults and eggs of <u>O.turkestanicum</u>, from naturally infected Cattle 3-5 years old

No.	Sex	Worms Recovery			Tissue egg counts /gram		
		Female	Male	Total	Liver	Small intestine	Large intestine
1.	Male	196	271	467	120	300	0
2.	"	780	1133	1913	200	15000	0
3.	"	3002	4372	7374	500	24000	0
4.	"	20	34	54	140	260	0
5.	"	1966	2327	4293	260	28000	0
6.	Female	438	1739	2177	285	0	0
7.	"	10	68	78	40	80	0
8.	"	140	156	296	120	5280	0
19.	"	30	23	53	0	350	0
10.	"	454	1078	1532	0	120	0
Mean (S.E.)		703 (316)	1120 (441)	1823 (749)	168 (48)	7339 (3455)	0

Table, 5

Recovery of adults and eggs of O.turkestanicum, from naturally infected Cattle 5-7 years old

No.	Sex	Worms Recovery			Tissue egg counts /gram		
		Female	Male	Total	Liver	Small intestine	Large intestine
1.	Female	204	1356	1560	115	220	0
2.	"	82	278	360	50	2550	0
3.	"	396	654	1050	400	1100	0
4.	Male	226	487	713	50	3250	0
5.	"	30	109	139	0	100	0
Mean (S.E.)		188 (63)	577 (215)	765 (252)	123 (71)	1444 (628)	0

Table, 6

Recovery of adults and eggs of O. turkestanicum, from naturally infected Sheep 1-3 years old

No.	Sex	Worms Recovery			Tissue egg counts /gram		
		Female	Male	Total	Liver	Small intestine	Large intestine
1.	Female	317	340	657	300	4470	0
2.	"	41	52	93	0	120	0
3.	"	140	218	358	180	1800	0
4.	"	26	57	83	50	200	0
5.	Male	28	57	85	110	220	0
6.	"	100	152	252	260	1010	0
Mean (S.E.)		108 (45)	146 (47)	254 (94)	150 (48)	1303 (686)	0

Table, 7

Recovery of adults and eggs of O.turkestanicum, from naturally

infected Sheep 3-5 years old

No.	Sex	Worms Recovery			Tissue egg counts /gram		
		Female	Male	Total	Liver	Small intestine	Large intestine
1.	Female	241	471	712	300	5600	0
2.	"	90	150	240	200	3850	0
3.	"	20	26	46	0	100	0
4.	"	75	124	199	40	530	0
5.	MMle	76	124	200	180	1000	0
6.	Male	155	325	480	430	1500	0
Mean (S.E.)		110 (31)	203 (66)	313 (98)	192 (65)	2097 (88)	0

Fig. 2a

Histogram showing the decline with age in the densities of O. turkestanicum eggs in the tissues of naturaly infected calves.

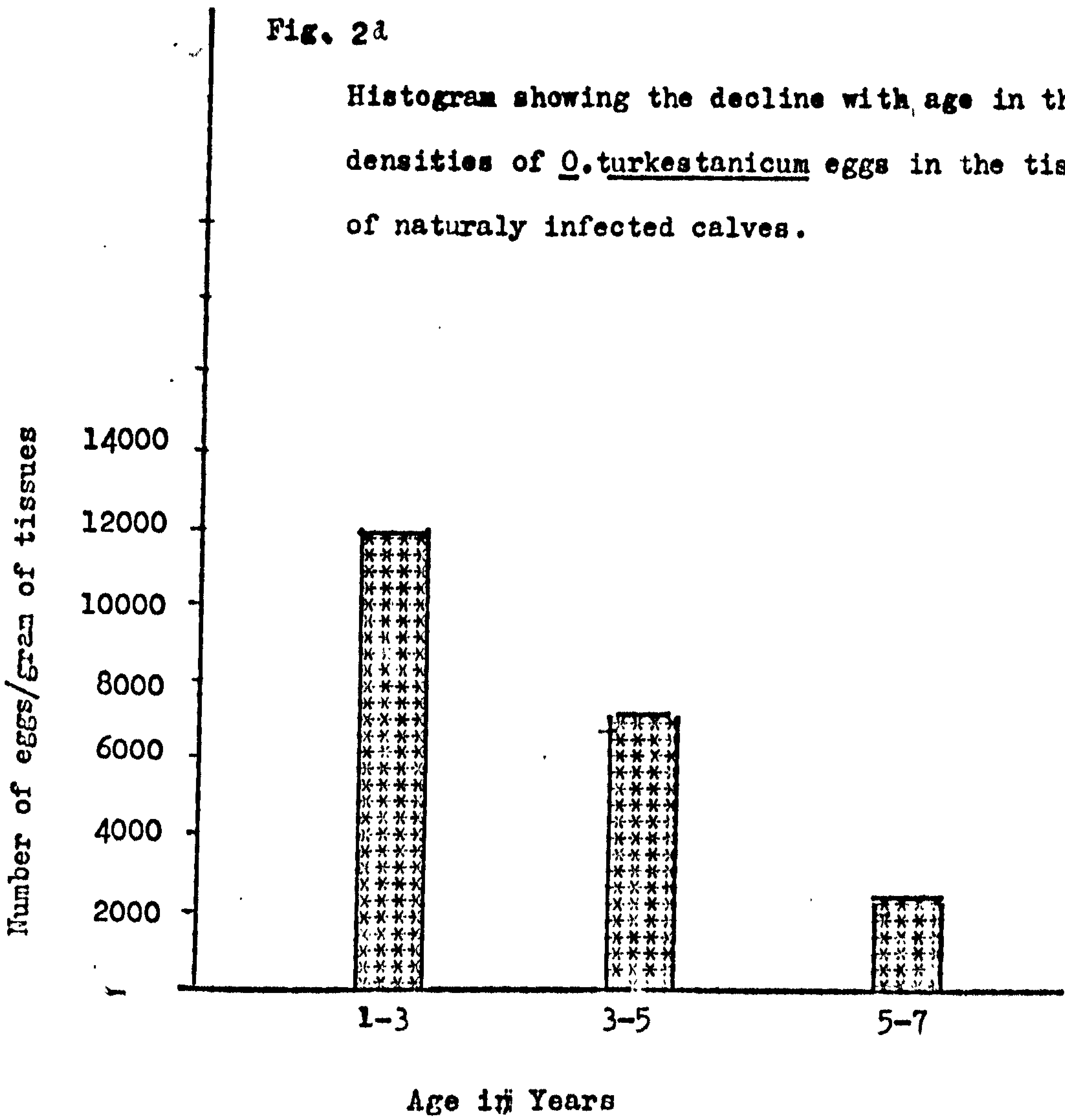
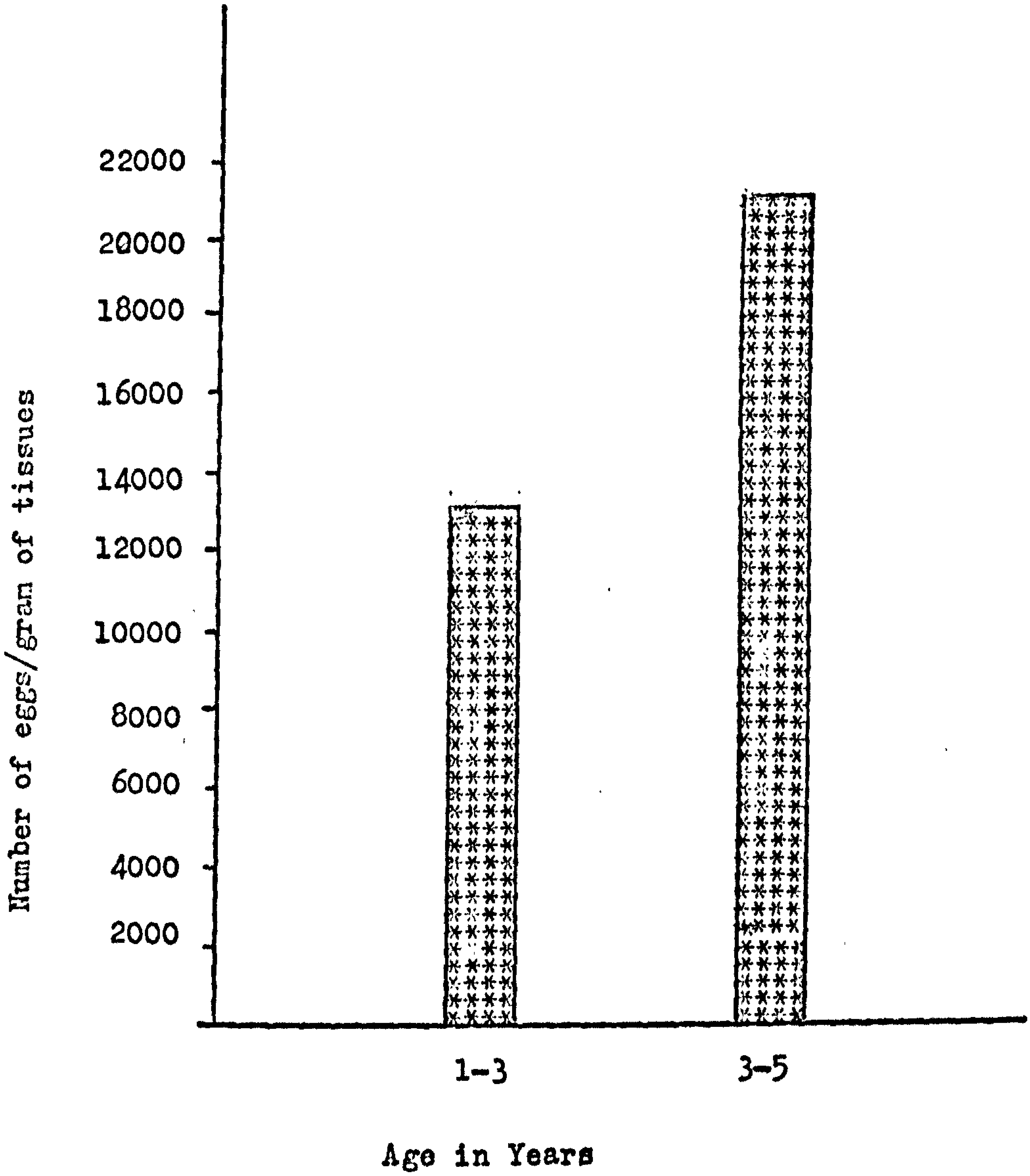


Fig. 3a

Histogram showing the increase with age in the densities of *O. turkestanicum* eggs in the tissues of naturally infected sheep.



CHAPTER 3

PARASITOLOGICAL OBSERVATIONS ON EXPERIMENTALLY INFECTED LARGE ANIMALS.

INTRODUCTION,

During the past few years increasing interest has been taken in the study of schistosomiasis of ruminants because of the economic importance of the disease. Previous studies on O.turkestanicum have been concerned either with the snail hosts (Dutt et al, 1964 and Azimov et al, 1968), or with the epidemiology and pathology of naturally infected animals (Azimov, 1965 and 1966, Lavrov et al, 1967). But the full life-history of the parasite had not been adequately studied in the laboratory, the following observations were made to elucidate the behaviour of the parasite in the definitive hosts.

Material and Methods,

Eight calves, 5 sheep, 2 goats, one buffalo-calf and one young wild pig were exposed to O.turkestanicum cercariae in the

/laboratory....

laboratory, to determine the infectivity and pathogenicity of parasite. At the same-time some of the animals were used for immunological studies (see Part, III).

The animals were obtained locally and were 7-9 months old. Repeated examination of the faeces were made to ensure that they were free from schistosome infection.

The calves and sheep were divided into 2 groups. Group I included 5 calves and 3 sheep which they were autopsied 9 weeks after exposure to O.turkestanicum cercariae. Group II included 3 calves and 2 sheep autopsied 18 weeks after exposure to the initial exposure of O.turkestanicum cercariae. Two goats, one buffalo-calf and one young wild pig were exposed to O.turkestanicum cercariae and were autopsied 9 weeks later.

The prepatent periods of the infections were determined by the faecal egg hatching technique of Standen (1949). The daily egg output in the faeces was expressed as (eggs per day) and also as (eggs per female worm per day) by collecting approximately overnight faecal specimens from each animal kept individually in a clean boxing room. The total daily egg production divided by the number of female worms recovered giving approximately the number of eggs in the faeces per female worm.

/Results....

RESULTS,

Prepatent periods,

The prepatent periods of O.turkestanicum in the different hosts (calves, sheep, and goats) are shown in Table, 8. The prepatent period appeared to be unaffected by the type of host and varied from 43-46 days. The numbers of eggs in the first days of their passage (as judged by the number of hatching miracidia) were very low, then increased gradually. Throughout the prepatent period the animals were healthy, but with the appearance of eggs in the faeces there was a rapid detrioration in their condition.

Infectivity,

The infectivity of O.turkestanicum to the different types of host were determined from the adult worms recovered. In calves the mean recovery rate was 37.6 %, in sheep 53.9 % and in goats 22.5 % (see Tables, 9-12).

In the buffalo the worm return was only 9.6 %; in the wild pig infected with 5,000 cercariae, only 26 immature worms were recovered. Furthermore, the worms recovered from the buffalo and pig were smaller than normal worms.

/Distribution....

Distribution of adult worms,

Some differences were noticed in the distribution of the worms in the differeht hosts (Table, 13). Very low number of worms were recovered from the hepatic veins (3.1 % in calves, 8.3 % in sheep and 5.2 % in goats) compared with a large number in the mesenteric veins, particularly the veins from the duodenum (96.9 % in calves, 91.7 % in sheep and 94.8 % in goats). No worms were detected from the inferior mesenteric veins. The unpaired worms in the liver were smaller than paired males in the mesenteric veins.

Faecal egg counts,

The daily egg output of O.turkestanicum in different hosts are given in Table 8, indicating that the mean number of eggs per gram of faeces per day was higher in cattle than sheep and goats, 179 ± 42, 37 ± 4 and 59 ± 1 respectively. The daily egg output in the faeces per individual female worm was also higher in calves than in sheep and goats (283 in calves, 52 in sheep and 82 in goats at 63 days after exposure to cercarias, Table, 14). No significan decrease of egg output was observed at 128 days after exposure to the cercariae, but with S.bovis there was a considerable decline in egg output in faeces as the duration of infection was prolonged (see Part, II).

/distribution of eggs....

Distribution of eggs in the visceral organs,

The duodenum was the portion of the alimentary tract most intensly involoved as a site of egg deposition (Tables 15, 16, 17). The density of eggs declined gradually in the jejunum and few eggs were found in the ileum. The large intestine was entirely free from eggs. As are shown in Table,13, approximately 97.7 %- 99 % of eggs in calves, 89 %- 90.8 % in sheep and 79.4 % of eggs in goats were found in the small intestine particularly the duodenum. The liver showed very low egg counts in calves (1 % - 2.3 %), but much higher egg counts were recovered in sheep (9.2 % - 11 %) and in goats (20.6 %).

The distribution of eggs in the different parts of the liver (left lobe, right lobe and portal tract area) showed no obvious differences. The other organs (lung, spleen, rumen, reticulum, omasum and abomasum) were free from eggs. In the buffalo only a few non-viable eggs were detected.

DISCUSSION,

The prepatent periods in the small and large ruminants varied from 43 to 46 days but it was 62 days in *Tatera indica* (a wild local rodent).

/The calves....

The calves and sheep showed a higher worm return than goats, buffaloes and wild pigs were poor experimental hosts for O.turkestanicum. Our prevalence studies showed also the same differences in the naturally infected animals.

Male worms always outnumbered female worms and since the cercariae for infection were pooled from at least 100 heavily infected L.gedrosiana, this probably shows that male cercariae outnumbered female cercariae in the original suspension, persumably there were more male larval stages in the snails. The predominance of the male worms in experimental animals is well known. Girges(1934) found excess male worms in the portal system of human cases in Egypt and Cheever(1968) found a slight predominance of male worms of S.mansoni in humans. Wright and Bennett(1967) found more male worms in hamsters infected with S.haematobium. Fairley et al(1930) found the male worms twice as many as the females in goats infected with S.spidale. Our observations on experimentally and naturally infected animals with O.turkestanicum showed a marked predominance of male worms in different hosts. The higher number of male worms in nature may be due to more resistance and therefore longer survival of male cercariae than female cercariae or alternatively the male miracidia may be more common or more infective to the snails.

Most of the worms were found in the duodenum, declining in numbers from there down the intestine. No worms or eggs were found in the large intestine. The number of worms in the liver was very low, particularly in calves.

Studies on the egg distribution of various strains of S.japonicum, S.mansoni and S.haematobium, in the different organs of infected mice, hamster and rhesus monkey have shown that the pattern of egg distribution is a strain characteristic (Hsu et al,1960; Saoud,1966; Wright and Bennett,1967; Nelson et al,1968). Hsu et al(1960) also suggested that distribution of eggs of schistosomes in the visceral organs of its host are influenced by the species of the host and the intensity of the infection in the host.

In our experiments the natural hosts of O.turkestanicum(calves, sheep and goats) were infected with the same number of cercariae but the resulting egg densities differed, in particular the livers of goats contained many more eggs than sheep and calves. The pattern of distribution of eggs along the alimentary canal was the same in different hosts, and the eggs were mostly deposited in the mucous membrane of the intestine in the duodenum.

/There...

There was also a close correlation between the egg densities per gram of liver and the pathological changes which were more severe in sheep and goats than in calves (see Chapter, 5).

The number of eggs in the faeces per individual female worm was very high in calves (283 but lower in sheep and goats (52 and 82 respectively). This differences are mainly due to the large amount of faeces produced by calves (average 2,000 gram) compared with sheep and goats (average 700 gram per day).

The daily egg output of O.turkestanicum per gram of faeces are given in Table, 8 (179 in calves, 37 in sheep and 59 in goats) which calves passed more eggs than sheep and goats.

Table, 8

Parasitological behaviour of O.turkestanicum

in Calves, Sheep and Goats

Type of animals	No. of animal	No. of cercariae	Total worms recovered	Prepatent period (days) ±S.E.	Eggs per gram of faeces per day(63 days after exposure
Calves	1	8,000	2272	44	83
	2	"	2550	44	215
	3	"	1256	43	150
	4	"	1862	46	125
	5	"	5450	43	372
	6	"	3031	44	130
Mean		8,000	2737±569	44±0.4	179±42
Sheep	1	5,000	1134	46	50
	2	"	2342	45	40
	3	"	1847	43	30
	4	"	2219	44	25
	5	"	929	46	40
Mean		5,000	1694±248	44.8-0.5	37±4
Goats	1	5,000	1165	46	58
	2	"	1086	46	60
Mean		5,000	1125±39	46±0	59±1

Table, 9

The distribution of adults worms of O.*turkestanisum* in experimentally infected calves.

No.	No. of cercariae	Duration of inf. (weeks)	Liver			Mesenteric veins			Total	Recovery rate %
			F.	M.	total	F.	M.	total		
1.	8000	9	4	4	8	1018	1416	2434	2442	30.3
2.	"	9	1	5	6	833	1023	1856	1862	23.2
3.	"	9	20	104	124	2357	2965	5326	5450	68.1
4.	"	18	33	13	46	538	672	1210	1256	15.6
5.	"	18	9	25	34	963	1275	2238	2272	28.4
6.	"	18	25	129	154	896	1981	2877	3031	37.9
7.	5000	9	32	232	264	781	1180	1961	2225	44.5
8.	5000	9	30	20	50	1242	1351	2593	2643	52.8
Mean (S.E.)			19 (4)	66 (29)	85 (31)	1078 (196)	1483 (248)	2562 (434)	2647 (441)	37.6 (6)

Table, 10

The distribution of adult worms of O.turkestanicum in experimentally infected sheep.

No.	No. of cercariae	Duration of inf. (weeks)	Liver			Mesenteric veins			Total	Recovery rate %
			F.	M.	total	F.	M.	total		
1.	5000	9	50	40	90	464	580	1044	1134	22.6
2.	"	9	15	27	42	950	1350	2300	2342	46.8
3.	"	9	105	217	322	525	1000	1525	1847	36.9
4.	"	18	15	207	222	536	1461	1997	2219	44.9
5.	"	18	76	96	172	339	418	757	929	18.6
Mean (S.E.)			52 (17)	117 (40)	170 (49)	562 (102)	962 (205)	1524 (286)	1694 (284)	33.9 (5.7)

Table, 11

Distribution of adult worms of *O. turkestanicum* in experimentally infected goats.

No.	No. of cercariae	Duration of inf. (weeks)	Liver			Mesenteric veins			Total	Recovery rate %
			F.	M.	total	F.	M.	total		
1.	5000	9	50	48	98	454	613	1067	1165	23.3
2.	5000	9	16	2	18	480	588	1068	1086	21.7
Mean (S.E.)			33	25	58	467	600	1067	1125	22.5

Table, 12

Recovery of adults and eggs of O. turkestanicum, from large animals experimentally infected.

Type of animals	No. of animals	Mean no. of cercariae	Duration of infection (weeks)	Mean worm recovery				Mean tissue egg counts per gram		
				F.	M.	total	% Recovery	Liver	Small intestine	Large intestine
Calves	3	8000	9	1411	1840	3251	40.6	404	17567	0
	3	8000	18	821	1365	2186	27.3	112	10300	0
	2	5000	9	1042	1392	2434	48.7	120	12000	0
Sheep	3	5000	9	703	1071	1774	35.5	1095	8880	0
	2	5000	18	483	1091	1574	31.5	705	6985	0
Goats	2	5000	9	500	625	1125	22.5	1665	6425	0
Buffalo	1	8000	9	222	551	773	9.6	25	55	0
Wild pig	1	5000	10	16	10	26	0.5	0	0	0

Table, 13

Percentage distribution of adults and eggs of O. turkestanicum in different organs of Calves, Sheep and Goats experimental infected.

Type of animal	Duration of infection (weeks)	Egg distribution			Worm distribution	
		Liver	Small intestine	Large intestine	Portal veins	Mesenteric veins
Calves	9	2.3	97.7	0	3.1	96.9
	9	1.0	99.0	0		
	18	1.0	99.0	0		
Sheep	9	11.0	89.0	0	8.3	91.7
	18	9.2	90.8	0		
Goats	9	20.6	79.4	0	5.2	94.8

Table, 14

Eggs production of _O. turkestanicum_, in large animals as estimated by faecal egg counts during the period of observations.

Type of animals	No. of animals	Days post infection	Mean no. of female worms	Mean no. of eggs per gram of faeces per day	Mean no. of eggs per female per day in faeces *
Calves	5	63	1263	179	283
	3	126	821	100	243
Sheep	3	63	703	37	52
	2	126	483	60	86
Goats	2	63	500	59	82

* Based on the over night faecal output.

Table, 15

The distribution of O.turkestanicum eggs in tissues of

Calves in infections of different duration

Duration of inf. (weeks)	No. of female worms	Liver per gram				Small intestine per gram				Large intestine
		R.Lobe	L.Lobe	P.T.	Mean	Duodenum	Jejunum	Ileum	Mean	
9	1022	260	300	280	280	63000	5450	50	22830	0
9	834	110	140	100	116	21500	1700	120	7773	0
9	2377	810	800	840	816	58000	8300	0	22100	0
9	813	230	130	120	160	14400	13800	0	9400	0
9	1272	60	100	80	80	35000	8800	0	14600	0
Mean	1263	294	294	284	290	38380	7610	34	15341	0
18	571	0	100	50	50	33600	1500	0	11700	0
18	972	100	150	100	117	33500	650	0	11380	0
18	921	160	100	250	170	11000	12100	360	7820	0
Mean	821	87	117	133	112	26030	4750	120	10300	0

(P.T.) Portal Tract Area

Table, 16

The distribution of O. turkestanicum eggs in tissues of Sheep in infections of different duration.

Duration of inf. (weeks)	No. of female worms	Liver per gram				Small intestine per gram				Large intestine per gram
		R. Lobe	L. Lobe	P.T.	Mean	Duodenum	Jejunum	Ileum	Mean	
9	514	1470	1506	1400	1458	17100	33000	0	16700	0
9	965	1712	1365	514	1197	2200	15042	105	5780	0
9	630	680	520	690	630	11650	780	50	4160	0
Mean	703	1287	1130	868	1095	10316	16274	50	8880	0
18	551	300	310	1100	570	17500	6500	60	8020	0
18	415	1022	725	775	840	15500	2100	260	5950	0
Mean	483	661	517	937	705	16500	4300	160	6985	0

(P.T.) Portal Tract Area.

Table, 17

The distribution of _O. turkestanicum_ eggs in tissues of goats in infections of different duration.

Duration of inf. (weeks)	No. of female worms	Liver per gram				Small intestine per gram				large intestine per gram
		R. Lobe	L. Lobe	P.T.	Mean	Duodenum	Jejunum	Ileum	Mean	
9	504	2490	2240	1950	2227	11650	4440	0	5370	0
9	496	1050	1160	1100	1103	15400	7040	0	7480	0
Mean	500	1770	1700	1525	1665	13535	5740	0	6425	0

(P.T.) Portal Tract Area

CHAPTER 4

EXPERIMENTAL STUDIES ON THE SUSCEPTIBILITY OF RODENTS, SMALL CARNIVORES AND BIRDS TO O.TURKESTANICUM.

INTRODUCTION,

These studies were made in anx effort to find a susceptible animals for the laboratory maintenance of O.turkestanicum and to evaluate the usefulness of various hosts for investigation of different phases in the biology of the parasite under laboratory conditions. At the same time it was hoped that the study might indicate which if any of the wild mammals and birds in Khuzestan might be capable of transmiting O.turkestanicum in nature.

MATERIAL AND METHODS,

Most of the wild mammals used in this experiments are common throughout the Khuzestan provinces

Tatera indica (gerbil) is very common in the cultivated areas and near houses; Nesokia indica (bandicoot) is found in

/ the cultivated..

the cultivated areas near water sources; *Rattus rattus*, the common house rat is found throughout the Khuzestan; *Mus musculus*, the common house mouse is found in human habitations, and *Herpestes* spp. (mongoose), inhabits canal banks in cultivated areas throughout the irrigation systems in Khuzestan.

Common stray dogs and cats and different laboratory rodents (albino mice, hamster, rabbit and multimammate rats (*Mastomys natalensis*) were also used. Common domestic ducks and chicken were also tested.

The mammals were exposed to different number of cercariae by immersion in cercarial suspensions. Among those animals only the mongoose was anaesthetized (with chloroform) before exposure to cercariae. The birds were exposed to cercariae by paddling and also cercarial suspension were administered through the mouth. Animals were autopsied at various intervals to allow the parasite to develop.

RESULTS,

The results of experimental infections of small mammals and birds are shown in Tables, 18, 19.

/The only....

The only wild rodent that developed a fair good infection was <u>Tatera indica</u> the local gerbil, this rodent lives very close to houses and farms. A moderate percentage of the worms were recovered as fully developed adults about 15.1 % , with prepatent period of 62 days. Aproximately 15 % of the total eggs in the tissues contained fully developed miracidia 10 weeks after exposure to cercariae.

In the other wild rodents the numbers of worms recovered varied from 1.8 % in <u>Mus musculus</u> to 16.4 % in <u>Nesokia indica</u> (Table, 18). In other species numerous immature eggs were deposited in the liver, but no viable eggs were observed. <u>Rattus rattus</u> and <u>Mus musculus</u> were very poor hosts with the very immature worm returns of only 3.7 % and 1.8 % respectively.

Of the usual laboratory animals, white mice, hamsters, multimammate rats and rabbits were all infected with parasite with recovery rates of 26.9 % , 34.2 % , 25.8 % and 21.0 % respectively, but non of them produced viable eggs.

One young dog 2 months old, 2 kittens, 3 mongooses, 6 ducks and one chicken were also experimentally exposed to

/different....

different number of O.turkestanicum cercariae. The cats died 20 days after exposure to cercariae due to some food toxicity. The dog, mongooses and birds were autopsied 10 to 12 weeks later, no any O.turkestanicum worm were detected. These animals were therefor considered as insusceptible to infection with O.turkestan- (Table, 19).

DISCUSSION,

It has been known since the earlier investigations on schistosomiasis that many domestic and wild animals are readily susceptible to infection with schistosomes infecting man and such animals serve as maintenance hosts and specially for S.japonicum and so enhance transmission to man (see Review by Nelson 1969 and 1971). A similar situation occur with schistosomes of veteri- importance, for example S.mattheei and S.bovis in Africa of which may antelopes are natural hosts (Le Roux, 1930).

The experimental infections reported here show that Tatera indica is a moderately susceptible host for O.turkestanicum as shown by number of viable eggs in the tissues and faeces. Witenberg and Longy (1966) reported O.turkestanicum from an

/unidentified....

unidentified field rat naturally infected in south Turkey in 1930 (preserved material) and Arfaa et al (1965) recorded that Tatera indica was susceptible to infection producing viable eggs with a prepatent period of 80-82 days. It is unlikely that this animal is a true mammalian host in nature in Khuzestan. MacHattie (1936) reported the developing of O.turkestanicum in rabbit, guinea pig and white mouse but only a few pairs of immature worms were produced with no eggs in tissues or faeces. Azimov et al(1968) also reported that O.turkestanicum from Amu Darya in Uzbekstan were developed to maturity in the rabbit.

The laboratory maintenance of O.turkestanicum in Tatera indica proved rather difficult. This wild rodent does not breed easily in laboratory and stocks had to be provided from animals trapped in the field. It proved easier to maintain the parasite on calves or sheep.

The results of the survey of mammals in Khuzestan and the laboratory studies suggest that the wild game play little part in maintaining this infection in nature and that the only significant hosts are the domestic ruminants.

Table, 18

The result of exposing various species of rodents to cercariae of O. *turkestanicum*.

Host	No. of animals	No. of cercariae		Mean worm recovery				Mean tissue egg counts			average prepatent period
		Range	Mean	F.	M.	Total	% Recovery	Liver	Gut	Total	
Albino mice	15	50-400	300	43	37	80	26.9	3300	100	3400	-
Hamster	11	50-200	125	26	16	42	34.2	180	3500	3680	-
Mastomys natalensis	10	100-400	240	62	37	62	25.8	-	-	-	-
Rabbit	6	200-1500	750	32	125	157	21.0	24700	25600	50300	-
Tatera indica	10	200-1000	600	27	63	90	15.1	26400	4960	31360	62
Nesokia indica	7	200-1000	600	42	51	98	16.4	11400	5000	16400	-
Rattus rattus	6	200-1000	670	13	11	24	3.7	0	0	0	-
Mus musculus	8	100-400	250	3	2	5	1.8	0	0	0	-

Table, 19

The results of exposing miscellaneous animals and birds to the cercariae of <u>O.turkestanicum</u>

Host	No. exposed	Cercariae		Duration of infection (weeks)	Worms recovery and tissue egg counts
		Range	Mean		
Dog	1	5000	5000	10	0
Cat	2	1000-2000	1500	3	0
Mongoose	3	500-1500	1000	12	0
Duck	6	500-3000	1000	10	0
Chicken	1	3000	3000	12	0

CHAPTER 5

THE PATHOLOGY OF NATURALLY AND EXPERIMENTALLY INFECTED RUMINANTS

INTRODUCTION,

The present study was undertaken to assess the pathological consequences of *O.turkestanicum* infection in experimentally infected calves, sheep and goats. Observations were also made on naturally infected animals including 21 cattle and 12 sheep heavily infected with *O.turkestanicum* collected from Dezful abattoir.

RESULTS,

PATHOLOGY OF EXPERIMENTAL INFECTIONS.

CALVES,

Ante-mortem examination,

The clinical conditions observed in the 12 calves experimentally infected with *O.turkestanicum* could be divided into 3 stages, the first stage characterised by restlessness and emaciation at the early prepatent period; the second stage mostly diarrhoea with foetid faeces, harsh hair coat and hollow appearance

/ of the ....

of the abdomen; the third stage was the recovery stage in which the acute manifestations disappeared and the animals showed signs of recovery. In comparision with S.bovis infection, the acute stage in O.turkestanicum infection was very short, mild and most of the calves recovered, but the emaciation persisted.

Post-mortem examination,

There were slight serous effusions in the abdominal cavity, thorax and some degree of hydropericardium. Lymph nodes were sightly enlarged and pigmented. The liver showed no obvious changes with no evidence of any minute greyish granolumata seen on the surface in O.turkestanicum infections. The density of O.turkestanicum eggs per gram of liver was very low in comparision with S.bovis. The mesenteric blood vessels in the duodenum were the most favourable site for adult worms and showed some degree of dilatation. The duodenum slightly swollen and some red focal lesions were observed in the mucosa. The charactristic eggs of O.turkestanicum were readily demonstrated in mucosal scrapings of the affected bowel (Plate, 3).

Microscopic examination,

O.turkestanicum produced no severe pathology in the calves liver. In microscopic preparations there was some cellular

/infilteration....

infiltration in the intrahepatic spaces in early stages particularly eosinophil infiltration.

In the intestinal tract, the eggs were found chiefly in the mucous membrane and occasionally in the lumen, but not in the submucosa as charactristic of S.bovis infections (Plates, 4, 5). Many of the eggs in the intestinal mucousa showed peculiar degeneration. They were distended and contained dead miracidia and were surrounded by lymphoid cells (Plate, 6). In general the O.turkestanicum infection in calves was much less serious than in S.bovis infection. It seems O.turkestanicum is better adapted to cattle than any of the other bovine schistosomes.

SHEEP

Ante-mortem examination.

Sheep were more affected by O.turkestanicum than calves and the clinical manifestations were quite obvious with loss of wool, lack of appetite, progressive emaciation, mucoid faeces and occasionally haemorrhagic diarrhoea. In spite of a good quality diet the weakness and loss of weight was pronounced. In some cases mild pneumonia with dry cough was obvious.

/Post-mortem....

Post-mortem examination,

In post-mortem examination there was a small amount of serous effusion in the abdominal cavity but considerable hydrothorax. Lymph nodes in general showed enlargement. The liver was chiefly affected with numerous minute greyish granolumata on the surface of liver under Glisson's capsule, but there were no lymphoid nodules in liver such as were seen frequently in S.bovis infections. Large numbers of adult worms were readily seen in the mesenteric and portal veins by holding up loops of intestine to the light. The mesentry and portal veins showed considerable dilatation. The intestinal wall was friable and easily damaged on examination and in some cases the duodenum was a dark-grey colour with haemorrhagic foci in the mucousa.

Microscopic examination,

Frequent deposition of pigment and occasional thrombo-phlebitis was noted in the liver; there was also cellular infiltration in the intralobular spaces and in the neighbourhood of Glisson's capsule. The eggs arrested in the liver and intestine gave rise to a well-defined nodules with numerous eosinophils and lymphoid cells infiltration but the reactions were not so marked

/and serious....

and serious as seen in *S.bovis* infections.

In the intestine the eggs were mostly found in the mucous membrane with cellular reaction and heavy eosinophils infiltration between the villi. Thickening and nodule formation in the intestinal wall particularly in the duodenum was pronounced.

GOATS

Ante-mortem examination,

Goats showed better tolerance to *O.turkestanicum* infection than sheep, and the percentage of worms recovery was lower. Clinical manifestations started with restlessness, emaciation, coated faeces and occasional diarrhoea but the general condition of the goats was much better than in the sheep.

Post-mortem examination,

Thoracic, hepatic and mesenteric lymph nodes were slightly enlarged. The worms were mostly in the duodenal venules. The liver showed numerous minute greyish granolumata, the gall bladder was distended. there was no sign of lymphoid nodule formation.

/Microscopic....

Microscopic examination,

The liver of the goats showed well defined granolumata with cellular infiltration with degenerated eggs in the centre in intralobular spaces. The most striking picture in goats was the Hoeplli phenomenon with antigen-antibody deposition around the eggs producing acidophil rosette-formation. The eggs were prevalent in the mucous membrane of the duodenum with some cellular infiltration.

PATHOLOGY IN NATURAL INFECTIONS.

Observations on 21 cattle and 12 sheep heavily infected with O.turkestanicum showed that naturally infected cattle harboured more parasites than sheep and goats, but the pathological changes in the internal organs were more severe in sheep than in cattle.

CATTLE,

The clinical condition of the naturally infected cattle was poor with harsh hair coat and emaciation. In most cases

/O.turkestanicum....

<u>O.turkestanicum</u> was associated with heavy <u>Fasciola gigantica</u> infection, so that it was impossible to be sure of the relative importance of <u>O.turkestanicum</u> as a cause of the illness but the slender and narrow shoulders of cattle was characteristic with heavy <u>O.turkestanicum</u> infections.

In post-mortem examination in heavily infected cattle the mesenteric fat-bodies were replaced by oedematous effusions with considerable amounts of ascitie fluid in the abdominal cavity. General hypertrophy and enlargement of the lymph nodes was noticed. The liver was not changed in appearance except for hypertrophy of the hepatic lymph nodes, and distension of the gall bladder. In general the number of worms recovered from the liver was very low and the egg density was also low. Histological examination showed few changes mostly eosinophils infiltration and rare egg particles. The intralobular changes in liver were mixed with fibrous formation due to the fasciolasis. The mesenteric veins were distended and loaded with a large number of adult <u>O.turkestanicum</u>.

/The mucous....

The mucous membranes of the small intestine was seriously damaged with a mass of cellular infiltration and heavy egg deposition. The most interesting picture was the general eosinophils infiltration with dead eggs in intestinal mucousa without defined granoluma formation.

SHEEP,

In spite of the low worms burden in sheep compared with the high worm recovery in cattle, the sheep were more affected than cattle. The progressive emaciation and the paleness of the carcases of sheep suggested *O.turkestanicum* infections even on superficial inspection. In the abdomen there was considerable serous effusion, degeneration of the fat-body and oedematous reactions in the mesentry. Enlargement and pigmentation of the lymph nodes was common in most of the heavily infected sheep, particularly the medial lymph nodes in thorax. Dilatation of the mesenteric veins, tortuous appearance of the intestinal veins and the dark colour of duodenum were conspicuous.

/The liver....

The liver usually showed either mild or moderate pathological changes. Macroscopically, the most outstanding findings were the minute greyish pseudotubercles all over the liver surface and deep in the liver substance. Microscopically these greyish dots contained O.turkestanicum eggs in different stages of disintegration surrounded by a variety of cellular infiltration and granuloma formation. The eosinophils proliferation was pronounced in both liver and intestine. In the intestine the eggs were found chiefly in the mucous membrane and lumen of the duodenum. A striking feature was the intense eosinophil and lymphoid cell infiltration in the villi of the duodenum(Plate, 7). In very heavily infected sheep the intestine was dark in colour with a weak walls which was easily damaged in pulling it away from the mesentery.

DISCUSSION,

Yamagiwa (1931) for the first time described the pathological finding of O.turkestanicum in naturally infected Mongolian cattle and described the acidophil rosette-type reactions around the O.turkestanicum

/ ova a ....

ova, a phenomenon which was later described by Hoeppli (1932) in *S.japonicum* infection. According to Yamagiwa the pathogenicity of *O.turkestanicum* in cattle is much milder than *S.japonicum*. In our investigations we also found that *O.turkestanicum* produced a much milder disease than *S.bovis*. MacHattie (1936) reported serious effects of heavily infestations in sheep and goats with *O.turkestanicum* but he also mentioned the low pathogenicity of this parasite in cattle. Lavrov *et al*(1964, 1967) studied the pathological aspects of *O.turkestanicum* in Uzbekstan ruminants and the pathological changes of affected organs particularly enlargement of the lymph nodes were mentioned.

The conclusion from the present study of the pathology of *O.turkestanicum* is that although cattle are heavily infected, the disease is less severe in cattle than in sheep. Adult cattle are well adapted and can carry heavy loads of the parasite but sheep can be seriously ill with the disease. It is not known to what extend the lesions in the duodenum affect the nutrition of cattle, there is no

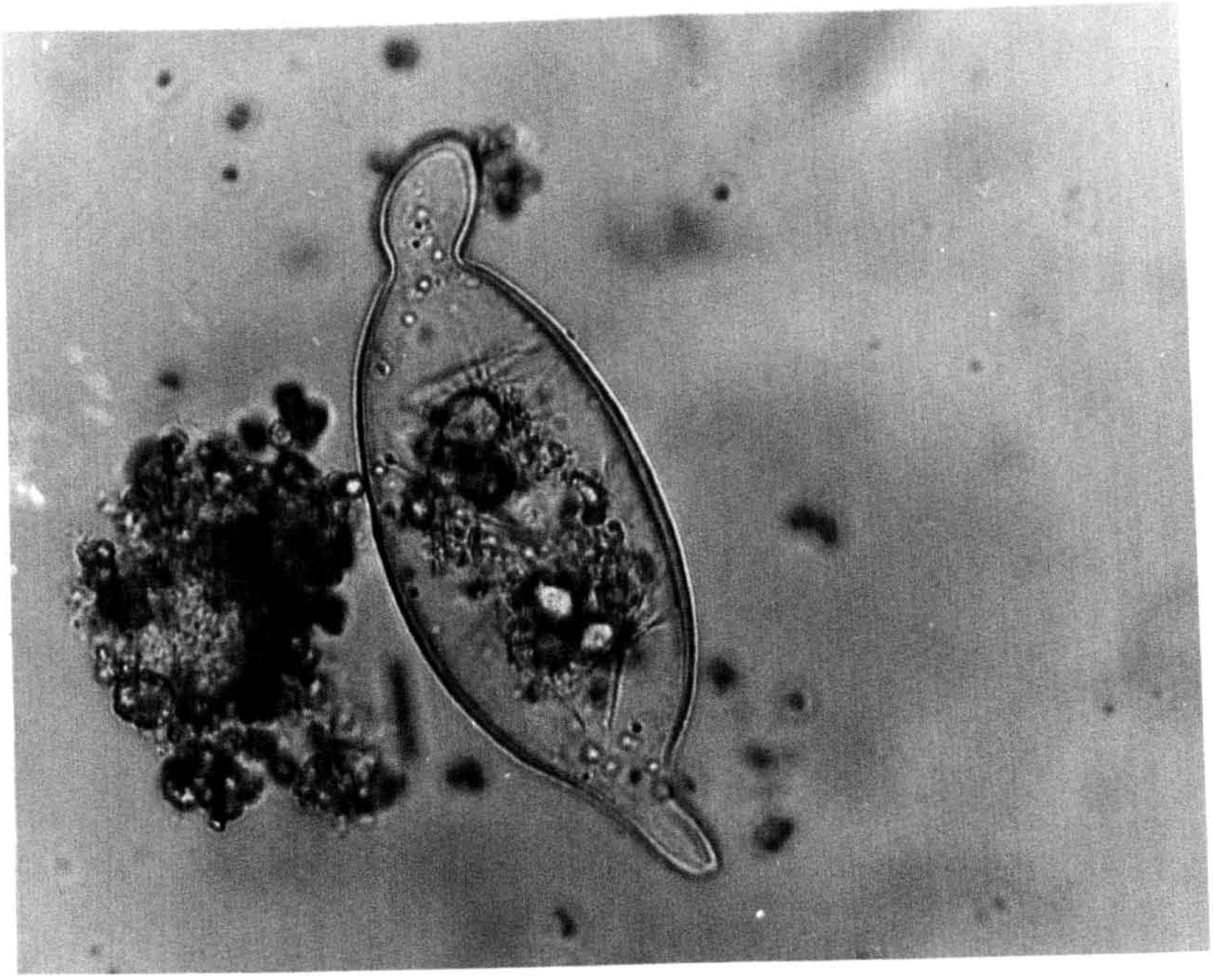
/ doubt....

doubt that O.turkestanicum in sheep is of considerable economic importance with severe losses in meat and wool; the damage in the intestine also makes them useless for processing as sausage skins.

In Iran and Iraq sheep and goats intestines are of considerable economic importance for the country by exporting to other countries for use as casings for sausages and also for some other industrial products. The intestines are examined for their ability to hold water and tested one by one by practiced workers and according to their quality are graded "high " or " low " or discarded entirely. Thus porous intestines known in the trade as " sprinklers " are discarded and intestines showing patchy thichness are of little value since they are not of uniform strength. The west part of Iran (Kermanshah and Kurdestan) produces excellent casings while the south-west part (Khuzestan) show a high proportion of " sprinklers ". MacHattie(1936) also reported a large number of discarded sheep intestines from the southern Iran particularly Basreh area, due to Schistosomiasis. Damages usually cause by deposition of eggs in intestinal walls and tissue reactions.

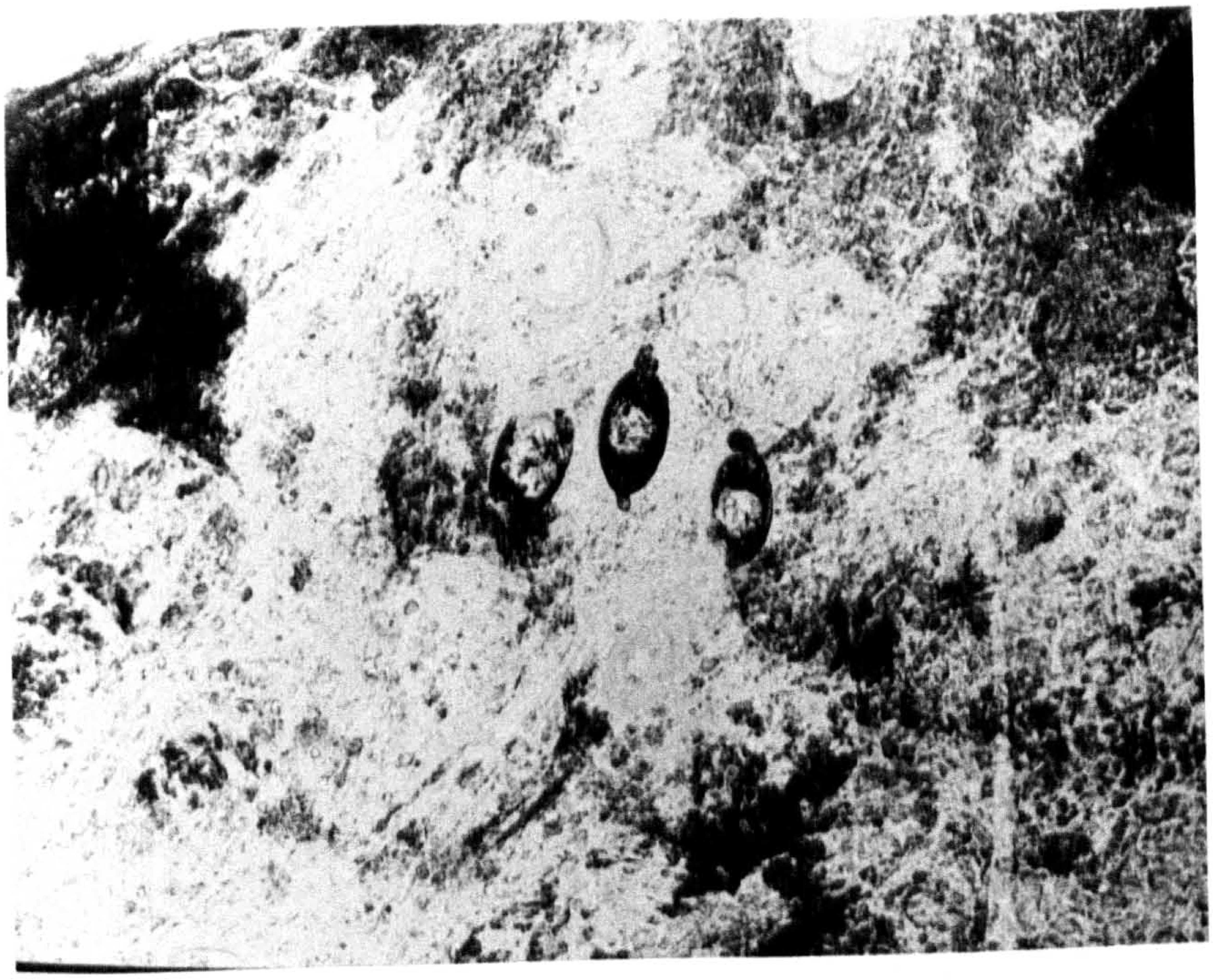
/Prophylactic....

Prophylactic measures against bovine schistosomiasis in the absence of a control program could include: keeping animals off pastures and water courses where surveys had revealed infected snails; providing clean water supply; sending the weak and infected animals to slaughter as soon as possible; keeping young stock and adults separate from the point of view of pastures.



Plate, 2

The egg of Ornithobilharzia turkestanicum



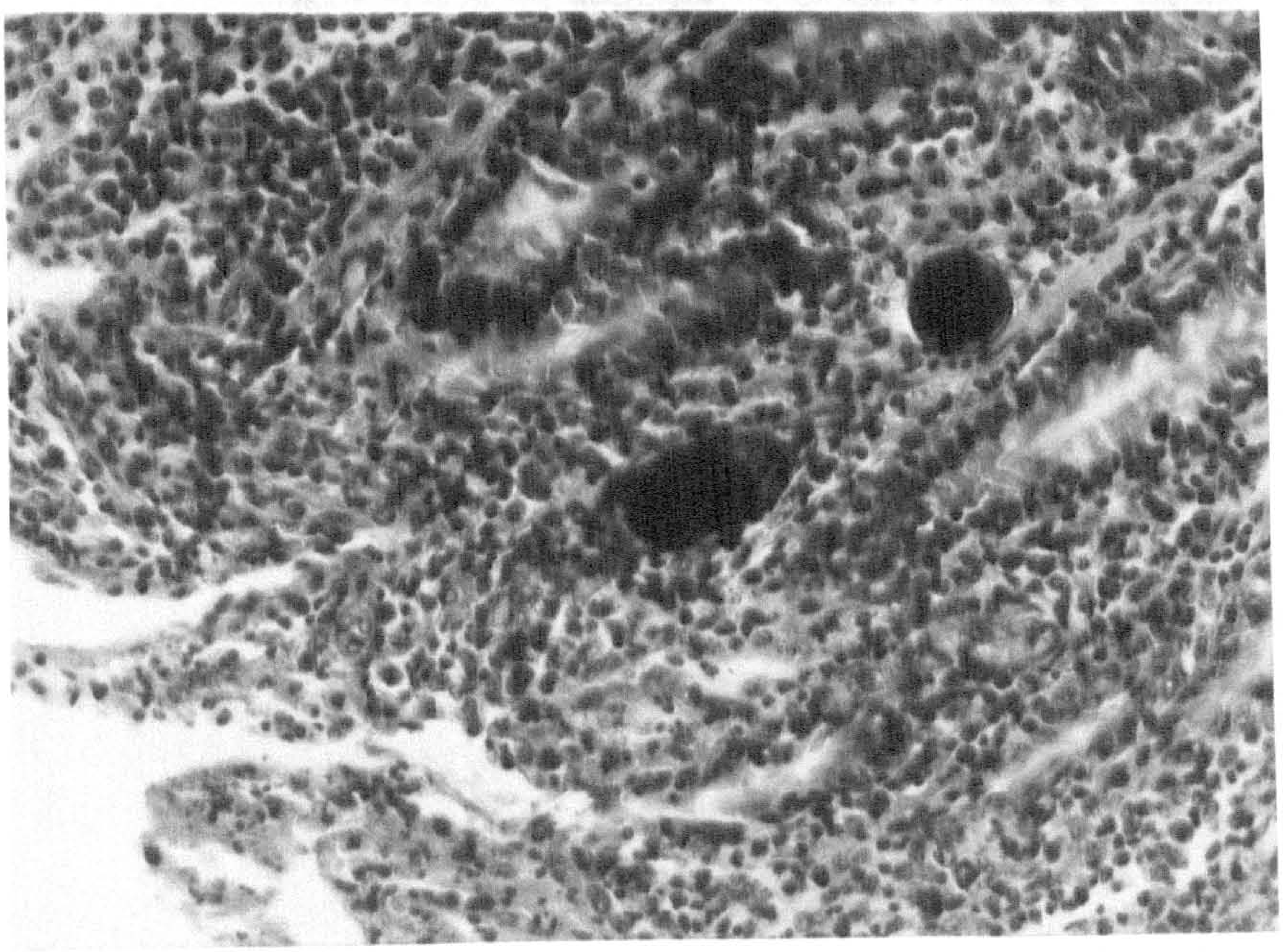
Plate, 3

The eggs of O. turkestanicum in a smear of a small intestine scraping.

Plate, 4

Duodenum of a calf showing numerous ova of O.turkestanicum

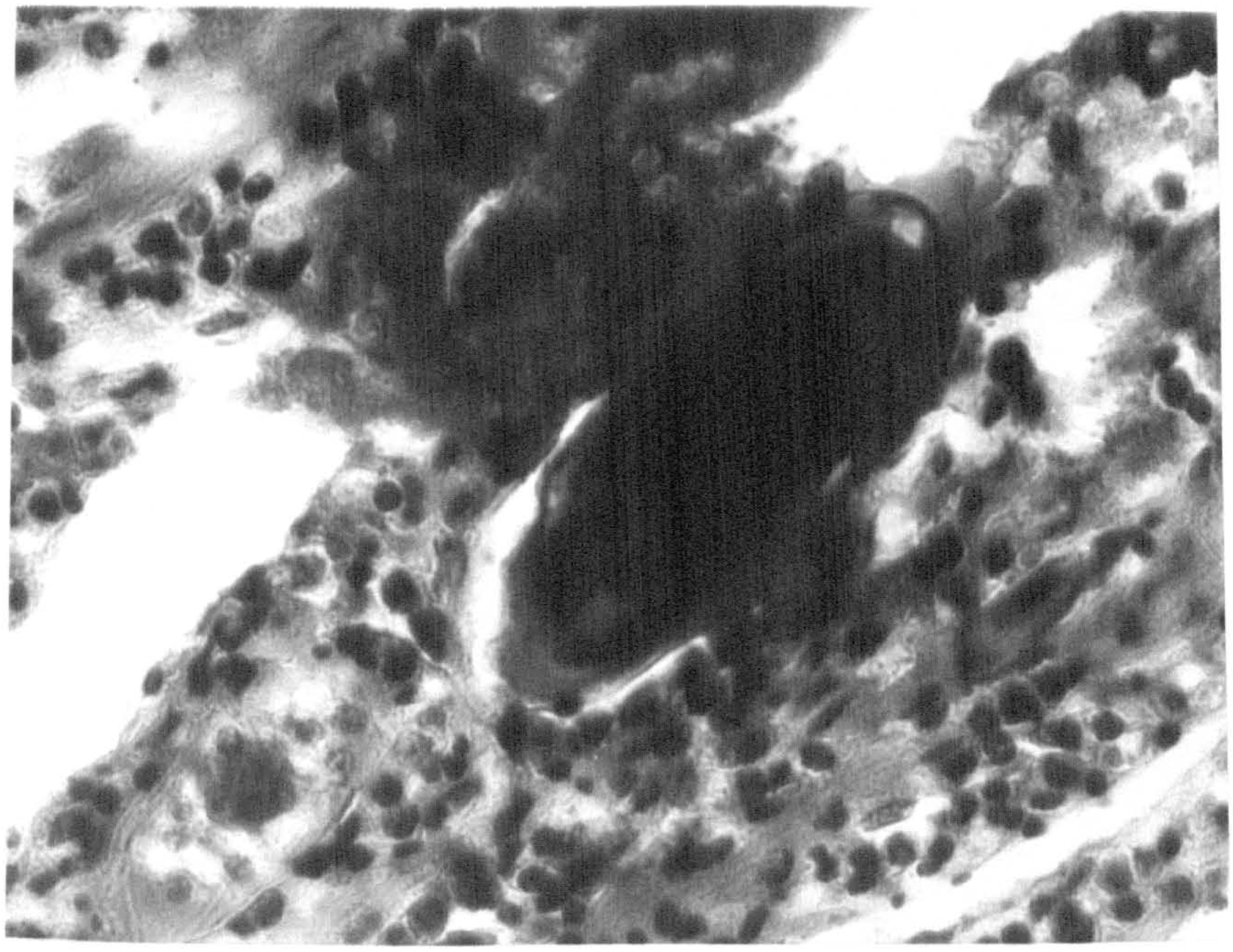
in the mucosa (H.E. 120 x).



Plate, 5

Typical O.turkestanicum eggs in the mucous membrane of the

intestine with diffused cellular infiltration(H. E. x 240).



Plate, 6

Distended egg with dead embryo of O.turkestanicum in intestine with tissue response (H. E. x 560).

Plate, 7

Degeneration of superficial mucosa of intestine. Ova are apparent in the mucosa with dense eosinophil infiltration (natural infected sheep with O.turkestanicum, H. E. x 120).

PART II

PART. II

SCHISTOSOMA BOVIS SONSINO, 1876.

INTRODUCTION,

Schistosoma bovis was first found by Sonsino (1876) in the portal veins of a bull in Zagazing, Egypt. Subsequent records are by: Blane and Desportes (1936) from Morocco; Anderson and Gobert (1934) from Tunisia; Van den berghe (1934) and Werry (1952) from various part of Congo; Dowdeswell (1938) from Kenya; Ugamda, Rhodesia, Angula and Mozambique (W.H.O. tech. series, 139, 1957); Edwards and Wilson (1958) from Ghana; Sobrero (1960) from Somalia; Marill (1961) from Mauritania; Cowper (1963) from Nigeria; Dinnik and Dinnik (1965) from Tanzania and Zambia; Soliaman (1956). Eisa (1963), Malek (1961, 1969) and Hussein (1969) from Sudan.

Outside Africa *S. bovis* exists in several Mediterranean countries including: Sicily (Grassi and Rovelli, 1898); Sardinia (San-Felice and Loi, 1897); Italy (Bertolini, 1908); Corsica (Brumpt, 1930)

/and Spain....

and Spain (Sanchez Botija, 1955). S.bovis is also a common parasite in the Middle-East and has been reported from animals in Iraq by MacHattie and Chawick (1932), MacHattie (1936); Israel by Witenberg (cited by Lengy, 1962) and Iran (Bijan et al,1958; Arfaa,1959; Arfaa et al,1965).

S.bovis is principally a parasite of cattle to which it is well adapted and sheep and goats, occasionally horses, donkeys and camels are definitive hosts of secondary importance. Faulkner and Epstein (1957) and Dinnik and Dinnik (1965) have suggested that African S.bovis originated from the Mediterranean area after A. D. 668 and that the parasite was widely disseminated by migration of domesticated animals with nomadic people into north-east Africa, and gradually to the south via the eastern coast of Africa. On the other hahd S.mattheei is indigenous species to central and south Africa where it is maintained in indigenous wild mammals and snails.

There is a very close morphological resemblance between S.bovis and S.mattheei and this give rise to considerable discussion

/ on their....

on their validity as separate species (MacHattie and Chadwick, 1932; Van den berghe, 1937; Alves, 1949; Pitchford, 1965; Dinnik and Dinnik, 1965).

The original morphological description of S.bovis by Khlil (1924) was largely modified by MacHattie and Chadwick (1932) after examination of over 4,000 specimens of S.bovis from different species of domestic animals in Iraq. Detail accounts of the morphology of S.bovis were also made by Lengy (1962b) in Israel and Dinnik and Dinnik (1965) from the Sudan.

S.bovis is very infective to cattle, sheep and goats. It has also been found in pigs (Malek, 1961); camels (Solaiman, 1956); antelopes (Van den berghe, 1937) and the rodent Lophuromys flavopunctus in Kenya (Nelson et al, 1962).

Experimental infection with S.bovis has been reported in various species of small laboratory animals, calves, sheep, a cat and

/donkey....

donkey (Brumpt 1930; Malek 1961,1969; Lengy 1962 a ; Hussein 1969). The most susceptible laboratory animals for *S.bovis* as reported by Lengy (1962 a) was the hamster. Lurie and DeMeillon (1956) reported that *Mastomys natalensis* was a good experimental host for *S.bovis*. A gerbil *Tatera indica* was reported to be a good experimental host for this parasite in Iran (Arfaa *et al*, 1965).

The objective of the present work was to study the behaviour of this parasite in different type of ruminants in parallel with the observations on *O.turkestanicum* (see Part,I). The immunological interactions of *S.bovis* with the other schistosomes occuring in the area have also been studied (see Part, III). Observations on *Bulinus truncatus* the snail "vector" of *S.bovis* and *S.haematobium* in Khuzestan have been reported elsewhere (see Chu, Massoud and Arfaa, 1968).

CHAPTER, 1

STUDIES ON THE PREVALENCE AND INTENSITY OF S.BOVIS IN DOMESTIC ANIMALS IN KHUZESTAN.

INTRODUCTION,

Bijan et al (1958) was the first to report this parasite in Khuzestan(Iran). The intermediate host of this parasite is Bulinus truncatus in Iran and the same snail is the intermediate host for S.haematobium . The study of this parasite in ruminants was considered worthwhile because of its economic and zoonotic significance. Infection of man with S.bovis seems rather unlikely but eggs resembling S.bovis have been found in man (Raper 1951, Kisner et al 1953, Solaiman 1956, Malek 1961, Blair 1966, McMahon 1969).

A study on S.bovis infection in animals in Khuzestan was carried out by Arfaa et al (1965) and revealed that 20.8 % of cattle and 14.0 % of sheep were infected with this parasite. This data were

/based on ....

based on the examination of mesenteric veins of animal visceras slaughtered in Dezful abattoir from 1962-1964 which was before the antibilharzia snail control measures against B.truncatus were undertaken.

In the present study the same technique were used in the same area in large number of animals from 1969-1970 approximately 5-6 years after extensive snail control measures were initiated (see Massoud et al, 1969).

RESULTS AND CONCLUSIONS,

Observations on 632 cattle, 607 sheep and 89 goats showed a dramatic reduction in the S.bovis infection rate in cattle from 20.8 % in 1964 to 0.8 % in 1970. The reduction in sheep was from 14.0 % in 1964 to nil in 1970 (see Table, 31). Only 5 cattle were found infected and the infection were very light and were mostly mixed infections with O.turkestanicum.

These results show that the prevalence of bovine schistosomiasis can be used as an index of the effectiveness of control measures against human schistosomiasis, particularly in

/an endemic....

an endemic area like Khuzestan where the intermediate host of S.haematobium (the only ' human ' schistosome) and S.bovis is the same (B.truncatus). The life-span of ruminants usually does not exceed more than 7-10 years and during this period if proper control measures against molluscan host are achieved the sharp reduction of infection rate among ruminants indicates that snail destruction and interruption of transmission in the area has been successful.

Our data collected from 1969-1970 on S.bovis after long snail control measures (Massoud et al,1969) compared with data collected by Arfaa et al(1965) from 1962-1964 before snail control measures in the same area on S.bovis shows a striking reduction in infection rate of S.bovis among ruminants (Table, 31).

The data on human urinary schistosomiasis in the same area of Khusestan before and after snail control measure and chemotherapy shows a considerable reduction of infection rate in human population from average 38 % (Bilharziasis Pilot Project Report,1964) to under 10 % (Arfaa et al,1970). The incidence studies on 606 negative children from 1966 showed only 3.5 % infected in 1968 (Arfaa et al 1970) most of the infected cases coming from the southern of Study Area. These results indicates that compaign, against the snail intermediate host are effective for the control of human and animal schistosomiasis.

Table, 31

Comparing the infection rate of S.bovis in cattle and sheep before and after snail control measures in Khuzestan

Type of animal	1962-1964			1969-1970		
	No. examined	No. infected	infection rate %	No. examined	No. infected	infection rate %
Cattle	250	52	20.8	632	5	0.8
Sheep	71	10	14.0	607	0	0.0

CHAPTER, 2

PARASITOLOGICAL OBSERVATIONS ON EXPERIMENTALLY INFECTED LARGE ANIMALS

The objective of this study was to ilustrate the behaviour of this parasite in the various ruminants in Khusestan. The animals used for this study were 7 calves, 5 sheep, 2 goats and one buffalo-calf, 7-9 months old. All the animals were exposed to 5,000 cercariae of S.bovis and were autopsied at 9 weeks or 18 weeks after exposure. The faecal examination were begun 35 days after exposure to cercariae.

RESULTS,

Prepatent Periods.

The prepatent period of S.bovis in calves was shorter than in sheep and goats (range 44-45 days, mean 44.6 ± 0.2 in calves; range 47-50 days, mean 48.6 ± 0.6 days in sheep; range 47-48 days, mean 47.5 days in goats (see Table, 20). The differences between the means of the prepatent periods in calves and sheep were statistically significant $P < 0.001$.

/ Infectivity....

Infectivity.

As can be seen in Tables, 21-23 the mean number of worms recovered from calves varied from 2693-3562 (mean 3104 $\pm$ 146) this represents a recovery rate of 62.0 % . In sheep the worm recovery varied from 1624-2496 (mean 2072 $\pm$ 193), representing a recovery rate of 41.0 % . The corresponding figures for the goats were 3077-3656, (mean 3366 $\pm$ 289), recovery rate of 67.0 % . Slightly more male worms were recovered than female, though the differences were less than those observed in O.turkestanicum infections (see Part, I).

Distribution of adult worms.

Most of the worms were recovered from the mesenteric veins (Table, 24). In calves the mean number of worms collected from the hepatic veins was 440 $\pm$ 158 and from the mesenteric veins 2664 $\pm$167 (total 14 % in liver); in sheep 158$\pm$72 worms were from the liver and 2072$\pm$193 from the mesenteric veins (total 7.6 % in liver); in goats 857 worms were from the liver and 2509 from the mesenteric veins (total 24 % in liver).

/ The distribution...

The distribution of worms in the mesenteric veins was entirely different from O.turkestanicum; worms were all evenly distributed in veins down the large and small intestine in S.bovis whereas they were all in veins of the small intestine (superior mesenteric veins) in O.turkestanicum infections (see Part, I).

Faecal egg counts.

The daily egg output of S.bovis are given in Table, 20. The number of eggs per gram of faeces per day varied from 40-135(75 ± 15) in calves, from 41-92 (60 ± 8) in sheep and from 75-76 in goats.

The number of eggs per female passed per day in the faeces was estimated as described for O.turkestanicum eggs. The number of eggs in calves declined from 106 eggs per day per female worms at 63 days after exposure to 67 at 126 days after exposure. In contrast in sheep this number increased from 52 eggs per female per day at 63 days after exposure to 112 eggs per day at 126 days after exposure (see Table, 25).

/ Distribution...

Distribution of eggs in the visceral organs.

The pattern of egg distribution of S.bovis differed from O.turkestanicum eggs in the same hosts (see Tables 26-28). Egg densities per gram of tissues had a fairly uniform distribution in the small and large intestine. The total mean number of eggs per gram of tissue was highest in sheep and goats (6466 ± 610, 7807 ± 215) and least in calves (3143 ± 383). The percentage of egg deposition in the liver, small intestine and large intestine was 23.8, 38.5, 37.7 in calves; 50.3, 23.9 and 25.8 in sheep; and 22.2, 45.2 and 32.6 in goats (Table, 24).

The most striking picture was the increasing number of eggs per gram of tissue in sheep with increasing duration of infection, but in contrast there was a decline in egg densities with increased duration of infection in calves (Table, 29). This suggests that there is a partial immune response in calves, but no response in sheep.

/The pathological...

The pathological manifestations particularly in the liver of sheep and goats also became more severe with increased duration of the infection.

A statistical analysis of the egg densities of *S.bovis* in different parts of alimentary tract in calves and sheep can be seen in Table, 30. The differences between egg densities in various organs at 9 weeks were significant only in the liver and ileum, but 18 weeks after exposure the results were striking and the differences in egg densities were highly significant in all parts of alimentary canal. This is illustrated in the Table, 30 and Fig. 4 and 5 .

Digestion of other organs including the lung, spleen, rumen, reticulum, omasum and abomasum showed that only the abomasum was affected but from pathological point of view the densities were insignificant.

The exposure of one buffalo-calf to 5,000 cercariae of *S.bovis* produced no traces of any schistosome infection at autopsy 9 weeks after exposure to cercariae.

/ Discussion....

DISCUSSION,

It is generally accepted that the normal mode of entry of S.bovis cercariae into the hosts body is via the precutaneous route. Lengy (1962 b) infected sheep successfully with this parasite by immersing the legs in contaminated water for 2 hours; McCully and Kruger (1969) similarly reported a satisfactory worm return of S. mattheei by infecting the sheep by immersing one of the front legs into the cercarial suspension in a large glass jar for 30 minutes. Malek(1969) used a glass ring with S.bovis cercarial suspension puting on the shaven skin of ruminants. Saeed and Nelson(1969) used partial tail immersion in calves with S.mattheei and S.mansoni cercariae. None of these workers produced such a high worm return as in the present study (67.3 % in goats, 62.1 % in calves and 41.4 % in sheep). The method used was a modification of Kruger's method, the cercariae being suspended in a polythene bag which was fitted to one of the front legs of animal for 45 minutes. This method has proved to be a convenient and satisfactory method for infecting large mammals and could be adopted for laboratory studies with other animals such as monkeys.

/ The duration....

The duration of the prepatent period in schistosomes has been used as a criteria for differenciating schistosomes species (Schwetz, 1951, 1954); and also to differenciate geographic strains Hsu and Hsu(1954); Saoud (1966); Nelson _et al_(1968).

Hussein (1969) reported that the prepatent period in calves experimentally infected with _S.bovis_ averaged 48 days. Malek(1969) reported that the prepatent period in the Sudan strain of _S.bovis_ in calf, sheep and goats were 60, 51 and 65 days respectively, which was considerably longer than our findings with Iranian strain of _S.bovis_ in the same hosts (44 days in calves, 48 days in sheep and 47 days in goats).

The results show that the prepatent period is longer in sheep and goats than in calves. The lower number of egg densities and shorter prepatent period in calves indicate that _S.bovis_ is well adapted to the cow which is probably the main host for the maintanence of _S.bovis_ in nature. Sheep and goats are probably of secondary

/importance....

importance as definitive hosts and are more seriously affected by *S.bovis* than cattle.

The experimental exposure of the buffalo to *S.bovis* and our epidemiological data on 96 buffaloes slaughtered from 1969-1970 and 65 buffaloes slaughtered from 1962-1964(Arfaa *et al*,1965) in Khuzestan suggest that this animal is naturally resisted to *S.bovis* infection. Faust (1924) noted that the water buffalo wades in area infected with cercariae of *S.japonicum* without developing infection and he suggested that the thick skin of these animals might explain their apparent immunity. But Fairley(1930) showed that buffaloes can be infected precutaneously with the cercariae of *S.spidale* . In the buffalo-calf exposure to *O.turkestanicum* cercariae by leg immersion technique 9.6 % of the worms developed (see Part, I). These observations conclude that the buffalo is naturally resistant to *S.bovis* and *S.japonicum* but not to *S.spidale* or *O.turkestanicum*.

The number of male worms in the *S.bovis* infections slightly outnumbered the females. The recovery of *S.bovis* was much higher that with *O.turkestanicum* infections in cattle, sheep and goats. This may probably explain the more wide-spread distribution of *S.bovis* although the distribution of the snail host is also more wide-spread.

/The most....

The most interesting finding in the faecal egg counts was the declining number of eggs per female per day with prolonged duration of infection in calves and in contrast to the increasing egg output in sheep.

Malek(1969) found that in S.bovis the number of eggs per gram of faeces in sheep was much higher than cattle, which coincided with our finding with longer infections(18 weeks). McCully et al(1969) reported the same decline of egg output in faeces of oxen and sheep experimentally infected with S.mattheei.

Distribution of eggs in liver, small and large intestine was more or less uniform in each species of animals. But the egg densities per gram of tissues in sheep and goats were much higher than in calves: in particular the liver in sheep and goats were more affected by eggs. The density of eggs again dramatically increased in sheep with longer duration of infection and in contrast declined in calves possibly due to acquired resistance in the calves. The worms in the calves appeared to produce many fewer eggs than those in sheep and goats.

Fig. 4

Histogram showing declining egg densities of S.bovis in calves per gram of tissues by duration of infection.

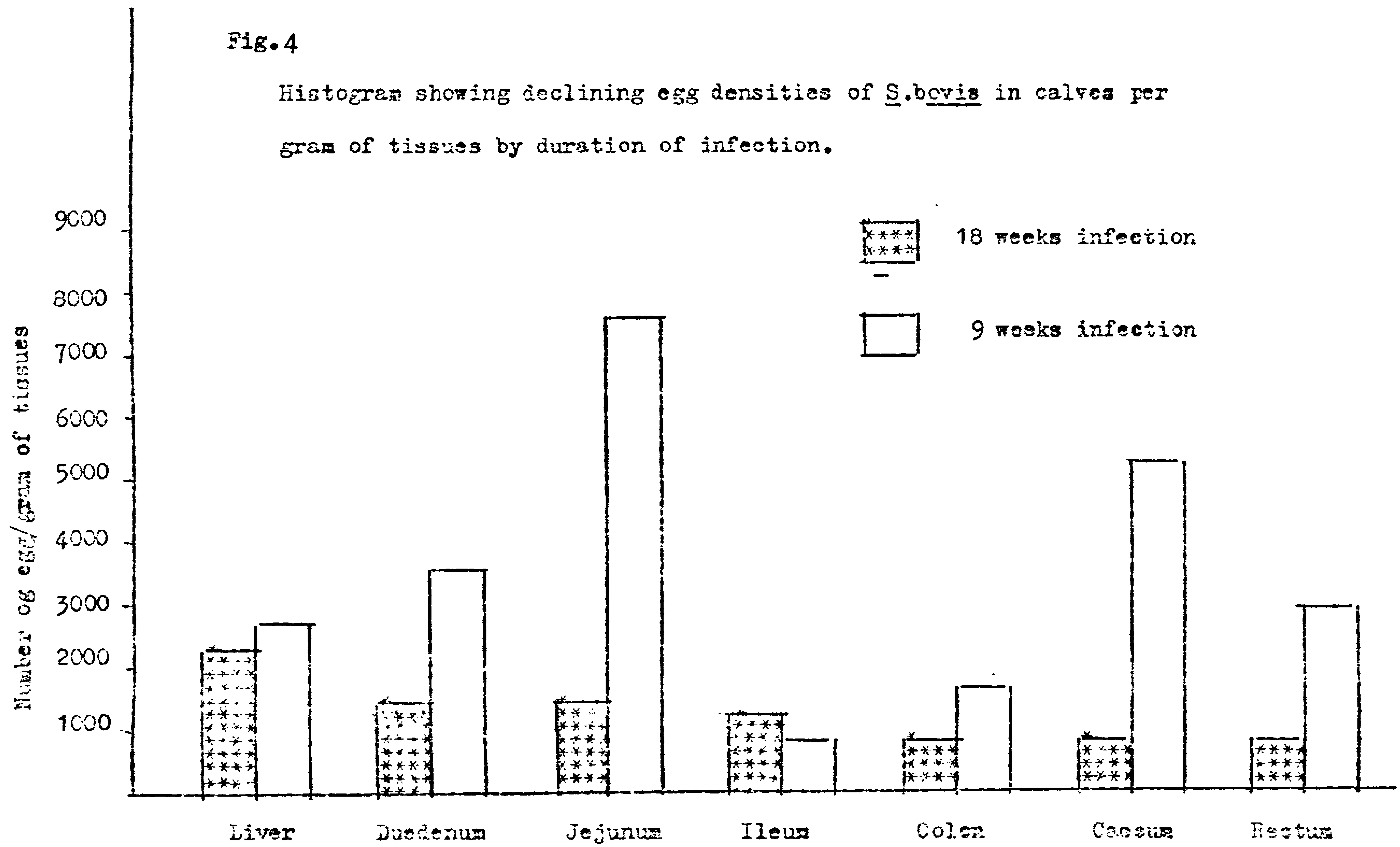
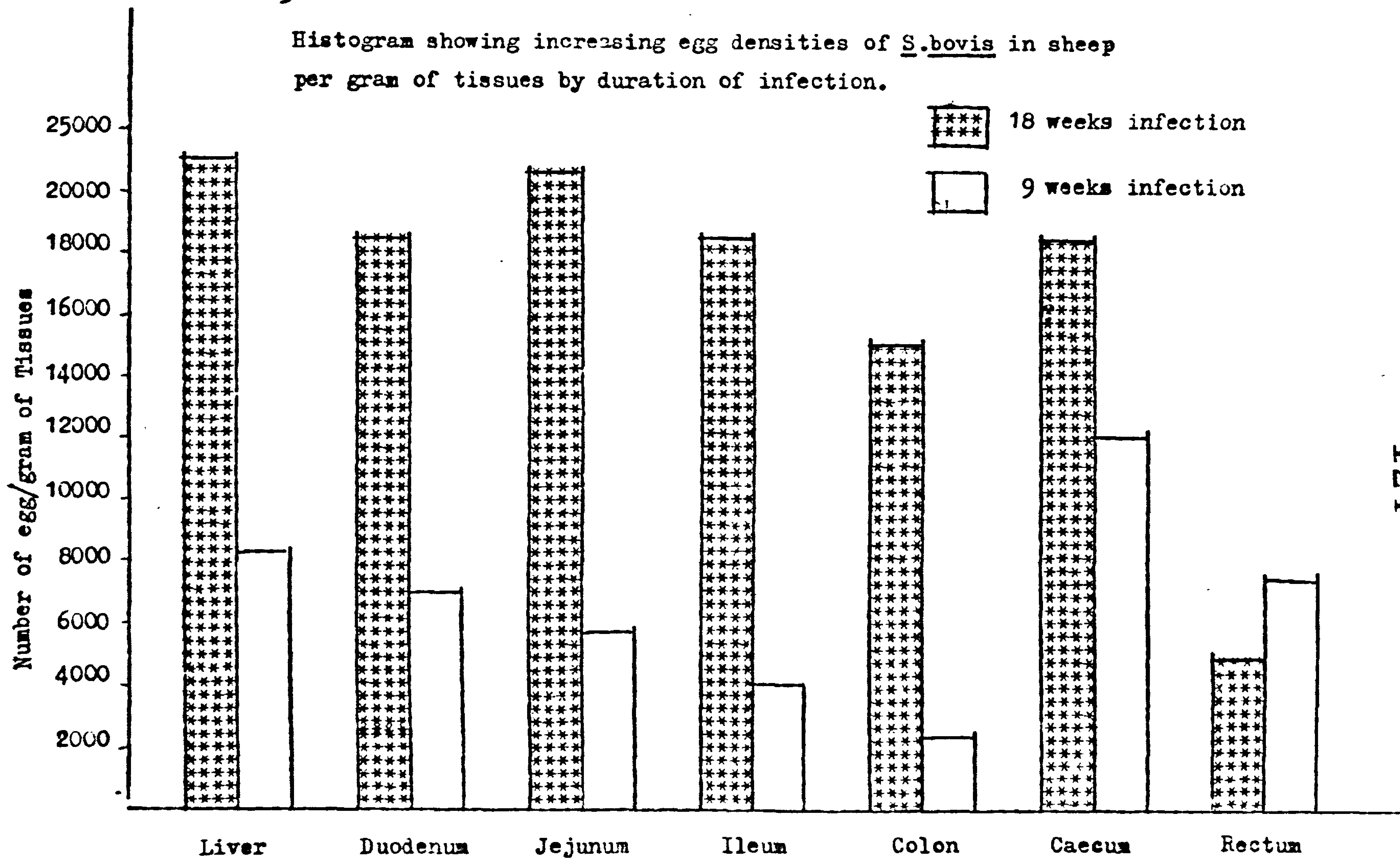


Fig.5

Histogram showing increasing egg densities of S.bovis in sheep per gram of tissues by duration of infection.



Table, 20

Parasitological behaviour of S.bovis in Calves, Sheep and Goats.

Type of animal	No. of animals	No. of cercariae	Total worms recovered	Prepatent period (days) ± S.E.	Eggs per gram of faeces per day (63 days after exposure)
Calves	1	5,000	2783	45	64
	2	"	3377	45	135
	3	"	3562	44	66
	4	"	3401	45	40
	5	"	2693	44	70
Mean		5,000	3163 (177)	44.6 (0.2)	75 (15.8)
Sheep	1	5,000	1940	48	57
	2	"	2496	50	92
	3	"	1806	47	41
	4	"	2296	48	62
	5	"	1689	50	48
Mean		5,000	2045 (151.8)	48.6 (0.6)	60 (8.7)
Goats	1	5,000	3656	47	76
	2	"	3077	48	75
Mean		5,000	3366	47.5	75.5

S.E Standard of Error

Table, 21

The distribution of adult worms of S.bovis in experimentally infected

Calves.

No.	No. of cercariae	Duration of inf. (weeks)	Liver			Mesenteric veins			Total	Recovery rate %
			F.	M.	Total	F.	M.	Total		
1	5,000	9	20	112	132	1230	1421	2651	2783	55.6
2	"	9	160	128	288	1501	1741	3242	3530	70.7
3	"	9	185	155	340	1158	1203	2361	2701	54.0
4	"	9	63	245	308	1317	1460	2777	3085	61.7
5	"	18	590	781	1371	947	1059	2006	3377	67.5
6	"	18	122	267	389	1419	1754	3173	3562	71.2
7	"	18	175	76	251	1222	1220	2442	2693	53.8
Mean (S.E.)			188 (70)	252 (92)	440 (158)	1256 (68)	1408 (101)	2664 (167)	3104 (146)	62.1% (2.9)

Table, 22

The distribution of adults worms of <u>S.bovis</u> in experimentally infected Sheep.

No.	No. of cercariae	Duration of inf. (weeks)	Liver			Mesenteric veins			Total	Recovery rate %
			F.	M.	Total	F.	M.	Total		
1	5,000	9	95	130	225	1096	1175	2271	2496	50.0
2	"	9	8	9	17	889	900	1789	1806	56.1
3	"	18	145	182	327	941	1028	1969	2296	45.9
4	"	18	41	24	65	774	850	1624	1689	33.7
Mean (S.E.)			72 (30)	86 (41)	158 (72)	925 (66)	988 (72)	1913 (138)	2072 (193)	41.4 (4)

Table, 23

The distribution of adult worms of <u>S.bovis</u> in experimentally infected

Goats

No.	No. of cercariae	Duration of inf. (weeks)	Liver			Mesenteric veins			Total	Recovery rate %
			F.	M.	Total	F.	M.	Total		
1	5,000	9	525	700	1225	1170	1261	2431	3656	73.1
2	"	9	195	295	490	1253	1334	2587	3077	61.5
Mean			360	497	857	1211	1297	2509	3366	67.3

Table, 24

Percentage distribution of adults and eggs of S.bovis in differeht organs of Calves, Sheep and Goats experimentally infected.

Type of animal	Egg distribution			Worm distribution	
	Liver	Small intestine	Large intestine	Portal veins	Mesenteric veins
Calves	23.8	38.5	37.7	14.1	85.9
Sheep	50.3	23.9	25.8	7.6	92.4
Goats	22.2	45.2	32.6	25.5	75.5

Table, 25

Egg production of S.bovis in large animals as estimated by faecal egg counts during the period of observations

Type of animals	No. of animals	Days post infection	Mean no. female worms	Mean no of eggs per gram of faeces per day	Mean no of egg per female per day in faeces *
Calves	[illegible]	63	1408	75	106
	3	126	1491	50	67
Sheep	2	63	1044	60	57
	2	126	947	180	112
Goats	2	63	1571	76	48

* Based on the over-night faecal output.

Table, 26

The dist-ribution of S.bovis eggs in tissues of Calves in infections of different duration

Duration of inf. (weeks)	No. of female worms	Liver per gram				Small intestine per gram				Large intestine per gram			
		R.Lobe	L.Lobe	PT.	Mean	Duodenum	Jejunum	Ileum	Mean	Caecum	Colon	Rectum	Mean
9	1250	3055	4545	3192	3597	250	4800	230	1760	8410	6400	7125	7311
	1661	2157	3280	2231	2550	4704	6704	200	3869	5550	1188	660	2466
	1343	600	1140	920	886	3500	7650	1100	4083	3500	1050	2500	2350
	1380	2070	2020	2150	2080	5750	9000	250	5000	3150	1000	1500	1883
Mean (S.E.)	1408	1970 (508)	2746 (743)	2123 (465)	2278 (562)	3551 (1192)	7038 (882)	445 (218)	3678 (684)	5152 (1207)	2409 (1330)	2946 (1442)	3502 (1257)
18	1537	3310	3130	4150	3530	2470	800	750	1340	1125	1150	1130	1135
	1541	1100	2230	1950	1760	1150	1200	2500	1616	600	560	600	587
	1397	1180	1000	1150	1110	720	3150	710	1526	1130	1300	900	1110
Mean (S.E.)	1491	1863 (723)	2120 (617)	2416 (896)	2133 (723)	1446 (526)	1716 (725)	1320 (590)	1494 (81)	952 (175)	1003 (225)	876 (153)	944 (178)

PT. Portal Tract Area

Table, 27

The distribution of S.bcvis eggs in tissues of Sheep in infections of different duration.

Duration of inf. (weeks)	No. of female worms	Liver per gram				Small intestine oer gram				Large intestine per gram			
		R.Lobe	L.Lobe	PT.	Mean	Duodenum	Jejunum	Ileum	Mean	Caecum	Colon	Rectum	Mean
9	1191	10650	15300	13650	13200	3200	5365	3267	3944	10690	2625	1900	5070
9	897	6000	5800	7145	6315	12300	7180	5700	8393	13630	625	563	4940
Mean	1044	8325	10550	10397	9757	7750	6272	4483	6168	12160	1625	1231	5005
18	1080	16250	16950	15750	16316	18600	23100	26000	23566	26800	1800	5000	11200
18	815	27500	35300	32700	31833	20000	25000	11000	18666	12100	1600	2000	5233
Mean	947	21875	26125	24225	24075	19300	24050	18500	20616	19450	1700	3500	8216

P T. Portal Tract Area

Table, 28

The distribution of S.bovis eggs in tissues of Goats 9 weeks after exposure to cercariae

No. of female worms	Liver per gram				Small intestine per gram				large intestine per gram			
	R.Lobe	L.lobe	PT.	Mean	Duodenum	Jejunum	Ileum	Mean	Caecum	Colon	Rectum	Mean
1695	9500	9200	11000	9900	14000	14600	5900	11500	13200	9400	2900	8500
1448	6300	5100	4500	5300	9450	16000	3700	9716	4840	960	10	1930
Mean (1571)	7900	7150	7750	7600	11725	15300	4800	10608	9020	5180	1450	5216

PT. Portal Tract Area

Table, 29

Recovery of adults and eggs of S.bovis from large animals experimentally infected in the laboratory

Type of animal	No. of animals	Mean no. of cercariae	Duration of infection (weeks)	Mean worm recovery				Mean tissue egg counts per gram		
				F.	M.	Total	% Recovery	Liver	Small intestine	Large intestine
Calves	4	5,000	9	1408	1616	3019	60.4	2278	3678	3502
	3	"	18	1491	1719	3210	64.2	2133	1494	944
Sheep	2	"	9	1044	1107	2151	43.2	9757	4635	5005
	2	"	18	947	1042	1989	39.8	24073	14050	8215
Goats	2	"	9	1571	1794	3365	67.3	7600	10608	5216
Buffalo	1	"	9	0	0	0	0	0	0	0

Table, 30

Comparision of tissue egg densities of <u>S.bovis</u> in calves and sheep.

		Calves			Sheep			
Duration of infection	Different organs	Range	Mean	S.E.	Range	Mean	S.E.	P. (probability)
		N = 5			N = 2			
9 Weeks	Liver	886-3597	2278	562.0	6315-13200	9757	3442.5	0.05
	Duodenum	250-5750	3551	1192.4	3200-12300	7750	4550.0	0.3 NS
	Jejunum	4800-9000	7038	882.4	5365- 7180	6272	907.5	0.2 NS
	Ileum	200-400	445	218.5	3267- 5700	4483	1216.5	0.001
	Caecum	3150-8410	5152	1207.9	10690-13630	12160	1470.0	0.02
	Colon	1000-6400	2409	1330.7	625- 2625	1625	1000.0	0.7 NS
	Rectum	660-7125	2946	1442.7	563- 1900	1231	668.5	0.5 NS
		N = 3			N = 2			
18 Weeks	Liver	1110-3530	2133	723.1	16316-31833	24074	7758.5	0.02
	Duodenum	720-2470	1446	526.5	18600-20000	19300	700.0	0.001
	Jejunum	800-3150	1716	725.9	23100-25000	24050	950.0	0.001
	Ileum	710-2500	1320	590.1	11000-26000	18500	7500.0	0.05
	Caecum	600-1130	951	175.8	12100-26800	19450	7350.0	0.05
	Colon	560-1300	1003	225.8	1600- 1800	1700	100.0	0.1 NS
	Rectum	600-1130	876	153.4	2000- 5000	3500	1500.0	0.1 NS

CHAPTER. 3

THE PATHOLOGY IN RUMINANTS EXPERIMENTALLY INFECTED WITH S.BOVIS WITH A NOTE ON A NATURALLY INFECTED COW

INTRODUCTION.

Very little has been published about the clinical and pathological manifestations of S.bovis in ruminants. The present report is an account of a comparative study on the pathological changes of S.bovis in large animals. The material forming the basis of this study was from different species of animals (calves, sheep and goats) experimentally exposed to 5,000 cercariae of S.bovis. A total of 12 calves, 5 sheep and 2 goats were experimentally infected. A heavily infected cow from Dezful abattoir was also studied.

/ Results....

RESULTS,

PATHOLOGY OF EXPERIMENTAL INFECTIONS,

CALVES,

Ante-mortem examination,

Slightly redness and minute haemorrhagic spots were seen at the exposure site on the legs skin which lasted for 2-3 days. After approximately 45-50 days when the first egg appeared in the faeces some change in the general condition of the animals were noticed; lack of appetite; hollow appearance of the abdomen; faeces becoming mostly mucoid with dark colour with blood and special smell; some times typical haemorrhagic diarrhea; loss of weight in some cases severe emaciation; loss of hair and harsh hair coat; pinched expresion an occasional dry cough. Usually after 2-3 weeks the calves a little passed the acute stage of infection and improved in condition but anaemia, weakness and harsh hair coat remained .

/Post-mortem....

Post-mortem examination,

Most of the calves showed a moderate amount of clear fluid in thorax and abdominal cavities with some degree of hydropreeardium.

Liver: There were many minute greyish foci (granolumata) on the surface of the liver as well as deep in the tissue substance. The Glisson,s capsule of the liver was often thickened particularly along the free edge of the liver. The surface of the liver particularly in 18 weeks old infections showed large yellowish or white spots, firm and quite distinct from the small granolumata(lymphoid nodules). Appearance of the left lobe in calves looked a little greyish with some fibrous streaks on the abdominak surface of liver along the intrahepatic veins. The colour of the liver was in general slightly darker than normal, but less so often in sheep and goats which were heavily pigmented. The main intrahepatic veins looked slightly disdended and contained several thrombi with numerous dead worms trapped in collagenous material.

Other organs: In parts of the small intestine there were slightly elevated red lesions, but the most severe pathology was seen in the

/caecum....

caecum. In most of the calves extensive haemorrhages and wide stripes of brownish pigments were seen in the mucous membrane of the caecum and some times in the rectum. There was also some degree of dilatation of the mesenteric veins which contained numerous adult schistosomes (Plate, 8).

Lymph nodes: The thoracic, portal and mesenteric lymph nodes were mostly enlarged with a dark appearance due to schistosomal pigment.

Microscopic examination,

Liver: The commonest lesions seen in the early stage of the infection (at 9 weeks after exposure) were the schistosome granolumas. There were also extreme perivascular inflamation with oedema and some degree of intralobular fibrosis (Plate, 9). The walls of the hepatic veins were mostly thickend with medial hypertrophy which is a distinct picture only seen in calves infected with schistosomes (Plate, 10).

Schistosome eggs either single or in groups were prevalent in the small intralobular veins producing granoluma with diffused reticulo-

/endothelial....

endothelial reactions which extended into the sinusoids (Plate, 11). The ova in the liver of calves were mostly in the portal areas where they provoked a granoluma and cellular infiltration in the parenchyma.

Ih calves with 18 weeks old infections the livers showed intravascular or extravascular lymphoid nodules with very dense lymphocites and eosinophils.

Other organs: Histological studies of the small and large intestine revealed eggs in all parts, particularly in the submucosa. Granoluma formation with extensive infiltration of lymphoid cell was prevalent in the submucosa engulfing the dead eggs. The caecum in some calves showed a very heavy infiltration and granoluma formation with numerous disintegrating eggs surrounded by different type of lymphoid cells; lymphoid nodule formation was also prevalent (Plates, 12, 13).

SHEEP.

Ante-mortem examination.

The ante-mortem conditions of the sheep depended upon the

/intensity....

intensity and duration of infection. After the prepatent period the clinical manifestations were obvious in almost all the experimentally infected sheep. Lack of appetite, progressive emaciation, weakness, paleness of mucous membranes and broken wool were all features of this stage of the infection. The sheep ingested large amount of wool daily so that wool was frequently mated in the faeces. Foetid faeces with mucous and sometimes haemorrhagic diarrhea and coughing was common.

Two sheep died during the experiment. One of them died 79 days after exposure to S.bovis cercariae with severe emaciation and secondary respiratory infection (pneumonia). At autopsy the liver was seen to be badly damaged and fibrous with high densities of eggs and granulomata.

The infected sheep never showed any sign of recovery as seen in calves, and they remained in critical condition up to time of autopsy.

/The relatively....

The relatively high numbers of egg per gram of tissue, the degree of liver damage and dramatic clinical conditions showed that *S. bovis* was much more serious in sheep than in calves. The amount of pathology produced was proportional with duration of the infection.

Post-mortem examination,

There was a considerable loss of mesenteric fat-body, ascitic fluid and serous effusion in the abdominal cavity with some degree of hydothorax and hydroprecardium.

Liver: Liver was dark in colour and had numerous small greyish spots in the parenchyma beneath the capsule and on the cut surface of the liver substance. In sheep with longer infection (18 weeks) lymphoid nodules were seen frequently all over the liver and they were quite distinct from the other parts (Plate, 14). In examination and perfusion of intrahepatic veins large number of living adult schistosomes were discovered. The gall bladder in most cases was distended. Some thrombi in response to parasites were observed in hepatic veins in which numerous dead worms were encapsulated.

/In sheep....

In sheep with 18 weeks old infections of S.bovis which were challenged with O.turkestanicum the liver was more seriously damaged by deposition of fibrous tissue and eggs.

Other organs; By holding the mesentery against a light a coiled mass of adult schistosomes could be seen in the mesenteric veins. The small intestine in some parts contained several prominent red and congested foci. The rectum and caecum were also haemorrhagic with some brownish stripes but not so intensely as in the calves.

Lymph nodes: Thoracic, hepatic and mesenteric lymph nodes showed some degree of enlargement and pigmentation.

Microscopic examination,

Liver: Schistosome eggs were mostly found in the intralobular branches of the portal veins and were surrounded by lymphoid cells, the eggs in the sheep livers rarely escaped from the portal veins to the parenchyma. In some cases may lymphoid cells took part in the reaction. A zone of intensive eosinophilic cells surrounded some of the ova and infiltrated

/to the centre....

to the centre of the granulomas and producing a mass of inflammatory reactions (Plate, 15).

In sheep with older infections (16 weeks), some of the intrahepatic branches of portal veins exhibited severe proliferative endophlebitis and thrombophlebitis with numerous eosinophils on the villous projections and around the veins in surrounding tissues(Plate,16). This type of pathological picture was only observed in sheep and not in calves or goats. Lymphoid nodules were more frequent in sheep with intensive and packed cellular proliferation. There was a very slight response to the living worms but strong response to the dead worms and thrombi in intralobular portal veins.

The fibrous formation in advance cases in sheep liver was outstanding. As a result of these lesions, the lumens of veins were either partially or totally obstructed with hyperplasia, hypertrophy and thrombophlebitis. Pigmentation of the liver was intense with most of the pigment in the Kuppfer cells. The intestine showed numerous eggs and granuloma formations all over the small and large intestine.

/ Goats....

GOATS,

Ante-mortem examination,

Goats were more affected than sheep and calves with S.bovis infections with considerable loss of weight, weakness, anaemia and abnormal faeces (mostly covered with a coat of mucous) and some times haemorrhagic diarrhea which was more severe in goats than in sheep.

Post-mortem examination,

A severe loss of mesenteric fat-body, the presence of ascitic fluid in the abdominal cavity were common. The lymph nodes were mostly enlarged and pigmented. The liver showed greyish minute spots. A large number of adult schistosomes were recovered from the liver by perfusion. Intestinal tract showed numerous red foci and granulomata in submucosa.

Microscopic examination,

Extensive host tissue reactions were observed in every intra-lobular areas in the liver. The reactions were a diffuse granulomatus

/proliferation....

proliferation with many eosinophils, and lymphoid cells surrounding numerous ova in intralobular spaces (Plates, 17, 18). A large number of eggs in the liver showed ' the Hoeppli phenomenon ' a very interesting antigen-antibody response. In some cases rod-shaped acidophil bodies were arranged side by side around the egg shell and made a rosette-form. This reaction was very common at this stage of the infection (9 weeks) in goats but not in sheep or calves (Plate, 18, 19).

THE PATHOLOGY IN A COW NATURALLY INFECTED WITH S.BOVIS

Among the different type of animals examined in the Khuzestan slaughter houses only a few cattle were found to be infected with *S.bovis* and these harboured very few adult worms. No sheep or goats were found to be infected. Most of the infected cattle showed mixed infections with *O.turkestanicum*: only one cow was found with a pure infection of *S.bovis*. This cow was over 7 years old with excessive

/emaciation....

emaciation and a large amount of ascitic fluid in the abdominal cavity, an oedematous serous membrane and markedly congestion of the intestines. All the mesenteric veins were blocked with dead worms, and showed thrombosis and severe pigmentation (Plate, 20). The intesinal veins were seriously injured with black and tortuous appearance on the outer surface of the intestine (Plate, 21). Scraping of the intestinal mucosa revealed very few eggs, but granuloma with acute, superficial haemorrhagic ulcerations were abundant(Plate, 22).

The histological sections of small intestine showed marked hyperplasia of the intima of veins and thrombi formation around the daed worms associated with clots (Plate, 23). Newly dead worms in veins showed milder reactions with eosinophil infiltration (Plate, 24); the living worms showed no considerable reactions (Plate, 25). Many dead worms and calcified eggs with granulomatus reactions were seen in the submucosa of intestine.

/discussion....

DISCUSSION,

Le Roux (1929) attributed the pathology of S.mattheei infections in sheep to egg and pigment deposition by the worms, and lesions caused by the dying worms. The number of eggs in liver is a good indication of the severity of the infection. In our experiments the density of the S.bovis eggs per gram of liver tissue in sheep and goats was much higher than in calves and the pathological pictures also was much more serious than in calves.

The amount of pigment in a liver was also useful indication of the intensity of the infection. Most of the pigments regurgitated from the gut of the schistosomes enters the liver by portal blood, but a small amount also enters the lymphatics and is phagocytised in the mesenteric lymph nodes. The reticulo-endothelial system in the liver espicially the Kupffer cells ingests the pigment and when the cells are overloaded the pigment pass into the hepatic veins and on to the heart in the venous blood and is eventually phagocytised in the lungs and

/lymph....

lymph nodes where it gives rise to a grey colour. The amount of pigment in above organs directly depends upon the number of worms and duration of infection. In our experiments the amount of pigment deposited in the livers and lymph nodes in sheep and goats was much higher than in calves and was heavier in those with the longer duration of infection (18 weeks). McCully and Kruger (1969) similarly reported the relatively higher pigmentation of livers in sheep than in cattle infected with S.mattheei.

In 9 weeks old infections with 5,000 S.bovis cercariae we found that more eggs and pigment were deposited in sheep and goats livers than in calves and that the pathological changes were also more serious in sheep and goats than in calves. This result showed that S.bovis is better adapted to calves than to sheep and goats.

The following distinctive pathological features were noticed in the different hosts:

Calves- The extensive medial hypertrophy of the intralobular hepatic

/veins.....

veins was not seen in sheep or goats. Hussein (1969) reported the same pictures of intrahepatic veins hypertrophy in cattle infected with S.bovis.

Sheep- The most characteristic feature was endophlebitis and intimal hypertrophy and hyperplasia of the intralobular veins in liver of sheep with longer duration of infection (18 weeks). Letulle (according to Dew, 1923) described a human case of endophlebitis of veins containing bilharzial adult worms and regarded it as being due to the local action of a toxin, but in general adult worms are thoughts to play very little role in the pathology of the human disease. McCully et al (1967, 1969) described a similar endophlebitis of the intrahepatic branches of the portal veins in the Hippopotamus and in sheep infected with S.hippopotami and S.mattheei, and they were attributed to the presence of live adult worms and the sensitivity of the hosts. In the present observations the endophlebitis and thrombophlebitis was a characteristic picture only in sheep.

Goats- The numerous eosinophilic antigen-antibody deposits around the S.bovis eggs in the liver of goats was the most striking picture

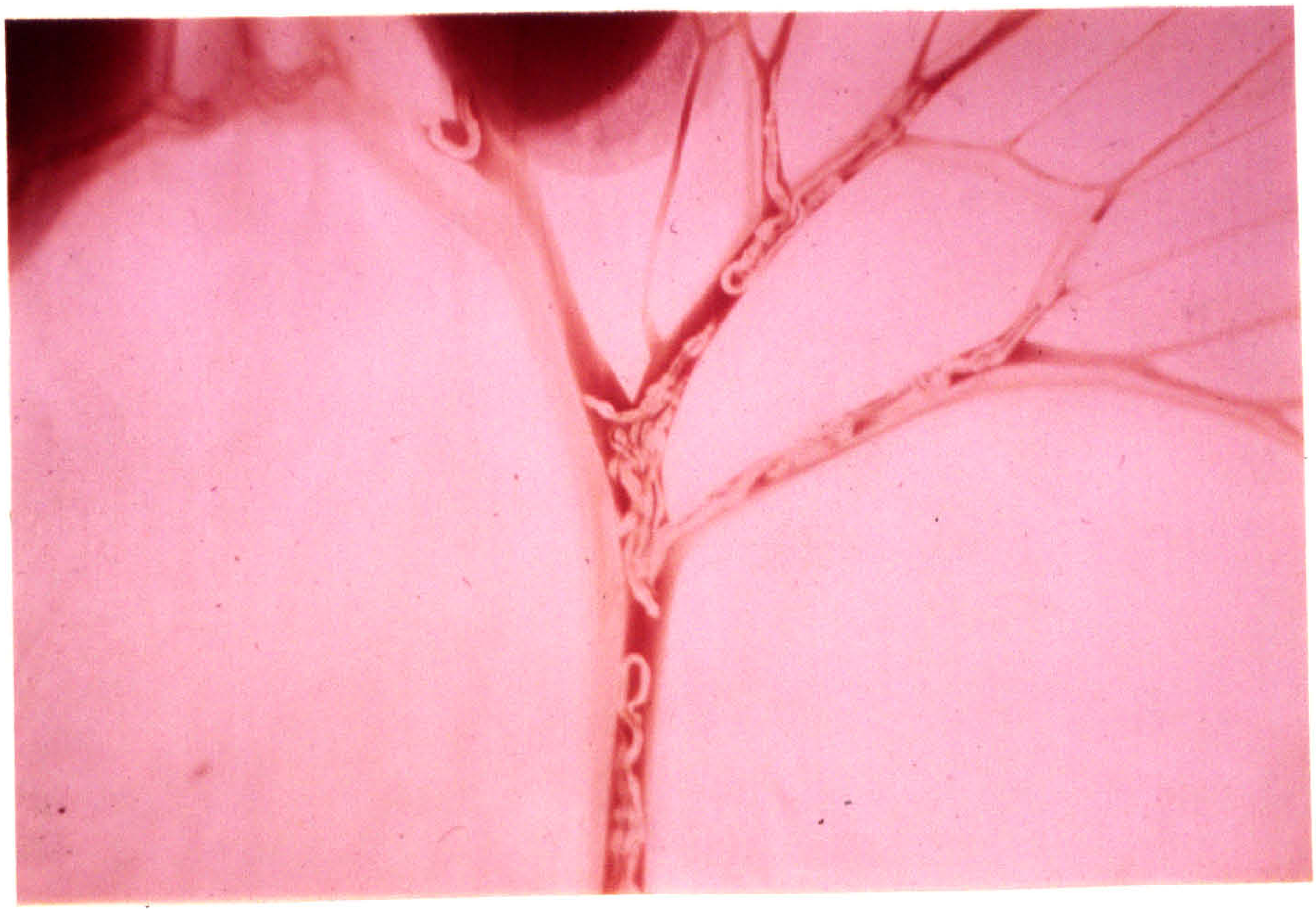
/ in this....

in this host. This reaction indicates a strong hypersensitivity to antigen associated with the eggs in this particular host. This phenomenon was first described by Yamagiwa (1931) in cattle infected with O.turkestanicum and later by Hoeppli (1932) in S.japonicum infection. Sadun et al (1962) observed this reaction in the liver of the Wood chuck infected with S.mansoni. McCully et al (1969) found the same phenomenon in cattle infected with S.mattheei. Verminous phlebitis was described by Fairley and Mackie (1930) as the most characteristic lesions in the liver of goats infected with S.spindale. The Hoeppli phenomenon was obseved also in goats liver infected with O.turkestanicum (see Part, I).

The different stages in the granuloma formation from the early reactions around the newly deposited eggs to the chronic granulomas with the fibrous tissue formation were seen in all the infected animals. The intense attraction of eosinophils to newly released eggs particularly in sheep and goats was interesting and may have been caused by delayed hypersensitivity as suggested by Meleney et al (1953) and Warren et al (1967).

/The occurence...

The occurence of lymphoid nodules and hyperplasia in tissues particularly in the livers of the ruminants with long standing infections was similar to that reported by Hussein (1969) and McCully and Kruger (1969) and is believed to be produce by both eggs and dead worms. This reaction is unusual and has not been reported from man or other experimental animals infected with schistosomes. It seems likely that further studies of the pathogenicity of schistosome infection in livestock will help to unravel some of the problems in the immunopathology of the schistosomiasis.



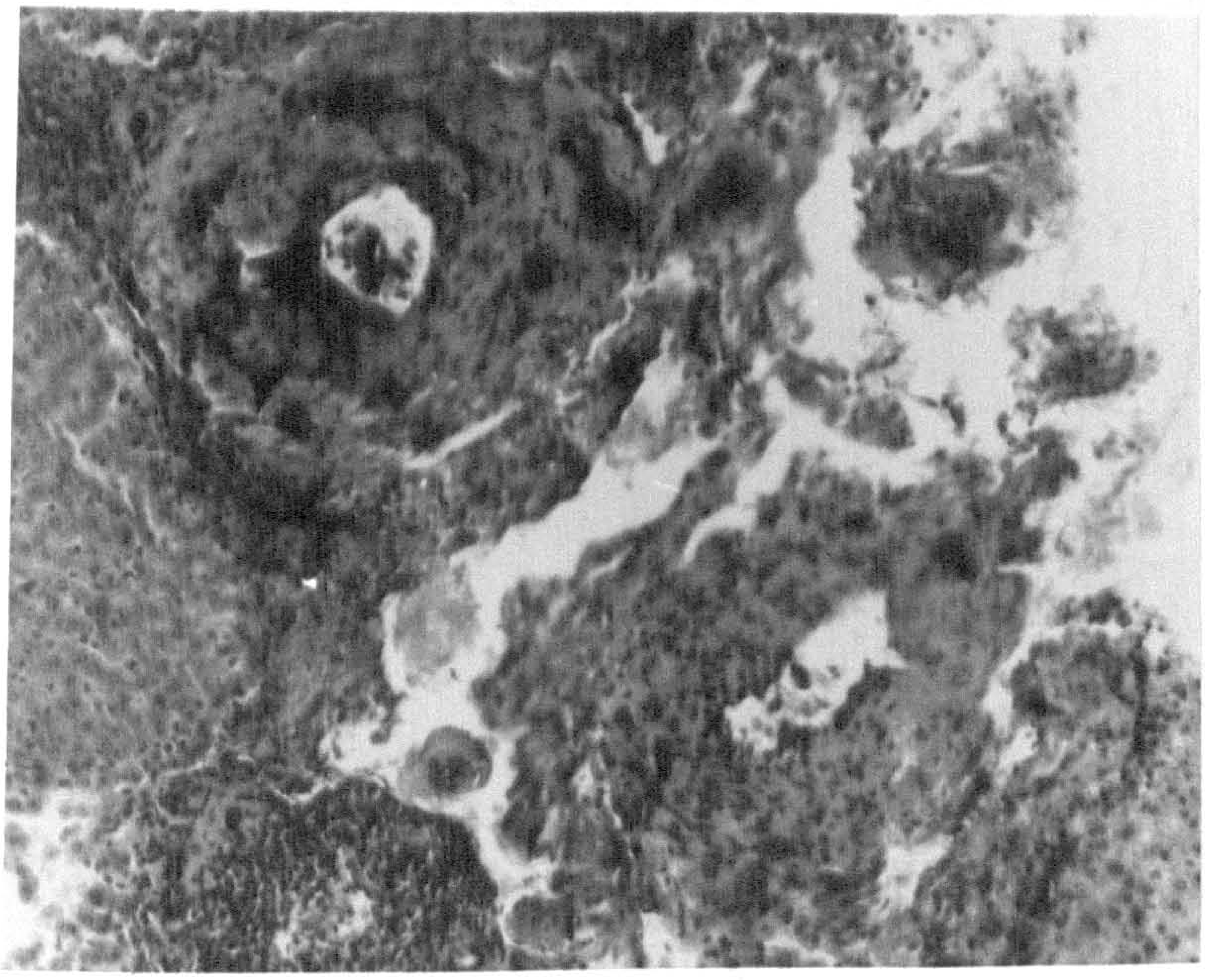
Plate, 8

<u>Schistosoma</u> <u>bovis</u> adults in mesenteric veins

(Calf, 9 weeks after exposure)

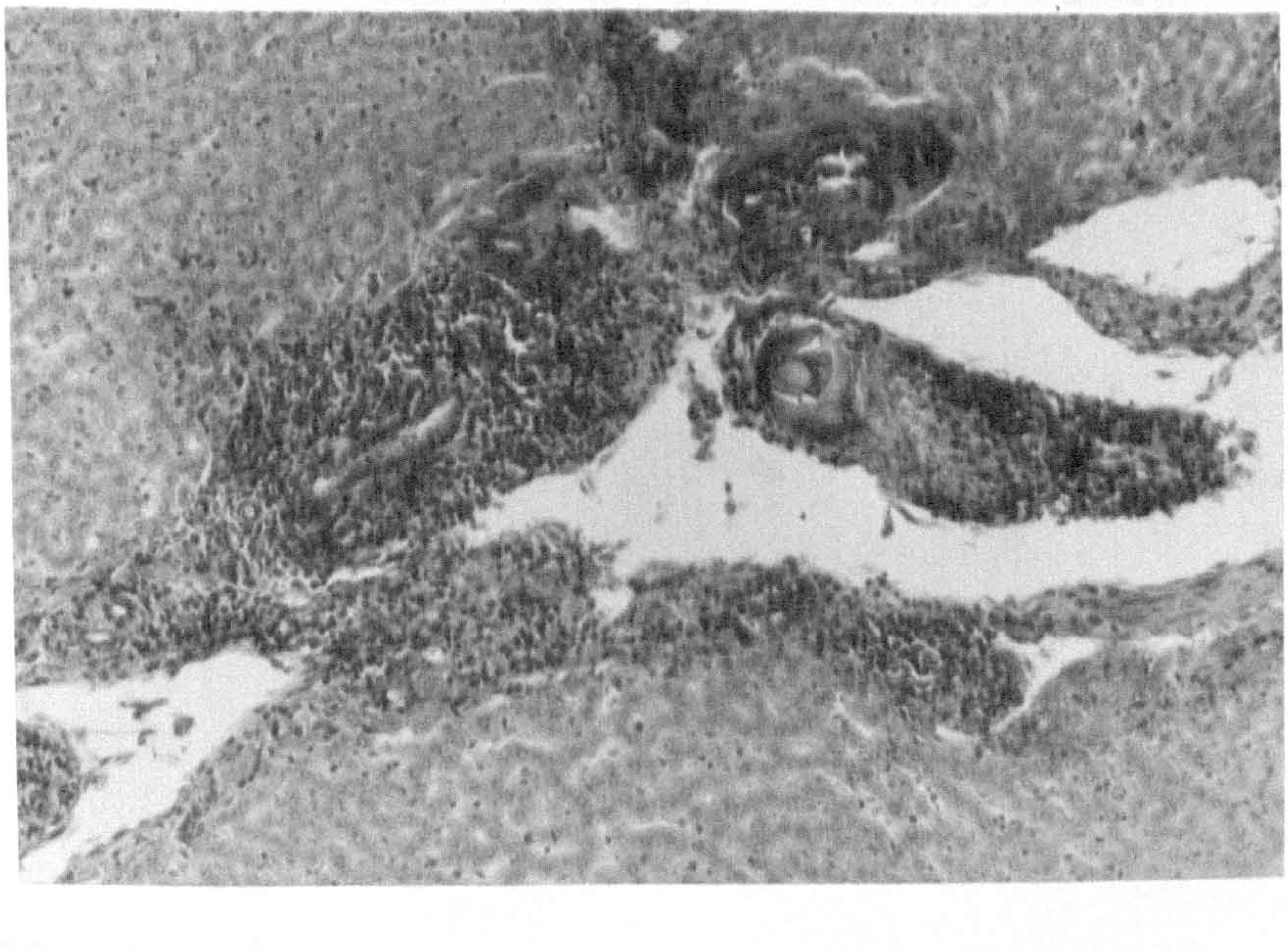
Plate, 9

Intralobular fibrosis, liver of calf (9 weeks after exposure to <u>S.bovis</u> cercariae, H. E. x 30).



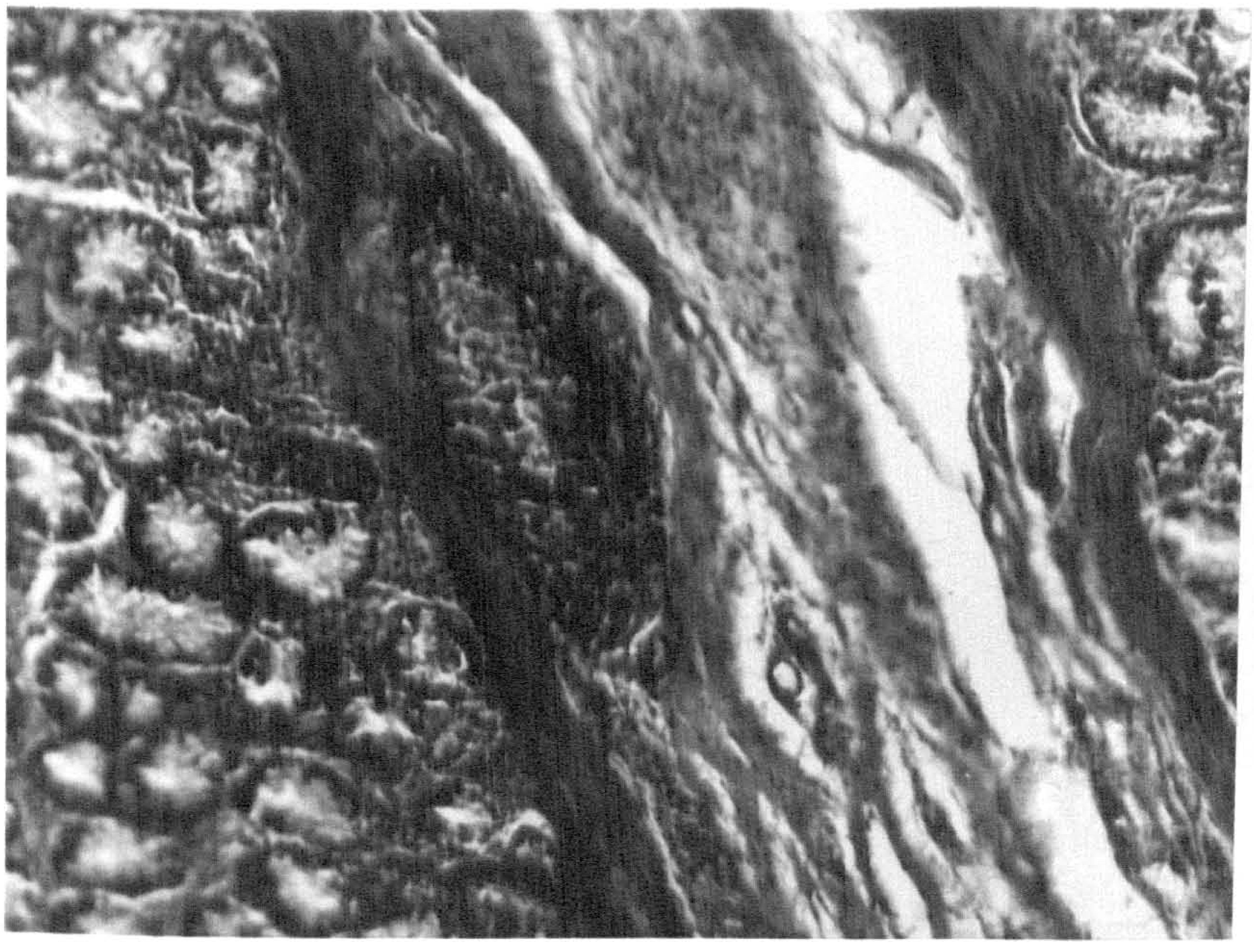
Plate, 10

Portal vein showing medial hypertrophy, with some cellular infiltration (Calf, 9 weeks after exposure to S.bovis cercariae, H. E. x 240).



Plate, 11

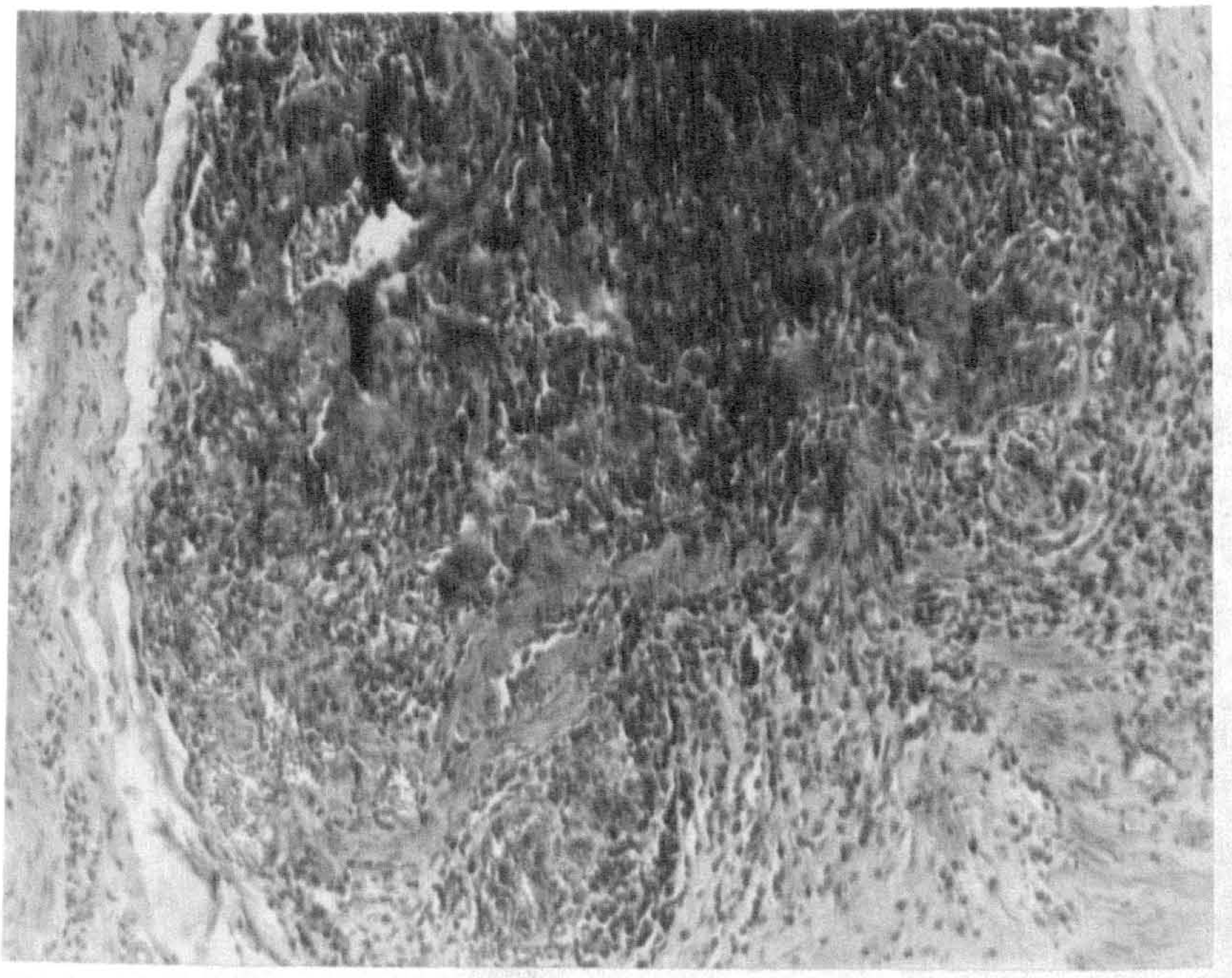
Intralobular granulomas in liver with medial hypertrophy of veins and dense cellular infiltration (calf, 9 weeks after exposure to S.bovis cercariae, H. E. x 120).



Plate, 12

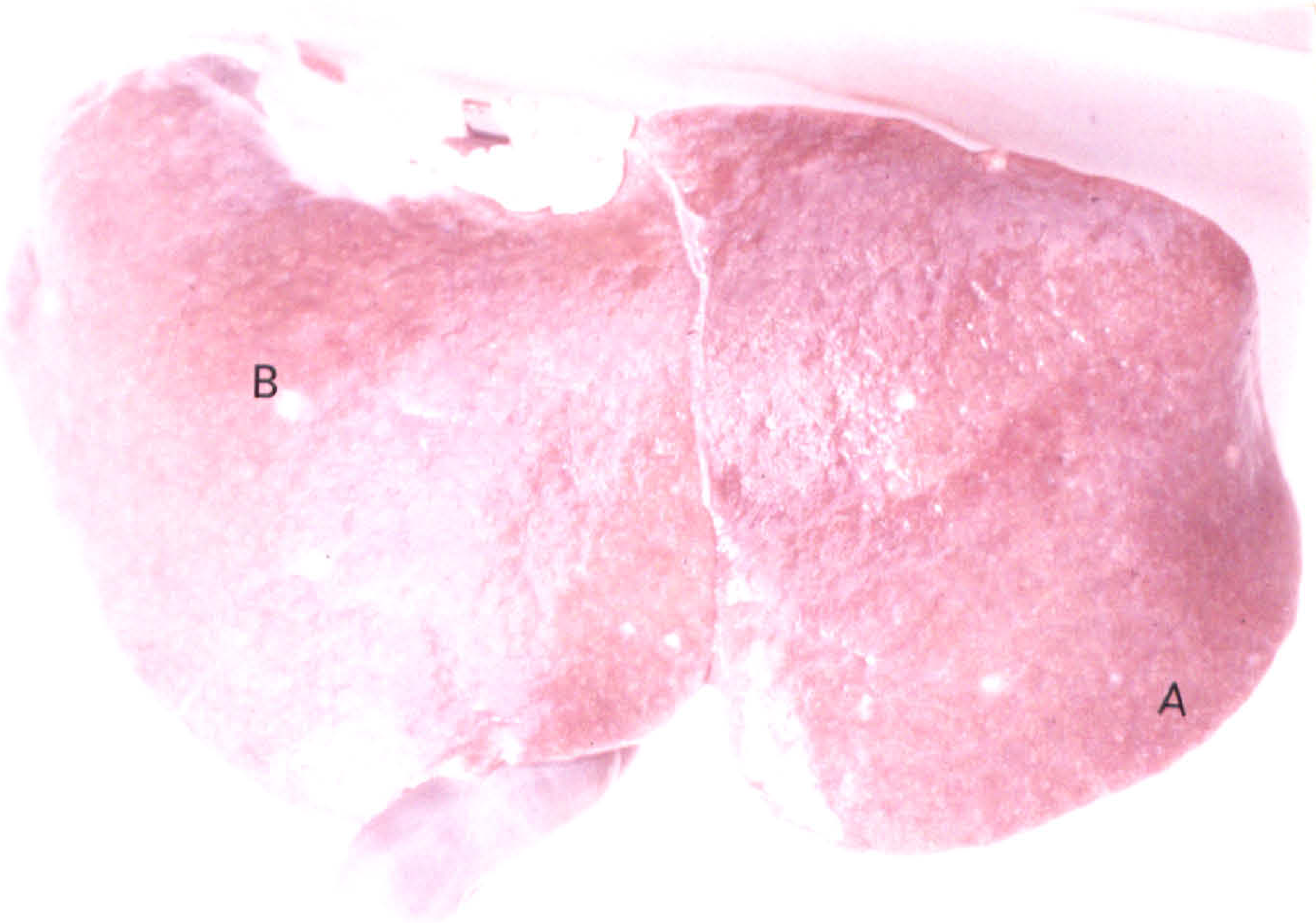
Lymphoid nodule in caecum of calf infected with S.bovis

(H. E. x 120).



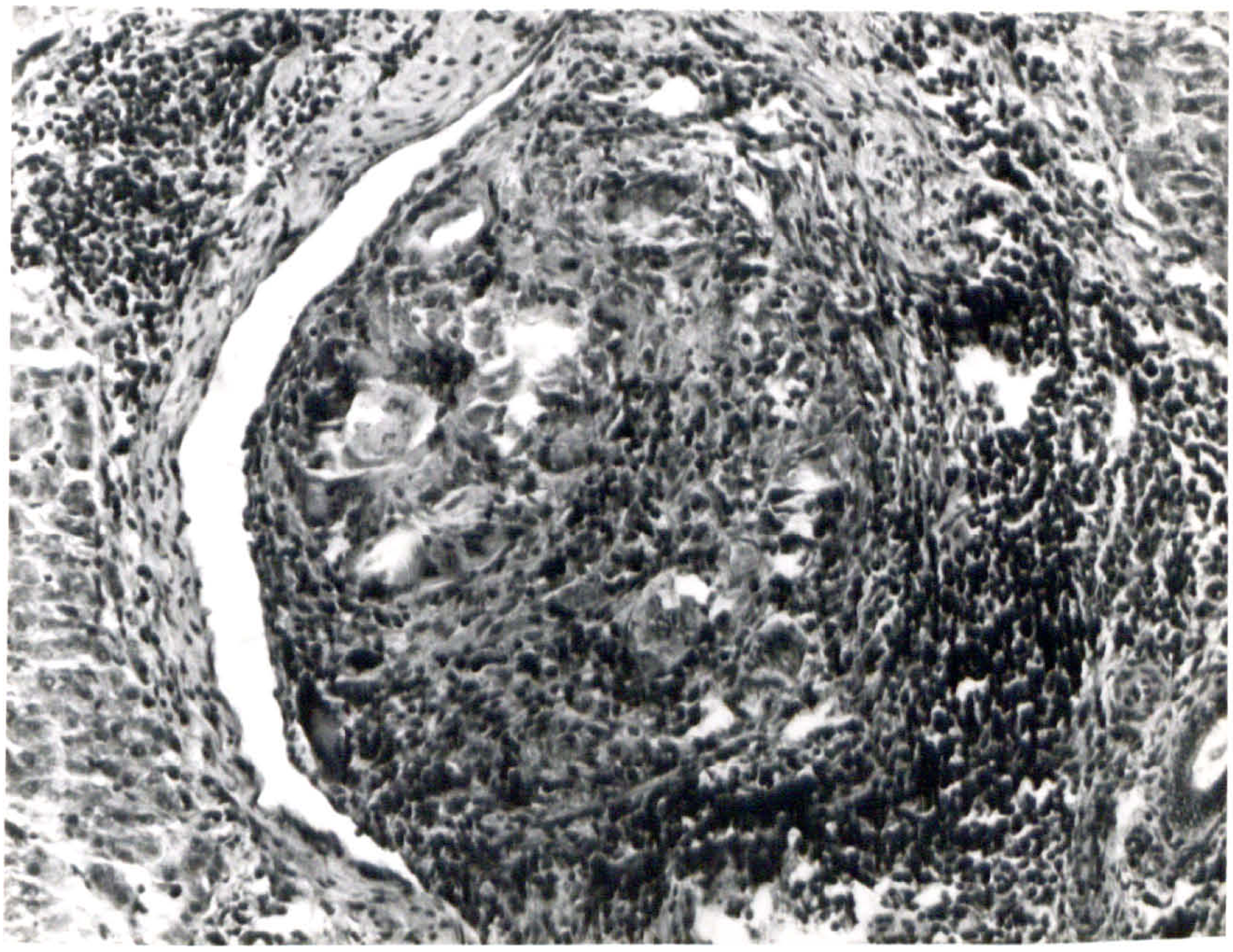
Plate, 13

Close structure of a lymphoid nodule in caecum of a calf with numerous destroyed egg particles (H. E. 240).



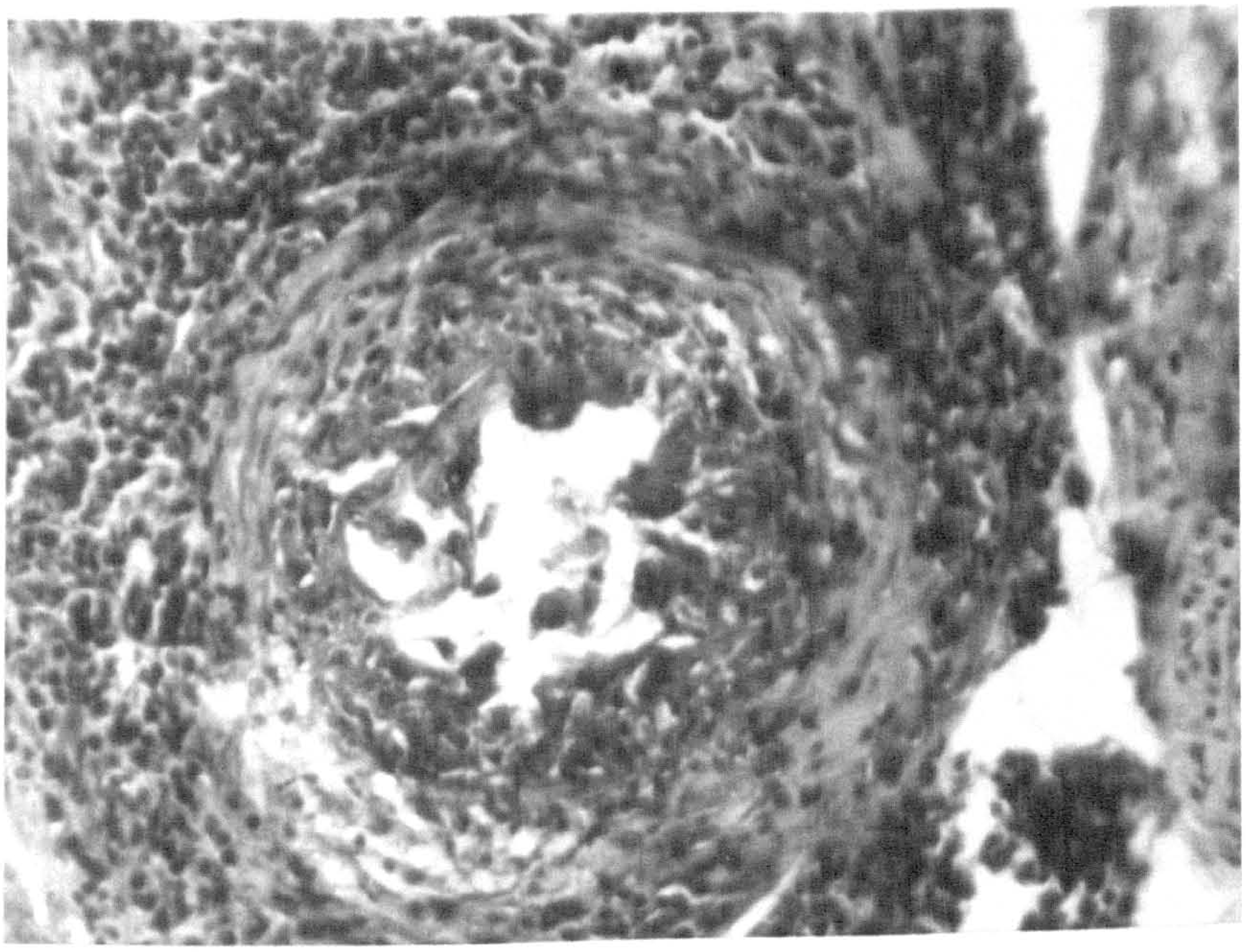
Plate, 14

Gross appearance of the liver of a sheep infected with S.bovis (18 weeks infection). Note: A, the numerous minute greyish nodules. B, large lymphoid nodules.



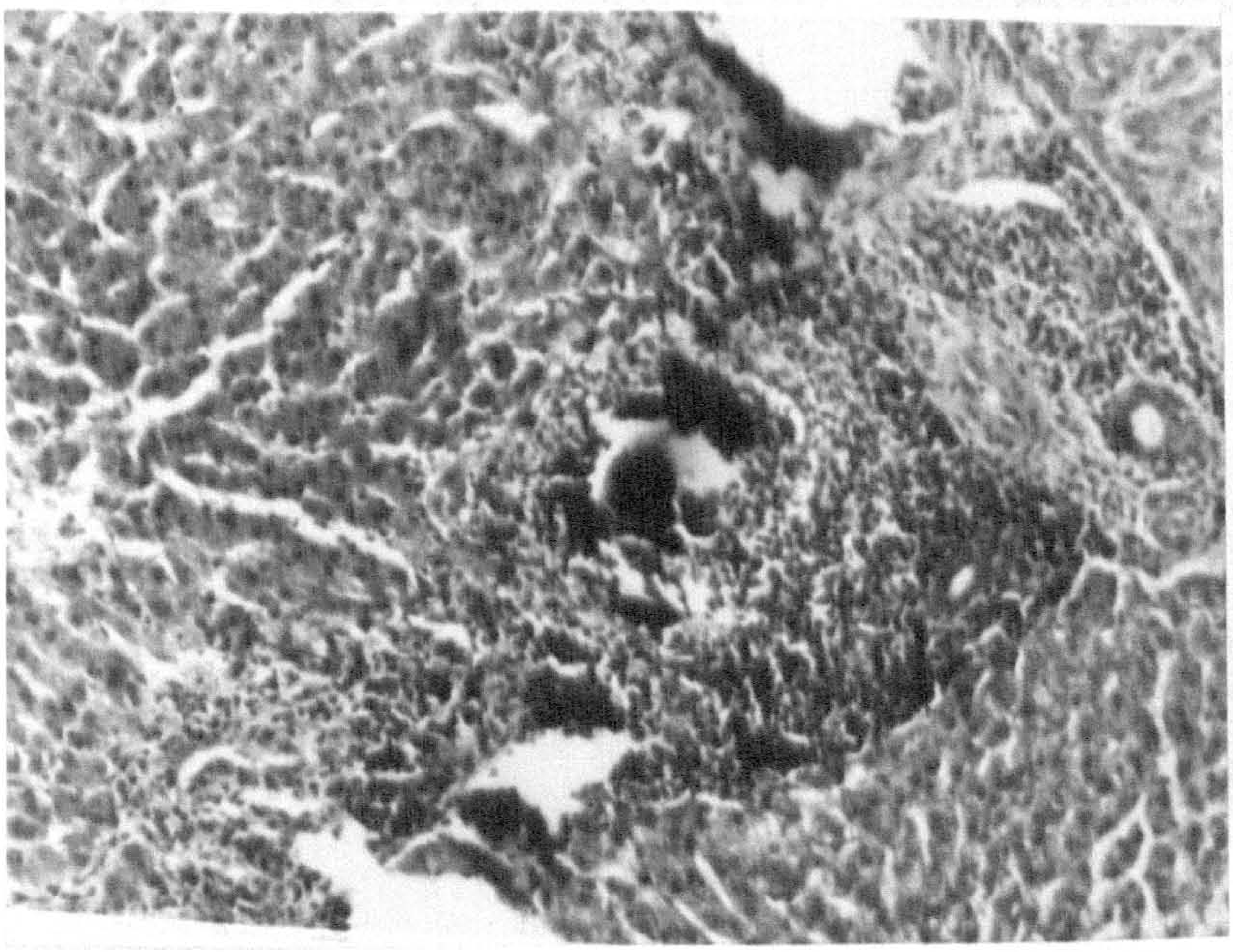
Plate, 15

Liver showing early stage of intravascular lymphoid nodule formation with some remnants of disintegrating eggs (sheep liver 18 weeks after exposure to S.bovis cercariae, H. E. x 240).



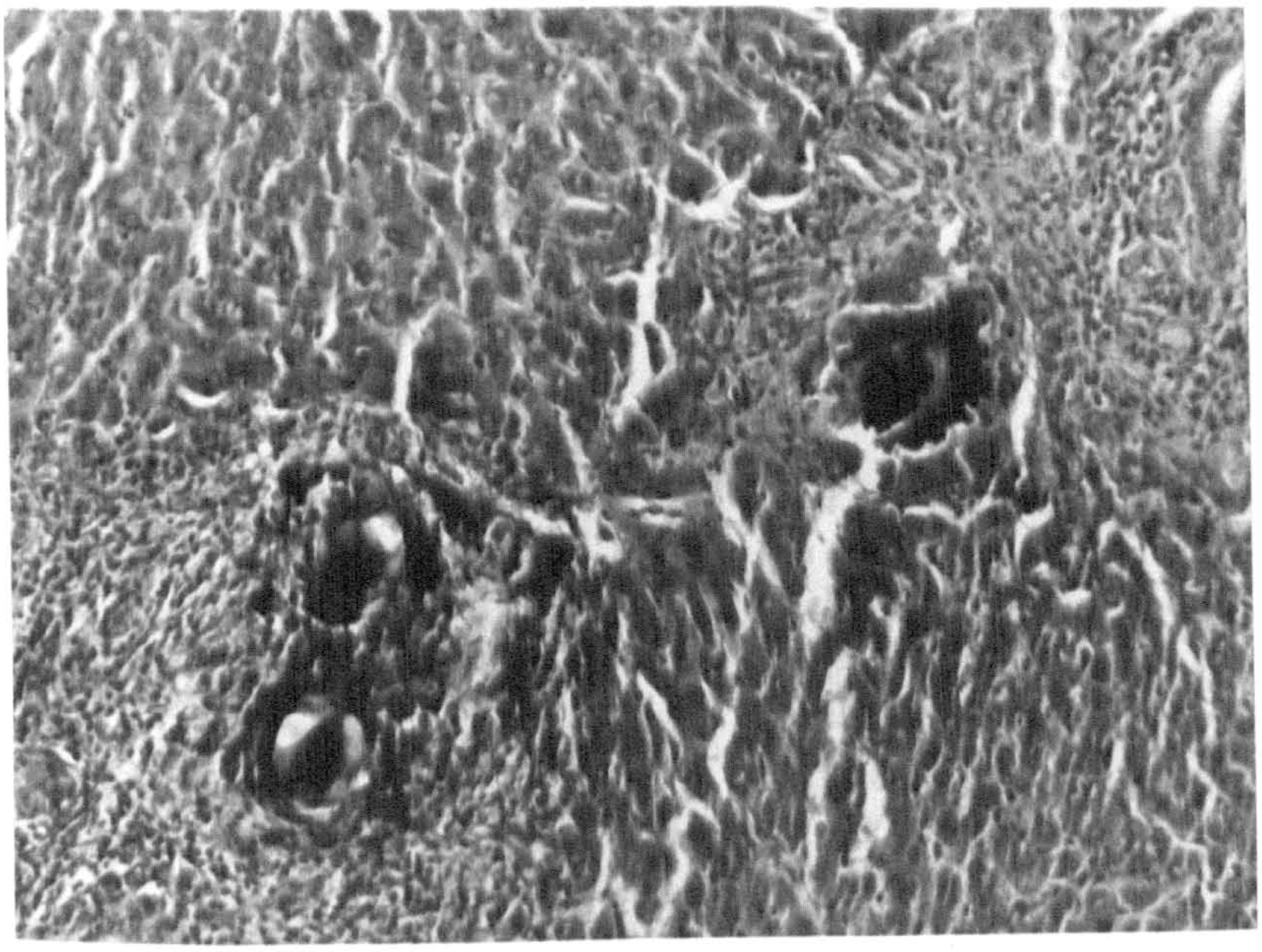
Plate, 16

Liver, branche of portal vein showing proliferative endophlebitis with many eosinophils within intimal projections and around veins. (sheep 18 weeks after exposure to S.bovis cercariae,H.E. x 240).



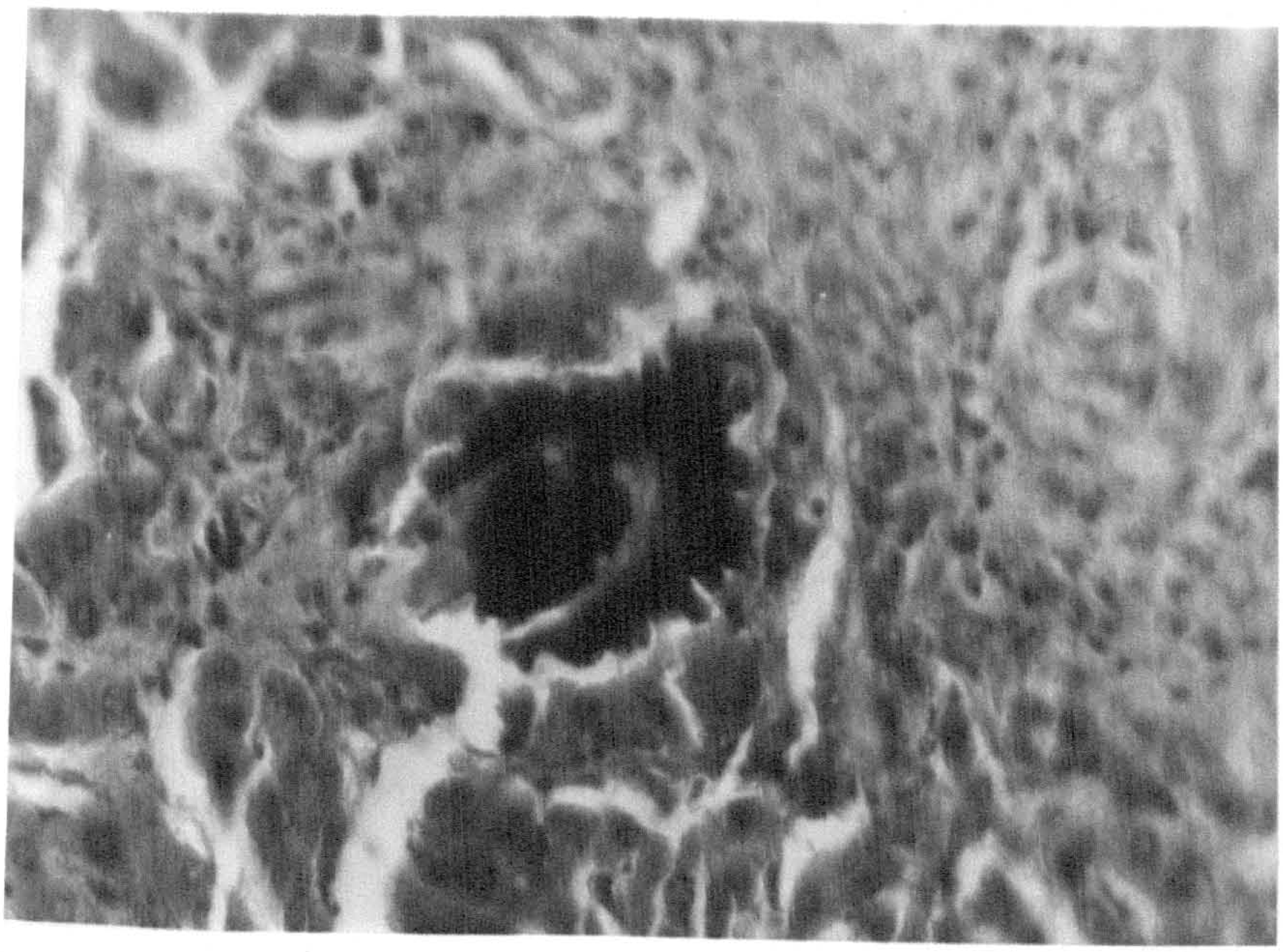
Plate, 17

Liver, intralobular granulomas with diffused cellular infiltration surrounding ova of S.bovis (goat, 9 weeks after exposure to cercariae, H. E. x 120).



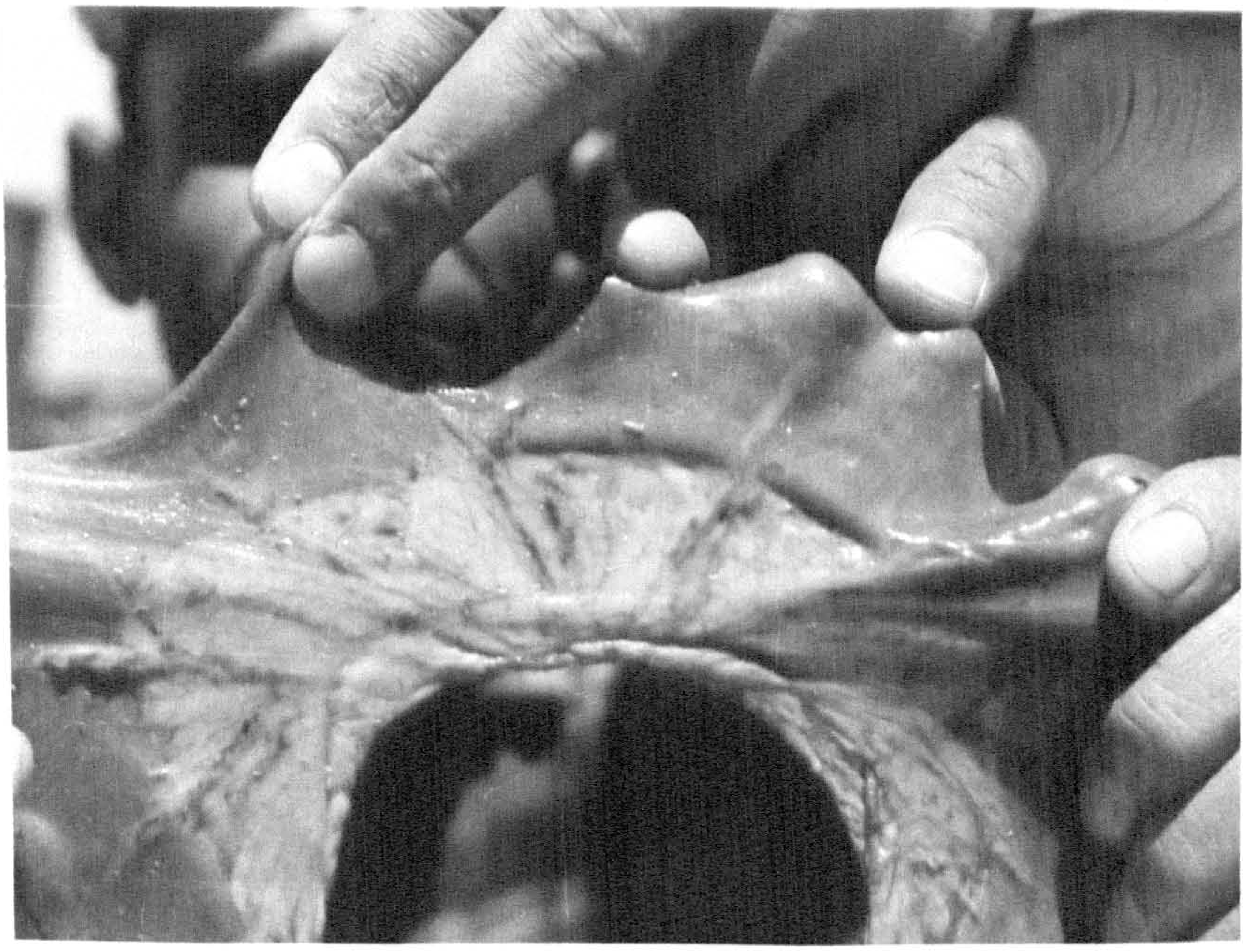
Plate, 18

Liver, intralobular granuloma and Hoeppli phenomenon (goat, 9 weeks after exposure to S.bovis cercariae, H. E. x 120).



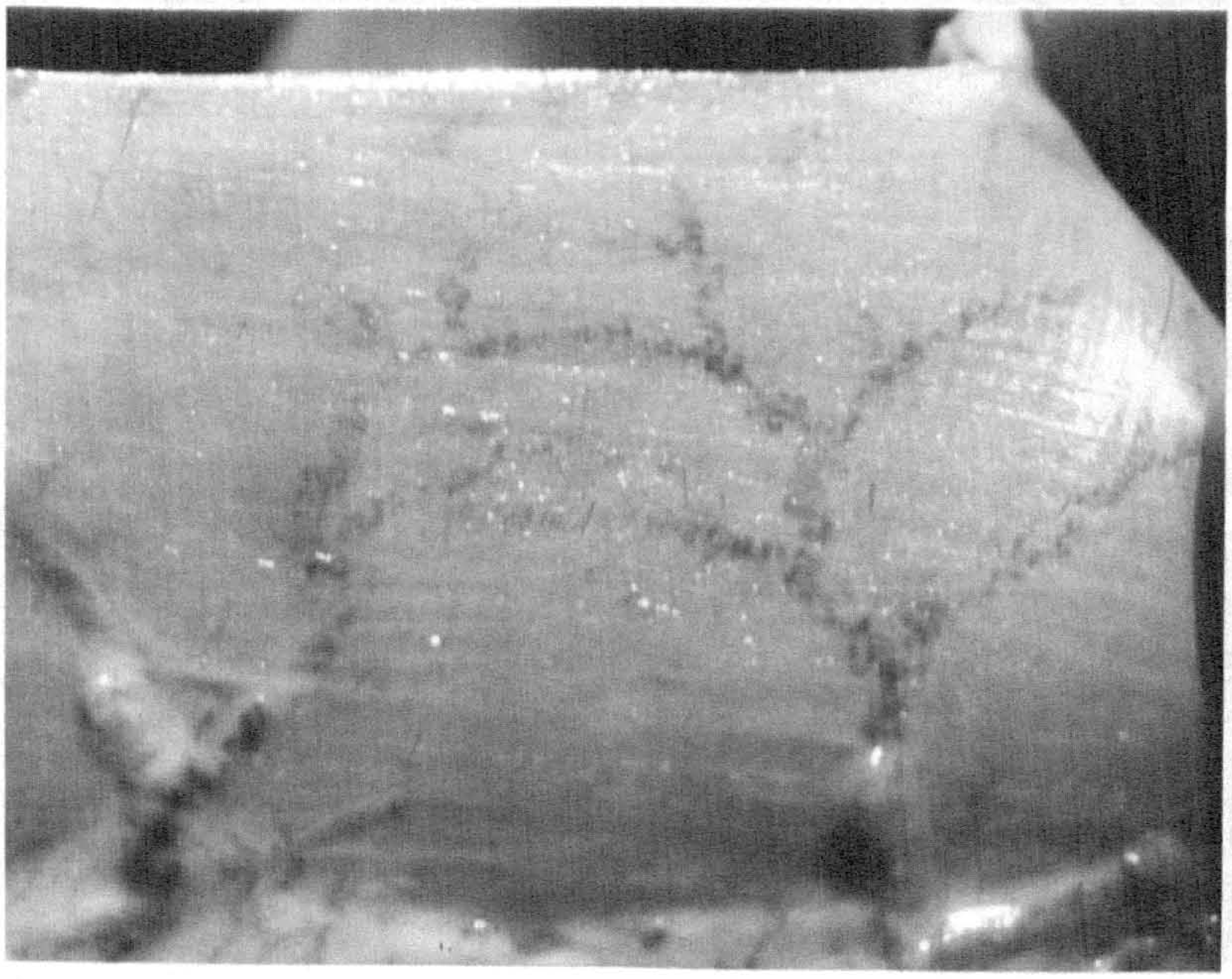
Plate, 19

Hoeppli phenomenon, a react-ion around S.bovis egg in the liver of a goat. Note the stellate-shaped accumulation of eosinophilic antigen-antibody material (9 weeks after exposure to cercariae H. E. x 560).



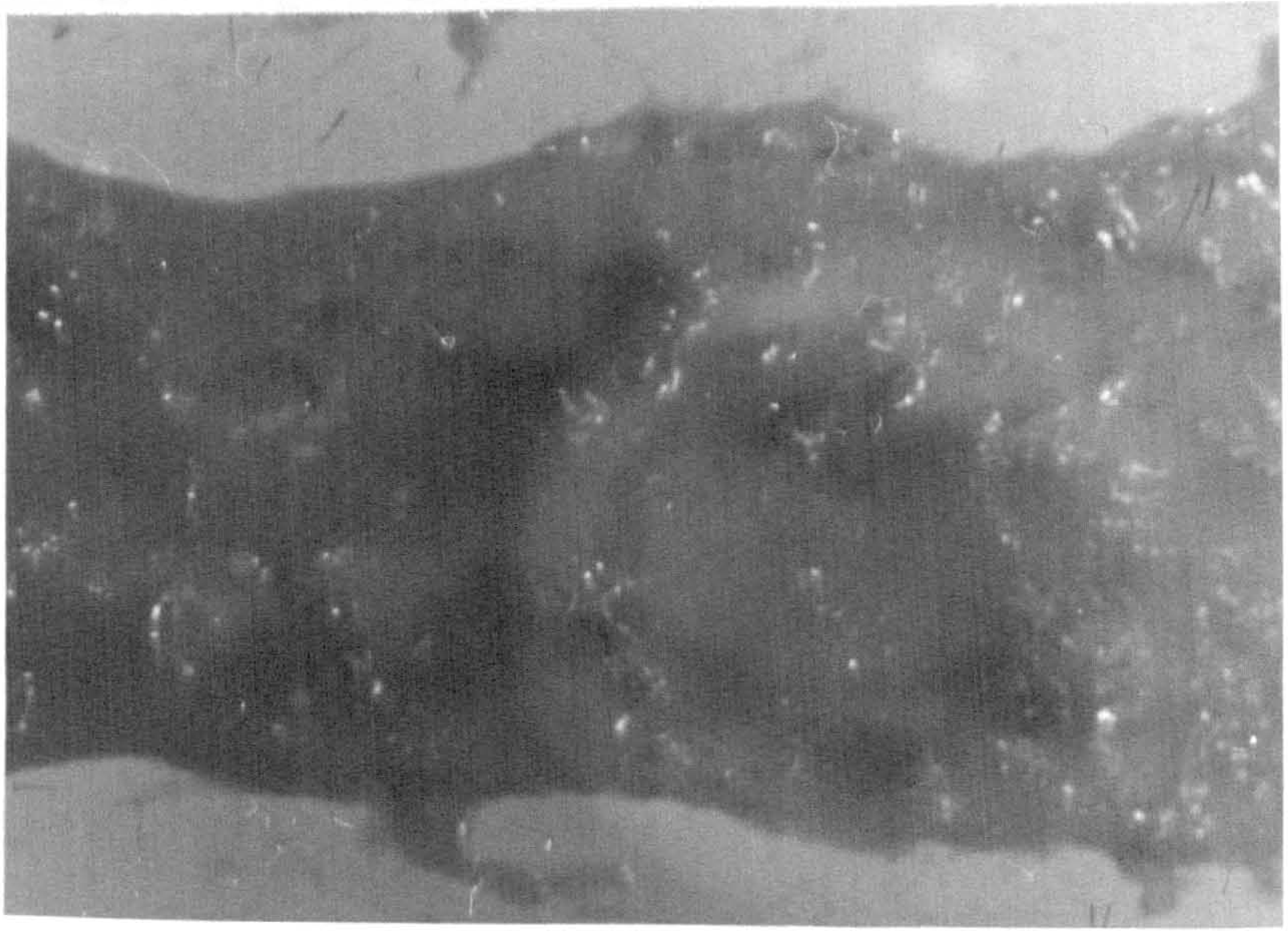
Plate, 20

Mesenteric veins contain dead worms with blockage and thrombus formation (naturally infected cow with S.bovis)



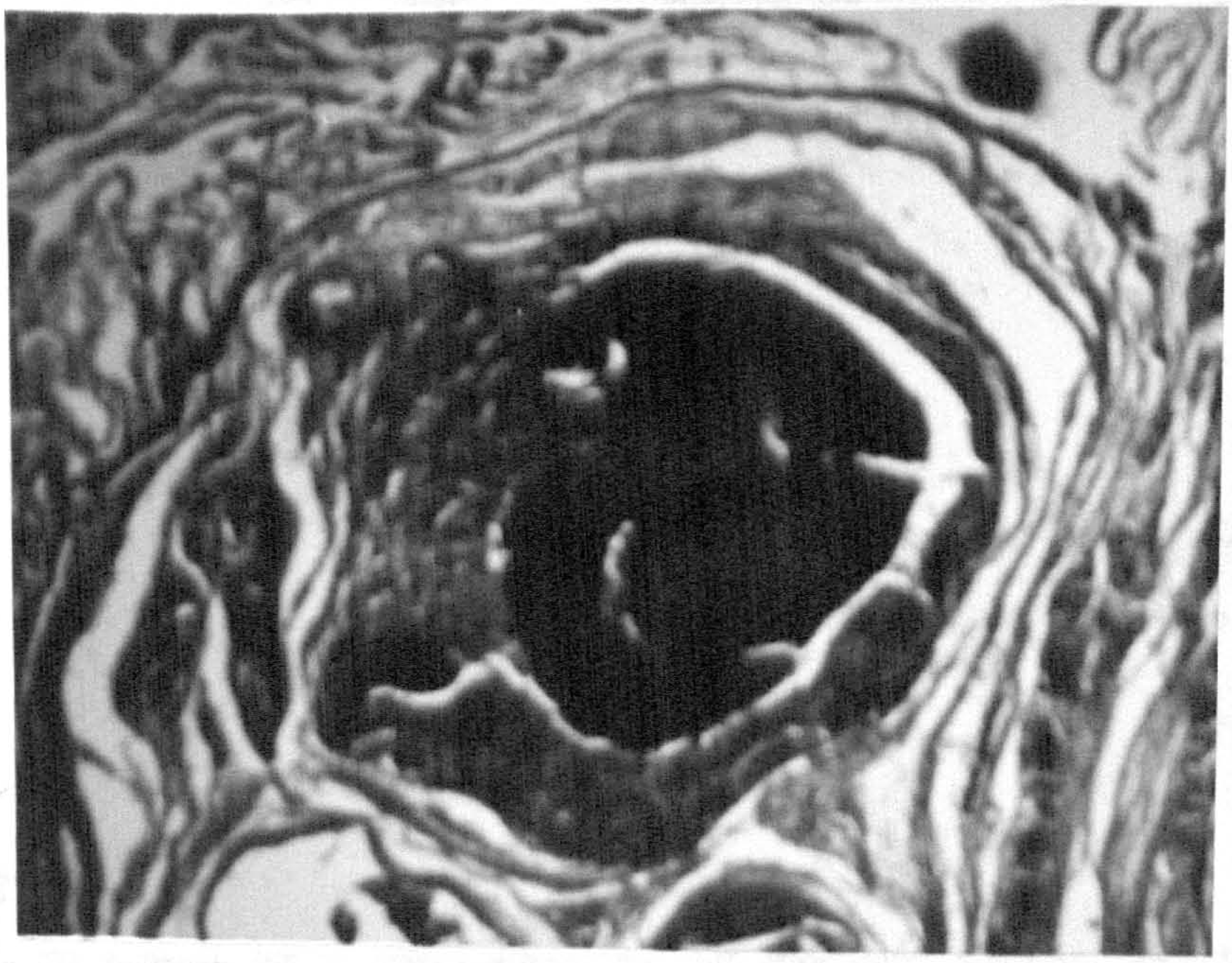
Plate, 21

The tortuous appearance of the intestinal veins(naturally infected cow with S.bovis).



Plate, 22

A chronic infection of S.bovis in the intestine of the naturally infected cow. Acute, superficial haemorrhagic ulcerations.



Plate, 23

Histological section of the affected mesenteric vein thrombus formation (H. E. x 120).

Plate, 24

Dead worms in a vein with mild reactions and lymphoid cell infiltration (naturally infected cow with S.bovis, H. E.x60).

Plate, 25

Live worms in a vein showing no obvious tissue reaction (naturally infected cow with S.bovis).

PART III

PART, III

EXPERIMENTAL STUDIES ON ACQUIRED RESISTANCE TO SCHISTOSOMIASIS USING HOMOLOGOUS AND HETEROLOGOUS SYSTEM

CHAPTER, 1

INTRODUCTION,

Epidemiological studies suggest that man and domestic animals can acquire resistance to schistosomes. The first observations were those of Fujinami(1917) in a S.japonicum endemic area in Japan who observed that children were more affected than adults and that calves brought in from the outside were seen to sicken and die some time after, although local animals were apparently relatively resistant to the severer effects and did not die. Subsequent observers have noted that the prevalence and intensity of S.haematobium infection decreases in adults as compared with children (Gerber,1952; Gothe,1963). The same was noted by Fisher (1934) with S.intercalatum. Nelson (1959)

/from studies....

from studies on the incidence and distribution of *S.mansoni* in West Nile district of Uganda found that the highest incidence was in the 5-15 years age group. The same pattern of the disease was observed with *S.japonicum* infection in China by Vogel *et al* (1953) and in the Philippines by Pesigan *et al* (1953). However, naturally acquired immunity may not be the only explanation for the apparent mildness of schistosomiasis in adults: Van den Berghe (1959) has suggested that there is age resistance in man and Lewert and Mandlowitz (1959) have shown that in old experimental animals relatively fewer cercariae penetrate. Nelson (1959) suggested that in addition to acquired resistance other factors might account for the differences in pathogenicity in different areas for example racial tolerance, different strains of the parasites and differences of nutritional levels.

Le Roux (1961) theorized that cercariae of the animal schistosome species *S.bovis* may immunize man against the human schistosome species *S.haematobium* and vice versa and that this could explain the distribution pattern of these infections, for example the presence of *S.bovis* and

/absence....

absence of S.haematobium from Sardinia and the reverse situation in Egypt. Nelson et al(1962) suggested that constant exposure to animal schistosome cercariae will modify schistosome infection in man by reducing the worm burden without necessarily affecting the prevalence rate, but in these areas the complications less would be severe. This type of heterologous immunity response in man was named 'Zooprophylaxis' which has been defined as the prevention or amelioration of disease in man as a result of previous exposure to heterologous infections of animal origin (Nelson,in press).

Apart from the epidemiological evidence most of which has been reviewd by Clarke (1966 b), there are numerous experimental studies on animals to suport the view that acquired immunity plays an important role in schistosome infections. But only three experiments have been carried out on man (Fisher 1934; Gothe 1963; Clarke 1966 a) and the results have been inconclusive.

Experimental data shows that mice and hamsters can acquire a partial resistance to reinfection with S.mansoni, S.japonicum and

/Schistosomatium....

Schistosomatium douthitti, and rat can develop partial immunity to S.mansoni and S.japonicum. Baboons and Cercopithecus monkeys can develop some resistance to S.mansoni and partial immunity has been induced in the horse, the dog and the rabbit after homologous immunisation with S.japonicum. Hamster can develop some resistance to reinfection with S.haematobium and rhesus monkeys can become completely resistant to reinfection with S.mansoni, S.japonicum, S.haematobium and Schistosomatium douthitti.

The experiments on heterologous immunity to schistosomes have recently been reviewed by Eveland et al (1969): mice can become partially resistant to S.mansoni after immunisation with S.rodhaini and S.bovis and S.mattheei and to Schistosomatium douthitti after immunisation with S.mansoni. Rhesus monkeys can acquire partial immunity to S.mansoni after exposure to S.japonicum, S.haematobium, S.bovis and S.mattheei; to S.haematobium after immunisation with S.bovis and to the 'J' strain of S.japonicum after immunisation with the 'CH' strain of S.japonicum, S.mansoni and Schistosomatium douthitti. Calves immunized with S.mansoni can partially resist challenge with S.mattheei and a chimpanzee with

/ a natural....

a natural infection of *S.mansoni* strongly resisted challenge with *S.japonicum*.

Studies on the mechanism of schistosome immunity have shown that inoculation with dead parasite material has a slight protective effect in mice but fails to induce any recognisable resistance in rhesus monkeys. Exposure to cercariae which are prevented ~~form~~ from maturing in the host by previous irradiation will provoke some resistance in mice and monkeys but only a partial resistance is developed in the rhesus monkey even after exposure to very large numbers of such irradiated cercariae (Smithers,1962).

The experiments of Smithers and Terry (1967) and Smithers *et al* (1969) on homologous immunity to *S.mansoni* in the rhesus monkey have shed considerable light on the stimulation of resistance. They have demonstrated that the adult worms provide the main immunogenic stimulus and in a series of transplant studies using worms that have matured in different hosts they have also shown that the adult worms are able to

/ disguise....

disguise their presence in the host by incorporating host antigens in their cuticle. This produces a state of ' Concomitant ' immunity where the adult worms are able to survive in an otherwise completely immune host. Recently Clegg and Smithers (1970) have demonstrated that in the presence of serum from highly immune rhesus monkey some schistosomula die within the first twenty-four hours in vitro and by the fourth day 95-100 % have died. This suggests the presence of a lethal factor in immune serum: this appears to be a specific antibody which is dependent for its action on complement.

The purpose of the present study was to investigate possible cross reactions between the animal, and human schstosomes that are prevalent in Khuzestan. In the first experiments in mice O.turkestanicum was used as the immunizing agent. In the second experiments calves and sheep were used for further investigation, using O.turkestanicum, S.bovis and S. haematobium. The results were quite striking, especially in calves which are the main natural host of bovine schistosomiasis in the Khuzestan endemic area.

CHAPTER. 2

MATERIAL AND METHODS.

Three species of experimental hosts were used in the present study:

1. Albino mice, 6-8 weeks old.
2. Calves, male 7-9 months old(local race).
3. Sheep, male 7-9 months old (local race).

Four species of schistosomes were used: the local strains of O.turkestanicum, S.bovis and S.haematobium, and a Puerto Rican strain of S.mansoni.

In mice each experiment was separately controled and followed a similar design as that used by Nelson et al(1968): group A, immunized and challenged; group B, challenged control; and group C, immunized but not challenged. Immunisation consisted of a single or triple exposure to various doses of O.turkestanicum cercariae and the challenge at 9

/ weeks....

weeks was with 300 cercariae of S.bovis or S.haematobium or 150 cercariae of S.mansoni. The cercariae were all taken from the same suspension obtained from a large number of infected snails.

The calves and sheep were divided into groups of 2-3 animals. The challenge control group in one experiment served as the control for the other experiments. In the heterologous immunity experiments immunizing and challenging doses of cercariae in large animals consisted of a single exposure of 8,000 cercariae of O.turkestanicum or 5,000 S.bovis in calves and 5,000 cercaraie of O.turkestanicum or S.bovis in sheep. Immunizing the calves with S.haematobium consisted of three inoculations of 7,000 cercariae in each inoculation with 4 weeks interval.

Autopsy was performed 9 weeks after the challenge exposure. The mean worm recoveries and tissue egg counts per gram were used as the criteria to evaluate the degree of resistance.

/ In homologous....

In homologous immunity experiments on calves the immunisation consisted of a single exposure of 1,000 cercariae of S.bovis or O. turkestanicum. The challenge was made with 4,000 cercariae of the same species, but the challenge control groups were exposed to 5,000 cercariae, which meant that each animal in the immunized and control groups finally received 5,000 cercariae.

The protective effect of immunity in schistosomiasis was measured by determining the effects on expected worm burden and egg counts of the challenge infection. Immunity may be complete in the sense that no developing or mature worms can be found or it may be partial in that the worms and eggs load of the immunized animals are reduced. Stunting of the worms with the production of fewer eggs by the females, is also considered as evidence of acquired immunity. Other criteria like distribution and viability of eggs and also eggs per female worm were considered.

CHAPTER, 3

RESULTS IN MICE,

A. Heterologous immunity studies.

Experiment,1: O.turkestanicum (100 cercariae) challenged with S.bovis(300 cercariae)

Analysis of the results of the schistosome recovery(Table,32) showed that the mean adult worm burden of S.bovis in the immunized group A, was lower than that of the challenge control group B, being 12 and 16.8 respectively, with a percentage reduction of 28.5 % .

S.bovis egg counts in the liver, gut and the total egg counts per mouse showed a more defined effect with a marked reduction in group A as compared with group B, (49.8 %). The differences between the means in the liver, gut and total egg counts were statistically significant ($P<0.05$; $P<0.01$ and $P<0.02$ respectively). The total

/ number....

number of eggs per female deposited in liver and gut was slightly lower in group A than in group B.

The mean adult worm burden of O.turkestanicum and eggs per mouse in the immunized group A was higher than that of group C, suggesting that the presence of the S.bovis stimulate a high egg output than occurs with the single infection. A similar phenomenon was reported by Taylor et al(1969) with S.mattheei in the presence of S.mansoni.

Experiment, 2: O.turkestanicum (50 x 3 150 cercariae) challenged with S.bovis (300 cercariae)

In this experiment immunisation was carried out by three repeated inoculation of 50, O.turkestanicum cercariae with 4 weeks interval between inoculations. The challenge exposure with S.bovis cercariae was carried out 4 weeks after the last exposure to O. turkestanicum cercariae.

/ As indicated....

As indicated in Table, 33, the mean recovery of S.bovis from the challenge infection was lower in group A than that in group B, with a reduction of 21.9 % .

Analysis of the egg counts in the tissues showed a considerable reduction in liver, gut and the total number of eggs per mouse in group A as compared with group B (total 55.8 %) . The student t-test showed that the differences between the means of the eggs in liver, gut and the total eggs of these two groups were significant ($P < 0.05$, $P < 0.02$ and $P < 0.02$ respectively). The total number of eggs per female was markedly lower in group A than in group B.

The mean burden of worms and eggs of O.turkestanicum in group A was again higher than group C but the differences was less marked than in the first experiment.

Experiment, 3: O.turkestanicum, (200 cercariae) challenged with S.bovis (300 cercariae).

Analysis of the results of this experiment (Table, 34) showed that there was a reduction of 30.1 % in worm burden of

/S.bovis ....

S.bovis in group A compared with group B. The egg counts in the tissues showed significant reductions in liver and total eggs per mouse ($P < 0.05$ and $P < 0.05$), but not in the gut. The number of eggs per female was also higher in non-immunized group B than that in group A.

Experiment, 4: O.turkestanicum (50 x 3 = 150 cercariae) challenged with S.haematobium (300 cercariae).

Analysis of the results of this experiment(Table, 35) showed that although there were no significant differences between the means of adults of S.haematobium(the reduction was only 8.7 %), the tissue egg counts in the liver were considerably reduced in challenge group A compared with control group B; a 41.1 % reduction was observed. There was a significant difference between the means of the liver egg counts and total eggs per mouse in two groups A and B ($P < 0.01$ and $P < 0.05$).

The number of eggs per female worm were lower in group A than that in group B. There were considerably more eggs per

/mouse....

mouse in group A than that in group C.

Experiment, 5: O.turkestanicum (50 cercariae) challenged with S.mansoni (150 cercariae)

Analysis of the results of this experiment (Table, 36) showed that there was no marked differences observed between the mean number of either adults or tissue egg counts of S.mansoni in the immunized and non-immunised control groups A and B.

The mean tissue egg loads of O.turkestanicum per mouse in the immunised and challenged group A was markedly higher than that in control group C.

Experiment, 6: O.turkestanicum(200 cercariae) challenged with S.mansoni (150 cercariae).

Analysis of the results of this experiment (Table, 37) showed that although there was only a slight reduction in adult S.mansoni burden, there was a statistically significant difference in the means of the total tissue egg counts with a 37.9 % reduction and P values of ($P < 0.05$, $P < 0.05$ and $P < 0.02$) in liver, gut and total eggs respectively.

/B.Homologous...

B. Homologous Immunity Studies,

Experiment, 7: *S. bovis* (100 cercariae) challenged with *S. bovis* (300 cercariae)

This experiment was designed to compare the immunizing effect of homologous infection with *S. bovis* in mice compared to the results previously obtained in calves. The analysis of the results in this experiment (Table, 38) showed a 66.0 % reduction in the adult *S. bovis* burden($P < 0.001$). Tissue egg counts in liver, gut and total eggs per mouse also showed considerable reductions (49.4 %). P values in liver, gut and total egg counts per mouse were $P < 0.2$, $P < 0.02$ and $P < 0.05$ respectively.

Table, 32

Experiment, 1: Mice immunized with O.turkestanicum and challenged with S.bovis,

Group	No. of cercariae in initial O.t. exposure	Interval (weeks)	No. of cercariae in S.bovis challenge exposure	Autopsy (weeks after initial exposure	No. of mice	Mean Worms Recovery						Reduction of S.bovis %	Tissue egg counts						Reduction in S.bovis egg load %
						P.		M.		total			Eggs/liver		Eggs/gut		Eggs/mouse		
						O.t.	S.b.	O.t.	S.b.	O.t.	S.b.		O.t.	S.b.	O.t.	S.b.	O.t.	S.b.	
A	100	10	300	19	8.	8.2	3.8	10.5	8.2	19.7	12	28.5	2187	9162	-	1000	2187	10162 (2674)*	49.8%
B	-	-	300	9	7	-	6.5	-	10.3	-	16.8		-	16971	-	3273	-	20244 (3114)*	
C	100	-	-	19	7	7.3	-	9.4	-	16.7	-		680	-	66	-	766	-	

* Egg per female

Table, 33

Experiment, 2: Mice immunized with O.turkestanicum and challenged with S.bovis.

Group	No. of cercariae in initial exposure O.t.	Interval (weeks)	No. of cercariae in S.bovis challenge exposure	Autopsy (weeks after initial exposure	No. of mice	Mean Worms Recovery F. O.t.	Mean Worms Recovery F. S.b.	Mean Worms Recovery M. O.t.	Mean Worms Recovery M. S.b.	Mean Worms Recovery total O.t.	Mean Worms Recovery total S.b.	Reduction of S.bovis %	Tissue egg counts Eggs/liver O.t.	Tissue egg counts Eggs/liver S.b.	Tissue egg counts Eggs/ gut O.t.	Tissue egg counts Eggs/ gut S.b.	Tissue egg counts Eggs/mouse O.t.	Tissue egg counts Eggs/mouse S.b.	Reduction in S.bovis egg load %
A	50x3 150	21	300	30	111	4.5	6.7	6.4	10.8	10.9	17.5	21.9	621	11581	27	1223	648	12804 (1911)*	55.8%
B	-	-	300	9	7	-	8.3	-	14.1	-	22.4		-	25630	-	3350	-	28980 (3492)*	
C	50x3 150	24	-	30	7	5.7	-	6.6	-	12.3	-		557	-	-	-	557	-	

* Egg per female

Table, 34

Experiment,3: Mice immunized with O.turkestanicum and challenged with S.bovis.

Group	No. of cercariae in initial O.t. exposure	Interval (weeks)	No. of cercariae in S.bovis challenge exposure	Autopsy (weeks after initial exposure	No. of mice	Mean Worms Recovery F. O.t.	F. S.b.	M. O.t.	M. S.b.	total O.t.	total S.b.	Reduction of S.bovis %	Tissue egg counts Eggs/liver O.t.	Eggs/liver S.b.	Eggs/gut O.t.	Eggs/gut S.b.	Eggs/mouse O.t.	Eggs/mouse S.b.	Reduction in S.bovis egg load %
A	200	11	300	20	10	20.1	5.5	24.3	6.8	44.4	12.3		1550	9550	55	680	1605	10230 (1860)*	
B	-	-	300	9	6	-	6.3	-	10.3	-	17.6	30.1	-	20700	-	516	-	21216 (2906)*	51.3%
C	100	-	-	20	9	24.6	-	24.6	-	49.2	-		1563	-	-	-	1563	-	

* Egg per female

Table, 35

Experiment,4: Mice imm unized with O.turkestanicum and challenged with S.haematobium.

Group	No. of cercariae in initial O.t. exposure	Interval (weeks)	No. of cercariae in S.hae. challenge exposure	Autopsy (weeks after initial exposure)	No. of mice	Mean Worms Recovery F. O.t.	F. S.h.	M. O.t.	M. S.h.	Total O.t.	Total S.h.	Reduction of S.haematobium%	Tissue Egg Counts Eggs/liver O.t.	Eggs/liver S.h.	Eggs/gut O.t.	Eggs/gut S.h.	Eggs/mouse O.t.	Eggs/mouse S.h.	Reduction in S.haematobium egg load %
A	50x3 150	12	300	25	10	11.5	6.7	9.2	13.1	20.7	19.8		5070	7725	95	1650	5165	9345 1394*	
B	-	-	300	13	10	-	7.6	14	14.1	-	21.7	8.7%	-	14180	-	1685	-	15865 2087*	41.1%
C	50x3 150	-	-	25	8	9	-	7.2	-	16.2	-		887		-	-	887	-	

* Eggs per female

Table, 36

Experiment,5: Mice immunized with O.turkestanicum and challenged with S.mansoni.

Group	No. of cercariae in initial O.turk. exposure	Interval (weeks)	No. of cercariae in S.mansoni challenge exposure	Autopsy (weeks after initial exposure	No. of mice	Mean Worms Recovery						Reduction of S.mansoni %	Tissue egg conts						Reduction in S. mansoni egg load %
						Female		Male		Total			Eggs/Liver		Eggs/gut		Eggs/mouse		
						O.t.	S.m.	O.t.	S.m.	O.t.	S.m.		O.t.	S.m.	O.t.	S.m.	O.t.	S.m.	
A	50	8	150	16	10	5.5	11.9	1.8	18	7.3	29.9		693	16060	280	31940	973	48000 (4033)*	
B	-	-	150	8	9	-	18,4	-	22.6	-	41	25.5	-	18600	-	33000	-	51700 (2872)*	7.1
C	50	-	-	16	18	7.9	-	2.6	-	10.5	-		20	-	-	-	-	-	

* Egg per Female

Table, 37

Experiment, 6: Mice immunized with O.turkestanicum and challenged with S.mansoni,

Group	No. of cercariae in initial O.turk. exposure	Interval (weeks)	No. of cercariae in S.mansoni challenge exposure	Autopsy (weeks after initial exposure)	no. of mice	Mean Worms Recovery						Reduction of S.mansoni %	Tissue egg counts						Reduction in % S.mansoni egg load
						Female		Male		Total			Egg/Liver		Egg/gut		Egg/mouse		
						O.t.	S.m.	O.t.	S.m.	O.t.	S.m.		O.t.	S.m.	O.t.	S.m.	O.t.	S.m.	
A	200	8	150	16	6	24.2	12.3	13.8	20.3	38	32.6	20.4	1370	13440	-	18660	1370	32100 (2610)*	37.9
B	-	-	150	8	9	-	18.4	-	22.6	-	41		-	18600	-	33000	-	51700 (2872)*	
C	200	-	-	16	7	26.7	-	21.1	-	47.8	-		2000	-	100	-	2100	-	

* Egg per Female

Table, 38

Mice immunized with S.bovis and challenged with S.bovis.

Group	No. of cercariae in initial S.bovis exposure	Interval (weeks)	No. of cercariae in S.bovis challenge exposure	Autopsy (weeks after initial exposure	Mice no.	Mean worm recovery			Reduction of S.bovis adult %	Tissue egg counts			Reduction in S. bovis egg load %
						Female	Male	total		Egg/liver	Egg/gut	Egg/mouse	
A	100	9	300	19	8	4.3	7.4	11.6		26,000	2,900	28,900 (2486)*	
B	-	-	300	10	8	14.6	19.6	34.2	66.1	47,800	9,337	57,137 (3893)*	49.4
C	100	-	-	19	4	2.5	4.7	7.2		7050	0	7050	

* Egg per Female.

CHAPTER. 4

RESULTS IN CALVES AND SHEEP,

1. CALVES,

A. Heterologous Immunity Studies,

Experiment, 1: O.turkestanicum versus S.bovis

The calves were divided into three groups. Group I consisted of 3 calves which were each exposed to 8,000 cercariae of O.turkestanicum but not challenged; they were killed 9 weeks after exposure to the cercariae. Group II consisted of 3 calves each exposed to 8,000 cercariae of O.turkestanicum and challenged 9 weeks later with 5,000 cercariae of S.bovis; they were killed after further 9 weeks. Group III consisted of 2 calves each exposed only to 5,000 cercariae of S.bovis, serving as the control for the calves in group II.

The results of this experiment are given in Table, 39 and 40. There was a reduction of 37.7 % adult S.bovis in the immunized calves compared with their challenge control group. The corresponding reductions in tissue egg counts were also

/considerable...

considerable, showing a reduction of 71.3 %, 65.6 % and 76.2 % in the liver, small intestine and large intestine respectively compared with the egg counts in control group III.

Experiment, 2: S.bovis versus O.turkestanicum,

The design of this study was the same as the previous experiment: there were 3 animals in each group except for group I which had 2 calves. The number of immunising S.bovis cercariae was 5,000, challenged by 8,000 cercariae of O.turkestanicum.

The results of this experiment are shown in Tables 41 and 42. The mean number of O.turkestanicum adults was reduced to 29.7 % of those in the challenge group II, and reduction of tissue egg counts per gram of liver and small intestine were 61.3 % and 77.0% respectively.

Experiment, 3: S.haematobium versus S.bovis,

The calves were divided into a non-immunized group I and immunized group II. In group I, calves were first immunized

/with....

with cercariae of S.haematobium and then challenged with cercariae of S.bovis. Immunisation of calves group I was by 7,000 S.haematobium cercariae for each exposure at 4 weeks interval giving a total of 21,000 cercariae per calf.

It was found that the mean worm recovery of S.bovis in immunized group I was much lower than that in the control group II, with a reduction of 42.3 % (Table, 43). Distinguishing S.haematobium from S.bovis was easy, since all the S.haematobium worms were very small and immature with undeveloped internal organs.

S.bovis egg counts in the liver, small intestine and large intestine were very reduced in group I compared with control group II: reduction of the 30.1 % in the liver, 45.0 % in the small intestine and 90.8 % in the large intestine (Table, 44).

Experiment, 4: S.haematobium versus O.turkestanicum,

The details of this experiment are given in Tables, 45 and 46. The mean recovery of adult O.turkestanicum was 31.4 % lower in

/challenge....

challenge group I than that in control group II. The corresponding reduction in tissue egg counts per gram of liver and small intestine was 17.5 % and 82.5 % respectively.

The results of the above experiment on calves showed that there is a strong interaction and cross-protection between S.bovis, O.turkestanicum and S.haematobium. This suggests that the immune phenomenon will occur in endemic areas like Khuzestan and that under natural condition this might reduce the severity of the disease in livestock.

B. Homologous Immunity Studies,

Experiment, 1: O.turkestanicum versus O.turkestanicum,

In this experiment 2 groups each of 2 calves were used. The results are given in Tables, 47 and 48. The mean number of O.turkestanic. adult worms recovered in group I was 1,400 while in the non-immunized control group II the mean recovery of adult worms were 2,434. The reduction in the immunized group was 42.4 % . Further evidence of

/protection...

protection can be seen in the tissue egg counts. In the immunized group I, the number of eggs in the liver was the same as in the non-immunized control group II, but in the small intestine there was a considerable reduction of egg densities (72.8 %).

Experiment, 2: S.bovis versus S.bovis,

Calves were divided into immunized and non-immunized groups.

Analysis of the results (Tables, 49 and 50) showed that there was no reduction in the worm recovery in the immunized group, but the tissue egg counts were reduced in the immunized group I animals compared with the non-immunized control group II by 21.2 % in liver, 46.9 % in small intestine and 76.0 % in large intestine.

/ 2. SHEEP, ...

2. SHEEP.

Heterologous Immunity Studies.

Experiment, 1: O.turkestanicum versus S.bovis.

The results of this experiment are given in Tables, 51 and 52. The reduction of worm burden was 8.2 % and the reduction of tissue egg counts was 19.8 % in liver, 15.4 % in small intestine and 26.3 % in large intestine.

Experiment, 2: S.bovis versus O.turkestanicum.

Analysis of the results of this experiment (Tables, 53 and 54) showed a reduction of 43.6 % in adult worms of O.turkestanicum burden and a reduction of tissue egg counts of 87.4 % in liver and 22.1 % in small intestine. The effect of immunisation in sheep in group II was variable: in sheep no. 2 low numbers of adult O.turkestanicum (432 worms) and no eggs were found in the liver digestion, with very low egg

/counts....

counts of 350 eggs per gram of small intestine, whereas sheep no.1 in the same group had 1569 worms and 13,475 eggs per gram of small intestine.

Table, 39

Experiment, 1. Immunisation against S.bovis with cercariae of O.turkestanicum in calves: Effect on adult worm counts.

Group	Calf no.	Number of cercariae			Autopsy (weeks after initial exposure)	Worms Recovery						Average number of S.bovis adults	Reduction in S.bovis adult loads %
		Initial exposure to O.turk.	Interval (weeks)	Challenge exposure with S.bovis		Female		Male		Total			
						O.t.	S.b.	O.t.	S.b.	O.t.	S.b.		
Group I O.turk. only	1	8,000	-	-	9	1022	-	1420	-	2442	-		
	2	8,000	-	-	9	1824	-	1028	-	1862	-		
	3	8,000	-	-	9	2377	-	3073	-	5450	-		
Average						1411		1840		3251			
Group II O.turk. x S.bovis	1	8,000	9	5,000	18	571	676	685	1136	1256	1812	1966	37.7
	2	8,000	9	5,000	18	972	660	1300	1586	2272	2246		
	3	8,000	9	5,000	18	921	744	2110	1098	3031	1842		
Average						821	693	1365	1273	2186	1966		
Group III S.bovis only	1	-	-	5,000	9	-	1250	-	1533	-	2783	3156	
	2	-	-	5,000	9	-	1661	-	1869	-	3530		
Average							1455		1701		3156		

Table, 40

Experiment, 1. Immunisation against S.bovis with cercariae of O.turkestanicum

in calves: Effect on tissue egg counts.

Group	Calf no.	Number of cercariae			Autopsy (weeks after initial exposure	Tissue egg counts per gram						Average number of S.bovis egg/gram	Reduction in S.bovis egg load (Mean) %
		Initial exposure to O.turk.	Interval (weeks)	Challenge exposure with S.bovis		Liver		Small intestine		Large intestine			
						O.t.	S.b.	O.t.	S.b.	O.t.	S.b.		
Group I O.turk. only	1	8,000	-	-	9	280	-	22830	-	-	-		
	2	8,000	-	-	9	116	-	7773	-	-	-		
	3	8,000	-	-	9	816	-	22100	-	-	-		
Average						404		17567					
Group II O.turk. x S.bovis	1	8,000	9	5,000	18	50	786	11700	580	-	1086		
	2	8,000	9	5,000	18	117	773	11380	1065	-	967	926	72.5
	3	8,000	9	5,000	18	170	1058	7820	562	-	1316		
Average						112	872	10300	735		1123		
Group III S.bovis only	1	-	-	5,000	9	-	3597	-	1350	-	7312	3312	
	2	-	-	5,000	9	-	2556	-	2927	-	2130		
Average							3076		2138		4721		
Reduction of S.bovis egg per gram of tissues %							71.6		66.0		76.2		

Table, 41

Experiment,2. Immunisation against O.turkestanicum with cercariae of S.bovis in calves: Effect on adult worm counts.

Group	Calf No.	Number of cercariae			Autopsy (weeks after initial exposure)	Worms Recovery						Average number of O.turk. adults	Reduction in O.turk. adult loads %
		Initial exposure to S.bovis	Interval (weeks)	Challenge exposure with O.turk.		Female		Male		Total			
						S.B.	O.t.	S.b.	O.t.	S.b.	O.t.		
Group I S.bovis only	1	5,000	-	-	9	1250	-	1533	-	2783	-		
	2	5,000	-	-	9	1661	-	1869	-	3530	-		
Average						1455		1701		3530			
Group II S.bovis x O.turk.	1	5,000	9	8,000	18	1357	952	1840	1198	3377	2150	2283	29.7
	2	5,000	9	8,000	18	1541	853	2021	1011	3562	1866		
	3	5,000	9	8,000	18	1397	1145	1296	1689	2693	2834		
Average						1491	983	1719	1300	3210	2283		
Group III O.turk. only	1	-	-	8,000	9	-	1022	-	1420	-	2442	3251	
	2	-	-	8,000	9	-	834	-	1028	-	1862		
	3	-	-	8,000	9	-	2377	-	3073	-	5450		
Average							1411		1840		3251		

Table, 42

Experiment, 2. Immunisation against O.turkestanicum with cercariae of S.bovis

in calves : Effect on tissue egg counts.

Group	Calf no.	Number of cercariae: Initial exposure to S.bovis	Number of cercariae: Interval (weeks)	Number of cercariae: Challenge exposure with O.turk.	Autopsy (weeks after initial exposure	Tissue egg counts per gram: Liver S.b.	Liver O.t.	Small intestine S.b.	Small intestine O.t.	Large intestine S.b.	Large intestine O.t.	Average number of O.turk. egg/gram	Reduction in O.turk. egg load (Mean) %
Group I S.bovis only	1	5,000	-	-	9	3597	-	1350	-	7312	-		
	2	5,000	-	-	9	2556	-	2927	-	2130	-		
Average						3076		2138		4721			
Group II S.bovis x O.turk.	1	5,000	9	8,000	18	3530	215	859	1230	1135	-	2078	76.8
	2	5,000	9	8,000	18	1760	183	1783	3423	587	-		
	3	5,000	9	8,000	18	1110	71	1177	7350	1110	-		
Average						2133	156	1266	4001	943			
Group III O.turk. only	1	-	-	8,000	18	-	280	-	22830	-	-	8985	
	2	-	-	8,000	18	-	116	-	7773	-	-		
	3	-	-	8,000	18	-	816	-	22100	-	-		
Average							404		17567				
Reduction of O.turkestanicum egg per gram of tissues %							61.3		77.1				

Table, 43

Experiment,3. Immu nisation against S.bovis with cercariae of S.haematobium in calves: Effect on adult worm counts.

Group	Calf no.	Number of cercariae			Autopsy (weeks after initial exposure	Worms Recovery						Average number of S.bovis adults	Reduction in S.bovis adult loads %
		Initial exposure to S.haem.	Interval (weeks)	Challenge exposure with S.bovis		Female		Male		Total			
						S.ha.	S.b.	S.ha.	S.b.	S.ha.	S.b.		
Group I S.haem. x S.bovis	1	21,000 (7000 x3)	13	5,000	22	251	1104	167	934	418	2038	1819	42.3
	2	"	13	5,000	22	111	794	139	806	250	1600		
Average						181	949	153	870	334	1819		
Group II S.bovis only	1	-	-	5,000	9	-	1250	-	1533	-	2783	3156	
	2	-	-	5,000	9	-	1661	-	1869	-	3530		
Average							1455		1701		3156		

Table,44

Experiment,3. Immunisation against S.bovis with cercariae of S.haematobium in calves: Effect on tissue egg counts.

Group	Calf no.	Number of cercariae			Autopsy (weeks after initial exposure	Tissue egg counts per gram						Average number of S.bovis egg/gram	Reduction in S.bovis eggload (Mean) %
		Initial exposure to S.haem.	Interval (weeks)	Challenge exposure with S.bovis		Liver		Small intestine		Large intestine			
						S.ha.	S.b.	S.ha.	S.b.	S.ha.	S.b.		
Group I S.haem. x S.bovis	1	21,000 (7000 x 3)	13	5,000	22	0	2990	0	1690	0	380	1232	62.2
	2	"	13	5,000	22	0	1400	0	750	0	480		
Average							2150		1175		430		
Group II S.bovis only	1	-	-	5,000	9	-	3597	-	1350	-	7312	3312	
	2	-	-	5,000	9	-	2556	-	2927	-	2130		
Average							3076		2138		4721		
Reduction of S.bovis egg per gram of tissue %							30.1		45.0		90.8		

Table, 45

Experiment, 4: Immunisation against O.turkestanicum with cercariae of S.haematobium in calves: Effect on adult worm counts.

Group	Calf no.	Number of cercariae: Initial exposure to S.haem.	Number of cercariae: Interval (weeks)	Number of cercariae: Challenge exposure with O.turk.	Autopsy (weeks after initial exposure	Worms Recovery: Female S.ha.	Worms Recovery: Female O.t.	Worms Recovery: Male S.ha.	Worms Recovery: Male O.t.	Worms Recovery: Total S.ha.	Worms Recovery: Total O.t.	Average number of O.turk. adults	Reduction in O.turk. adult loads %
Group I S.haem. x O.turk.	1	21,000 (7000x3)	13	8,000	22	186	1199	454	1530	640	2729	2230	31.4
	2	"	13	8,000	22	43	828	161	903	204	1731		
Average						114	1013	308	1216	422	2230		
Group II O.turk. only	1	-	-	8,000	9	-	1022	-	1420	-	2442	3251	
	2	-	-	8,000	9	-	824	-	1028	-	1862		
	3	-	-	8,000	9	-	2377	-	3073	-	5450		
Average							1411		1840		3251		

Table, 46

Experiment,46. Immunisation against O.turkestanicum with cercariae of S.haematobium : Effect on tissue egg counts (in calves).

Group	Calf no.	Number of cercariae: Initial exposure to S.haem.	Number of cercariae: Interval (weeks)	Number of cercariae: Challenge exposure with O.turk.	Autopsy (weeks after initial exposure)	Liver: S.ha.	Liver: O.t.	Small intestine: S.ha.	Small intestine: O.t.	Large intestine: S.ha.	Large intestine: O.t.	Average number of O.turk. egg/gram	Reduction in O.turk. eggload (Mean) %
Group I S.haem. x O.turk.	1	21,000 (700 x 3)	13	8,000	22	16	453	33	3040	40	-	1706	81.0
	2	"	13	8,000	22	0	212	0	3118	0	-		
Average						8	333	16	3019	20			
Group II O.turk. only	1	-	-	8,000	9	-	280	-	22830	-	-	8985	
	2	-	-	8,000	9	-	116	-	7773	-	-		
	3	-	-	8,000	9	-	816	-	22100	-	-		
Average							404		17567				
Reduction of O.turk. egg per gram of tissue %							17.5		82.4				

Table, 47

Experiment, 1. Immunisation against *O. turkestanicum* with cercariae of *O. turkestanicum* in calves: Effect on adult worm counts.

Group	Calf No.	Number of cercariae			Autopsy (weeks after initial exposure	Worms recovery			Average number of *O. turk.* adults	Reduction in *O. turk.* adults load %
		Initial exposure to *O. turk.*	Interval (weeks)	Challenge exposure with O. turk.		Female	Male	total		
Group I *O. turk.* x *O. turk.*	1	1000	9	4000	18	707	900	1607	1400	42.4
	2	1000	9	4000	18	481	712	1193		
Average						594	806	1400		
Group II *O. turk.* only	1	1000	-	5000	9	813	1412	2225	2434	
	2	-	-	5000	9	1272	1371	2643		
Average						1042	1392	2434		

Table, 48

Experiment,1. Immunisation against O.turkestanicum with cercariae of O.turkestanicum in calves: Effect on tissue egg counts.

Group	Calf No.	Number of cercariae			Autopsy weeks after initial exposure	Tissue egg counts/gram			Average number of O.turk. egg/gram	Reduction in O.turk. eggload % (mean)
		Initial exposure to O.turk.	Interval (weeks)	challenge exposure with O.turk.		Liver	Small intestine	Large intestine		
Group I O.turk. x O.turk.	1	1000	9	4000	18	150	5000	-	1686	72.8 %
	2	1000	9	4000	18	76	1520	-		
Average						113	3260	-		
Group II O.turk. only	1	-	-	5000	9	160	9400	-	6055	
	2	-	+	5000	9	80	14600	-		
Average						110	12000	-		
Reduction of O.turk.egg per gram of tissue %						0	72.8			

Table, 49

Experiment, 2. Immunisation against S.bovis with cercariae of S.bovis in calves: Effect on adult worm counts.

Group	Calf No.	Number of cercariae			Autopsy weeks after initial exposure	Worms recovery			Average number of S.bovis adults	Reduction in S.bovis adults load %
		Initial exposure to S.bovis	Interval (weeks)	challenge exposure with S.bovis		Female	Male	total		
group I S.bovis x S.bovis	1	1000	9	4000	18	1242	1804	3049	3242	0
	2	1000	9	4000	18	1508	1928	3436		
Average						1376	1866	3242		
Group II S.bovis only	1	-	-	5000	9	1343	1358	2701	2893	
	2	-	-	5000	9	1380	1705	3085		
Average						1361	1532	2893		

Table, 50

Experiment,2. Immunisation against S.bovis with cercariae of S.bovis in calves: Effect on tissue egg counts.

Group	Calf No.	Number of cercariae: Initial exposure to S.bovis	Number of cercariae: Interval (weeks)	Number of cercariae: challenge exposure with S.bovis	Autopsy weeks after initial exposure	Tissue egg counts/gram: Liver	Tissue egg counts/gram: Small intestine	Tissue egg counts/gram: Large intestine	Average number of S.bovis egg/gram	Reduction in S.bovis eggload %
Group I S.bovis x S.bovis	1	1000	9	4000	18	1466	1550	496	1170	50.2 %
	2	1000	9	4000	18	870	2115	520		
Average						1168	1832	508		
Group II S.bovis only	1	-	-	5000	9	886	3095	2350	2350	
	2	-	-	5000	9	2080	3812	1883		
Average						1483	3453	2116		
Reduction of S.bovis egg per gram of tissue %						21.2	46.9	76.0		

Table, 51

Experiment, 1. Immunization against S.bovis with cercariae of O.turkestanicum in sheep: Effect on adult worm counts.

Group	Sheep no.	Number of cercariae: Initial exposure to O.turk.	Number of cercariae: Interval (weeks)	Number of cercariae: Challenge exposure with S.bovis	Autopsy (weeks after initial exposure	Worms Recovery: Female O.t.	Worms Recovery: Female S.b.	Worms Recovery: Male O.t.	Worms Recovery: Male S.b.	Worms Recovery: Total O.t.	Worms Recovery: Total S.b.	Average number of S.bovis adults	Reduction in S.bovis adult loads %
Group I	1	5,000	-	-	9	514	-	620	-	1134	-		
O.turk.	2	5,000	-	-	9	965	-	1377	-	2342	-		
only	3	5,000	-	-	9	630	-	1217	-	1847	-		
Average		5,000				703		1071		1774			
Group II O.turk.	1	5,000	9	5,000	18	551	1045	1668	1251	2219	2296	1974	8.2
x S.bovis	2	5,000	9	5,000	18	415	775	514	878	929	1653		
Average						483	910	1091	1064	1574	1974		
Group III	1	-	-	5,000	9	-	1191	-	1305	-	2496	2151	
S.bovis only	2	-	-	5,000	9	-	897	-	909	-	1806		
Average							1044		1107		2151		

Table, 52

Experiment,1. Immunisation against S.bovis with cercariae of O.turkestanicum

in sheep: Effect on tissue egg counts.

Group	Sheep no.	No. of cercariae: Initial exposure to O.turk.	No. of cercariae: Interval (weeks)	No. of cercariae: Challenge exposure with S.bovis	Autopsy weeks after initial exposure	Tissue egg counts per gram: Liver O.t.	Liver S.b.	Small intestine O.t.	Small intestine S.b.	Large intestine O.t.	Large intestine S.b.	Average number of S.bovis egg/gram	Reduction in S.bovis egg load (mean) %
Group I O.turk. only	1	5,000	-	-	9	1458	-	16700	-	-	-		
	2	5,000	-	-	9	1197	-	5780	-	-	-		
	3	5,000	-	-	9	630	-	4160	-	-	-		
Average						1095		8880					
Group II O.turk. x S.bovis	1	5,000	9	5,000	18	570	8300	8020	1818	-	3960	5139	20.6
	2	5,000	9	5,000	18	877	7330	5950	6020	-	3408		
Average						723	7815	6950	3919		3684		
Group III S.bovis only	1	-	-	5,000	9	-	13200	-	2958	-	5070	6462	
	2	-	-	5,000	9	-	6375	-	6312	-	4940		
Average							9757		4635		5005		
Reduction of S.bovis egg per gram of tissues %							19.8		15.4		26.3		

Table, 53

Experiment,2. Immunisation against O.turkestanicum with cercaraie of S.bovis in sheep: Effect on adult worm counts.

Group	Sheep no.	Number of cercariae			Autopsy (weeks after initial exposure)	Worms Recovery						Average number of O.turk. adults	Reduction in O.turk. adult loads %
		Initial exposure to S.bovis	Interval (weeks)	Challenge exposure with O.turk.		Female		Male		Total			
						S.b.	O.t.	S.b.	O.t.	S.b.	O.t.		
Group I S.bovis only	1	5,000	-	-	9	1191	-	1305	-	2496	-		
	2	5,000	-	-	9	897	-	909	-	1806	-		
Average						1044		1107		2151			
Group II S.bovis x O.turk.	1	5,000	9	5,000	18	1086	656	1210	913	2296	1569	1000	43.6
	2	5,000	9	5,000	18	815	83	874	349	1689	432		
Average						950	369	1042	631	1992	1000		
Group III O.turk. only	1	-	-	5,000	9	-	514	-	620	-	1134	1774	
	2	-	-	5,000	9	-	965	-	1377	-	2342		
	3	-	-	5,000	9	-	630	-	1217	-	1847		
Average							703		1071		1774		

Table, 54

Experiment, 2. Immunisation against O.turkestanicum with cercariae of S.bovis

in sheep: Effect on tissue egg counts.

Group	Sheep no.	Number of cercariae			Autopsy weeks after initial exposure	Tissue egg counts per gram						Average number of O.turk. egg/gram	Reduction in O.turk. egg load (mean) %
		Initial exposure to S.bovis	Interval (weeks)	Challenge exposure with O.turk.		Liver		Small intestine		Large intestine			
						S.b.	O.t.	S.b.	O.t.	S.b.	O.t.		
Group I S.bovis only	1	5,000	-	-	9	13200	2958	2958	-	5070	-		
	2	5,000	-	-	9	6315	-	6312	-	4940	-		
Average						9757		4635		5005			
Group II S.bovis x O.turk.	1	5,000	9	5,000	18	16316	276	15455	13475	11200	-	3525	29.5
	2	5,000	9	5,000	18	31830	0	14050	350	5230	-		
Average						24073	138	14752	6912	8215			
Group III O.turk. only	1	-	-	-	9	1458	1458	-	16700	-	-	4987	
	2	-	-	-	9	-	1197	-	5780	-	-		
	3	-	-	-	9	-	630	-	4160	-	-		
Average							1095		8880				
Reduction of O.turkestanicum egg per gram of tissues %							87.4		21.0				

CHAPTER. 5

DISCUSSION.

The results of the experiments on mice are summarized in Table,55. Initial single or multiple exposures to O. turkestanicum cercariae induced a moderate partial protection against the challenge infection with S.bovis and S.haematobium, but to a less extent against S.mansoni. The immunity was evident from both the reduction of worm burdens and the tissue egg counts. Initial doses of 100 cercariae or more produced partial protection, but 50 cercariae produced very poor resistance. It seems likely that with this parasite there is a minimum immunizing exposure as was reported by Smithers and Terry(1965) for S.mansoni in the rhesus monkey. This threshhold in O.turkestanicum is less than 100 cercariae.

A single exposure to O.turkestanicum cercariae produced immunity as effectively as repeated exposures. Furthermore, the degree of resistance in mice showed no correlation with the number

/of cercariae...

of cercariae used since 100 cercariae of _O.turkestanicum_ produced the same degree of protection as 200 cercariae or 150 cercariae in 3 inoculations. Hunter _et al_(1962) also found that with _S.mansoni_ in albino mice multiple exposure was no more effective in producing immunity to a challenge infection than a single exposure to the same total number of cercariae. On the other hand Kagan (1952), Thompson (1963), Wang _et al_(1958), Hsu _et al_(1963), Amin and Nelson (1969) suggested that resistance to schistosome infections is increased by repeated immunisation.

The experiment on homologous immunity in mice with _S.bovis_ showed that there was a moderate protection against the subsequent infection after immunisation with this species.

The results of the heterologous immunity studies in calves are summarized in Table, 56 and show reductions of 29.7 % to 42.4 % in adult worms load and 50.2 % to 81.0 % in tissue egg counts per

/gram....

gram of tissues in immunized groups compared with the non-immunized control groups. A strong reciprocal protection was demonstrated between S.bovis and O.turkestanicum in calves, the natural host of these parasites. The immunity in sheep (Table, 56) was less effective.

Results from calves together with the previous study which show some degree of 'self cure ' in S.bovis and O.turkestanicum infections in calves suggests calves are useful animals for studying immunity in schistosomiasis particularly as they are the natural hosts of these parasites.

The only previous report of heterologous studies in calves was by Hussein et al(1970) who reported a high degree of partial protection in calves immunized with S.mansoni against challenge with S.mattheei.

S.haematobium in the calves failed to develop to maturity. Nevertheless immunisation with 3 inoculations of S.haematobium

/cercariae....

cercariae produced very strong cross-protection against _S.bovis_ and _O.turkestanicum_. This experiment was the reverse of those reported by Hsu _et al_(1966) who immunized rhesus monkeys repeatedly with Iranian _S.bovis_ cercariae and then challenged them with Iranian _S.haematobium_ and produced a strong protection.

In our homologous immunity experiment in calves with _S.bovis_ no reduction in worm burden was observed in the immunized group compared with the non-immunized group, but in homologous immunity with _O. turkestanicum_ in calves a 42.4 % reduction of worms load was observed. There was a considerable reduction in tissue egg counts in the immunized groups, 50.2 % in _S.bovis_ and 72.8 % in the _O.turkestanicum_ experiments. In our observations on cattle naturally infected with _O.turkestanicum_ the worms load and tissue egg counts also declined with increasing age, which could be attributed to the development of some degree of natural acquired immunity either homologous or heterologous.

/We conclude....

We conclude from these experiments that natural heterologous and homologous immunity between both the bovine parasites and the human parasite S.haematobium could be a great importance in protecting the animals from the severe effects of subsequent reinfections. A similar effect may be of general importance in schaitosomiasis of domestic animals in many endemic areas.

Table, 55

Summary of work done on heterologous and homologous immunity between schistosome species in albino mice, percentage of reduction in worm load and tissue egg counts.

Immunized Species		Challenged Species		
	No. of cercariae	*S.bovis*	*S.haem.*	*S.mansoni*
O.turk.	50	-	-	25.5 (7.1)
	100	28.5 (49.8)	-	-
	150 (50x3)	21.9 (55.8)	8.7 (41.1)	-
	200	30.1 (51.3)	-	20.4 (37.9)
S.bovis	100	66.1 (49.4)	-	-

( ) Reduction of the mean egg counts per gram of tissues.

Table, 56

Summary of work done on heterologous and homologous immunity between schistosome species in calves and sheep, percentage of reduction in worm load and tissue egg counts.

Immunized Species	Challenged Species			
	Calves		Sheep	
	O.turk.	S.bovis	O.turk.	S.bovis
O.turk.	42.4 (72.8)	37.7 (72.5)	- -	8.2 (20.5)
S.bovis	29.7 (76.8)	0 (50.2)	43.6 (29.5)	- -
S.haem.	31.4 (81.0)	42.3 (62.2)	- -	- -

( ) Reduction of the mean egg counts per gram of tissues.

ADDENDUM

THE FIRST RECORD OF EXPERIMENTAL INFECTION OF CALVES WITH S.HAEMATOBIUM

INTRODUCTION,

In the above study on heterologous immunity calves were exposed to a large number of S.haematobium cercariae;at autopsy,22 weeks after initial exposure immature worms were present. There are no previous records of S.haematobium infection in cattle. Kuntz and Malakatis (1955) exposed goats to S.haematobium cercariae and only a very few small worms were recovered; Leiper (1915); MacHattie(1933); Saeed(1970) failed to infect sheep with S.haematobium.

In the present experiments 4 calves 7-9 months old from Khuzestan were exposed to a total of 21,000 cercariae of S.haematobium each, in 3 inoculations at 4 weeks intervals (in each inoculation 7,000 cercariae were used). For each inoculation, cercariae were

/pooled....

pooled from 20-30 Bulinus truncatus which were infected from a human source in the laboratory.

After 13 weeks from initial exposure the calves were challenged with S.bovis or O.turkestanicum cercariae to study the cross-immunity against animal schistosomes.

RESULTS,

The red papules on the skin of the legs showed that the cercariae had penetrated, when the animals were killed 22 weeks after initial exposure to S.haematobium cercariae it was found that all the 4 calves had developed limited S.haematobium infections (Table, 55). The number of worms recovered by perfusion varied from 250 to 640 (378 ± 99), the percentage of worms recovered was 3.0 %, 0.9 %, 1.9 % and 1.2 % with a mean of 1.8 % .

In tissue digestion for egg counts only calf no. 1 revealed a few deformed, rudimentary black eggs with no miracidia (Table, 55).

/ Most....

Most of the worms were found in the portal veins, but in calf no. 1 some large worms with deformed intra-uterine eggs were recovered from the lower mesenteric veins of the large intestine, but no worms or eggs were found in the vesciсal plexus or bladder wall. The immature S.haematobium worms were easily distinguished from O. turkestanicum and S.bovis worms because they were smaller in size and the females had undeveloped vitellaria.

DISCUSSION,

Not only domestic animals but also various species of rodents have been known as reservoir of great epidemiological importance for S.japonicum, one of the three principal species of the schistosome parasite of the man. As for the other two species S.haematobium and S.mansoni, it was believed until recently that man was the only host of epidemiological importance. A wide variety of rodents have been found naturally infected with S.mansoni but it is generally considered that they are unlikely to be of any significance as maintenance hosts.

/But recently...

But recently it has been shown that baboons in East-Africa act as a reservoir host for S.mansoni (Nelson et al,1962; Fenwick,1966,1969). Barbosa et al(1962) found adult S.mansoni in cattle slaughtered in an abattoir in Brazil and Saeed et al(1969) successfully infected calves with S.mansoni but their significance as natural maintenance host is still unknown.

Records of S.haematobium infections in animals are rare. Nelson (1960) reported two cases in East Africa one in a baboon and the other in a Cercopithecus monkey. A natural infection of S. haematobium in a West African chimpanzee was reported by De Paoli(1965). Recently one baboon out of 24 from West Africa was found to be naturally infected with S.haematobium and was passed viable eggs in both the faeces and urine (Taylor, 1971 personal comunication).

Kuntz and Malakatis (1955) exposed 3 goats to about 200,000 cercariae of S.haematobium each, and succeeded in infecting 2 of them,

/however...

however, the number of parasites recorded was extremely small. Leiper(1915), MacHattie and Chadwick(1932); MacHattie,et al(1933); Saeed (1970) were unable to infect sheep with S.haematobium. Saoud(1966) also failed to infect pigs with S.haematobium cercariae in laboratory and disputed the report by Hill and Omabamiro(1960) who clamed to have found a natural S.haematobium infection in a pig in Nigeria.

Our experiments with S.bovis and S.haematobium and O. turkestanicum show that success in experimentally infecting calves depends largely on the technique used. Using an efficient technique calves were showed to take S.haematobium infection but the worms recovered were immature. This may also happen to calves in nature in endemic areas, since they live in very close contact with infected natural waters. The immune-response which developed in calves with S.haematobium infection was considerable and was discussed in the previous chapter.

Table, 55

Recovery of adults and eggs of S.*haematobium* in calves infected with total 21,000 cercariae in 3 inoculations at 4 weeks intervals.

No. of calves	No. of cercariae	Duration of infection (weeks)	Worms Recovery				Tissue egg counts/gram			
			Female	Male	Total	% Recovery	Liver	Small intestine	Large intestine	Mean
1.	7000x3 (21000)	22	186	454	640	3.0	16	33	40	29
2.	"	22	43	161	204	0.9	0	0	0	0
3.	"	22	251	167	418	1.9	0	0	0	0
4.	"	22	111	139	250	1.2	0	0	0	0
Mean (S.E.)			148 (45)	230 (75)	378 (99)	1.8 (0.5)	4	8	10	7

PART IV

FIELD AND LABORATORY OBSERVATIONS ON LYMNAEA GEDROSIANA THE INTERMEDIATE HOST OF ORNITHOBILHARZIA TURKESTANICUM IN KHUZESTAN

INTRODUCTION,

L.gedrosiana (Annandale & Prashad) is a molluscan host of 2 important parasites of domestic animals (*O.turkestanicum* and *Fasciola gigantica*) in Khuzestan. Therefore, control measures against the snail are of great economic importance. detailed biological and ecological studies are necessary before control measures can be directed against *L.gedrosiana*. This approach has been used in other parts of the world against the snail hosts of human schistosomiasis (Pesigan *et al*,1958; Shiff, 1964; Webbe, 1962 and Chu *et al*, 1968).

The present study records observations on the transmission of *O.turkestanicum*, the biology and ecology of the principal molluscan host, seasonal fluctuations in snail population densities and associated *O.turkestanicum* infection rates and laboratory experiments designed to study the " vector " potential of *L.gedrosiana*.

/ Chapter, 1 ....

CHAPTER, 7

OBSERVATIONS ON THE GEOGRAPHICAL DISTRIBUTION OF L.GEDROSIANA IN KHUZESTAN

The L.gedrosiana survey was started in 1967 in conjunction with the B.truncatus survey. L.gedrosiana was found to be distributed all over the irrigation systems of Khuzestan. Most of the primary and secondary canals, water-sheds, isolated breeding sites such as ponds, swamps and ditches were visited. Map,1 shows that B.truncatus and L.gedrosiana were mostly found in the same areas. The following accounts give detail of the study area:

The Main Focus,

This area is the largest and agriculturally the richest plain and the best irrigated area in the Khuzestan; it is bordered in the north by the slopes north of Dezful and Andimeshk, and in the west and east by the Karkheh and Karun rivers, respectively, and

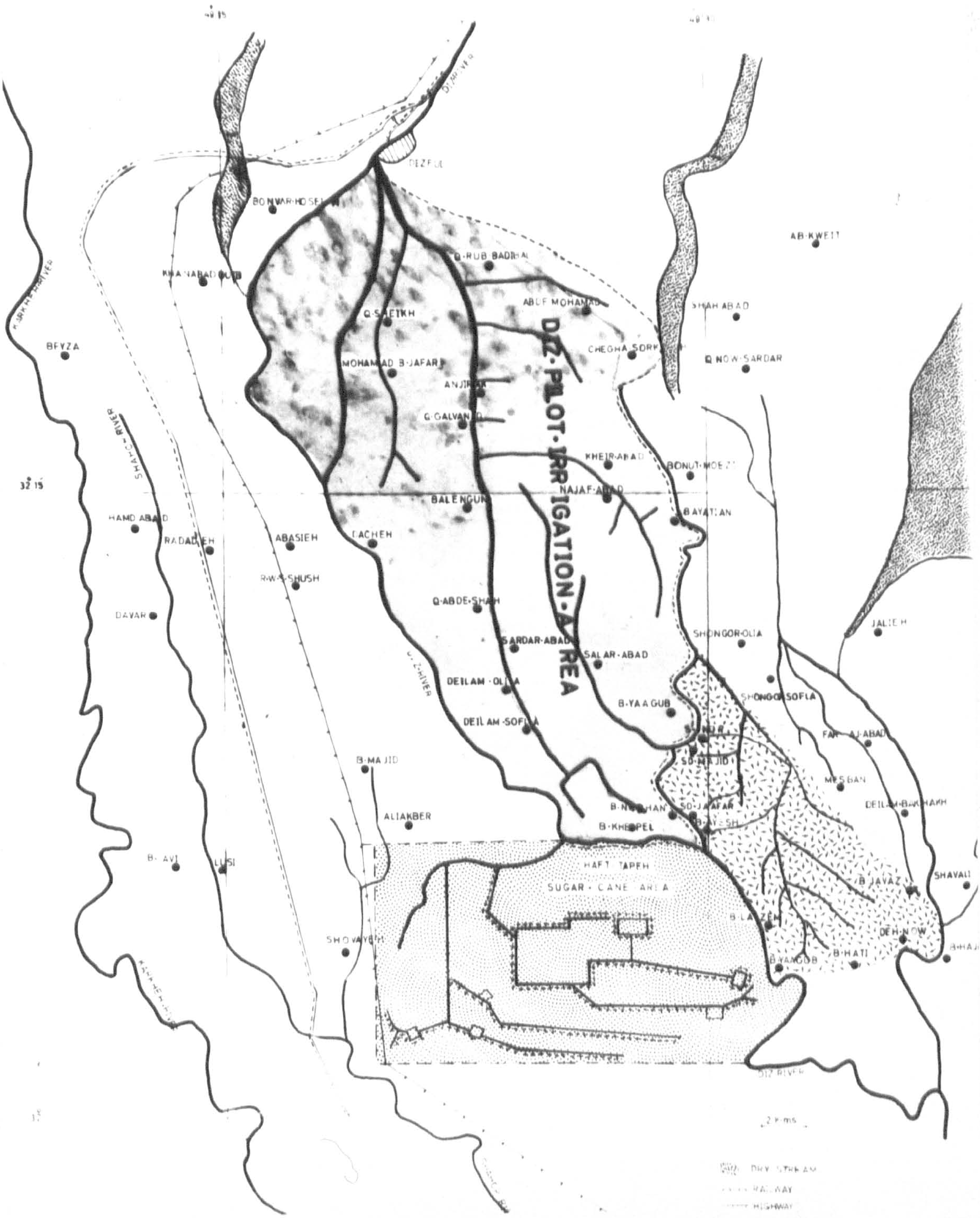
/terminates....

terminates in the south near Ahwaz. For convenience of description, the area has been divided into 3 namely, the northern area, the sugar cane area and the southern area.

The Northern Area (Map, 2)

The distribution of snails in this area during the period from 1959 to 1961 was described by Gaud *et al* (1962) and Chu *et al* (1968). In the centre covering an area of 22,000 ha, is a new irrigation system which was installed between 1962 and 1964; water is drawn only from the Dez river. Previously there had existed an old system of earth canals and flow of water in them was rapid. The new irrigation system includes main canals, lateral and tertiary canals; some of the canals are cement lined. The old canals are still used to distribute water to farming land and fruit gardens and as drains to collect water from the field and to discharge it into new large drains which finally empty themselves into the Abjirob stream and the Dez river. A number of canals are not in use and some of them dry out from time to time.

/The snails....



Map, 2

Details of rivers, streams, canals and villages, etc, in the Northern part of the main foci of <u>Lymnaea gedrosiana</u> and <u>Bulinus truncatus</u> in Khuzestan.

The snails were mostly found in canals, drains, swamps, ricefields, ponds and springs. From 1965 onwards, many new habitats were found-consisting of swamps and sidepools along new canals and new highways. The increased use of water in the ricefields may account for the further spread of the snails.

Between the lower ends of the new irrigation area and the Lureh area is the Abjirob area where a chain of irrigation canals has existed for some 20 years, the source of water here is the Abjirob stream, which also collects water from the new irrigation area and discharges through a dam into Dez river. B.truncatus and L.gedrosiana were found in most canals at their terminal ends in swamps adjoining the canals. The Lureh area is located in the northe part of the Abjirob area between the Shureh stream and the Abjirob stream. The Lureh stream is the only water source for this area. Schistosome infections in man and animals were wide spread.

In the west is an area receiving water from several sources: from the Karkheh river (Hermoshi canals); from the Dez river; from

/the Shahur....

the Shahur stream which also crosses this area. Snails were found in swamps, way-sidepools and canals. Recently, the Greater Dez Project was begun to install a new irrigation system in this area, with water taken from the Dez river.

<u>The Sugar Cane Area</u>,

This area located in the South part of Pilot Project Area and separated by wire mesh from the adjoining areas. It is a new irrigation system with numerous newly built canals, large water reservoirs and a deep drainage network, the water source is from the Dez river. <u>B.truncatus</u> and <u>L.gedrosiana</u> were wide spread all over the area. The slow water flow in the canals and the large water reservoirs with plenty of vegetation gave ideal conditions for snail breeding.

<u>The Southern Area</u>,

This area is bounded in the north by the Sugar Cane Area, in the east by the Dez-Karun river, in the west by the Karkheh river and the Ahwaz-Dezful Highway and in the south it extends to Ahwaz. The water in this area is derived from the Shahur river which divided into two branchs. The upper branch (Maleh) is wide, meandring and overgrown with heavy vegetation; during the dry season (summer and autumn) this branch becomes broken up into several swamps. During the wet seasons (winter and spring) snails are therefore carried to down stream areas. The lower branch of the Shahur river is about 60 km. long; it is the main branch and the water flows fast. In the course of the lowwer branch there are several small and large marshy swamps and snails were spread all over the area; man and animal schistosomes were abundant in this area.

Other Important Areas,

The lower Karkheh Area(Dasht-Mishan),

This area is bounded in the west by the Iraq frontier, in the north by the dry Dehloran area, in the east by Ahwaz and in the south by a desert. A dam was established at Hamidieh where a main irrigation channel feeds water to some canals in the east and south

/ part of....

part of this area. The lower Karkheh river divides into two branches at Kut Naim. The southern branch was empty of water for 7 km; below that point it receives water from the main Hamidieh channel. The northern branch further divides at Susangird and discharges into a large marsh (Susangird swamp) adjoining the Iraq border.

The area north of the northern branch of the Karkheh river was completely dry. On the south side, the water was pumped from the river into short irrigation canals along the bank. L.gedrosiana flourished in irrigation canals, but B.truncatus was found only in ponds and tertiary canals around a village called Ghadir. In the large swamp (Susangird swamp) between the Iran and Iraq border only dead shells of Lymnaea, Gitulus and few live Viviparus and Melanoides were found.

The Shushtar Area,

The water in this area is drawn partly from the Karun river and partly from natural streams which finally join the Karun river.

/ Lymnaea....

Lymnaea was abundant in canals and ponds around market gardens and irrigation canals, *B.truncatus* was found in a marshy drain near Boneh Arabha. Animal schistosomes was prevalent in this region.

Other Areas,

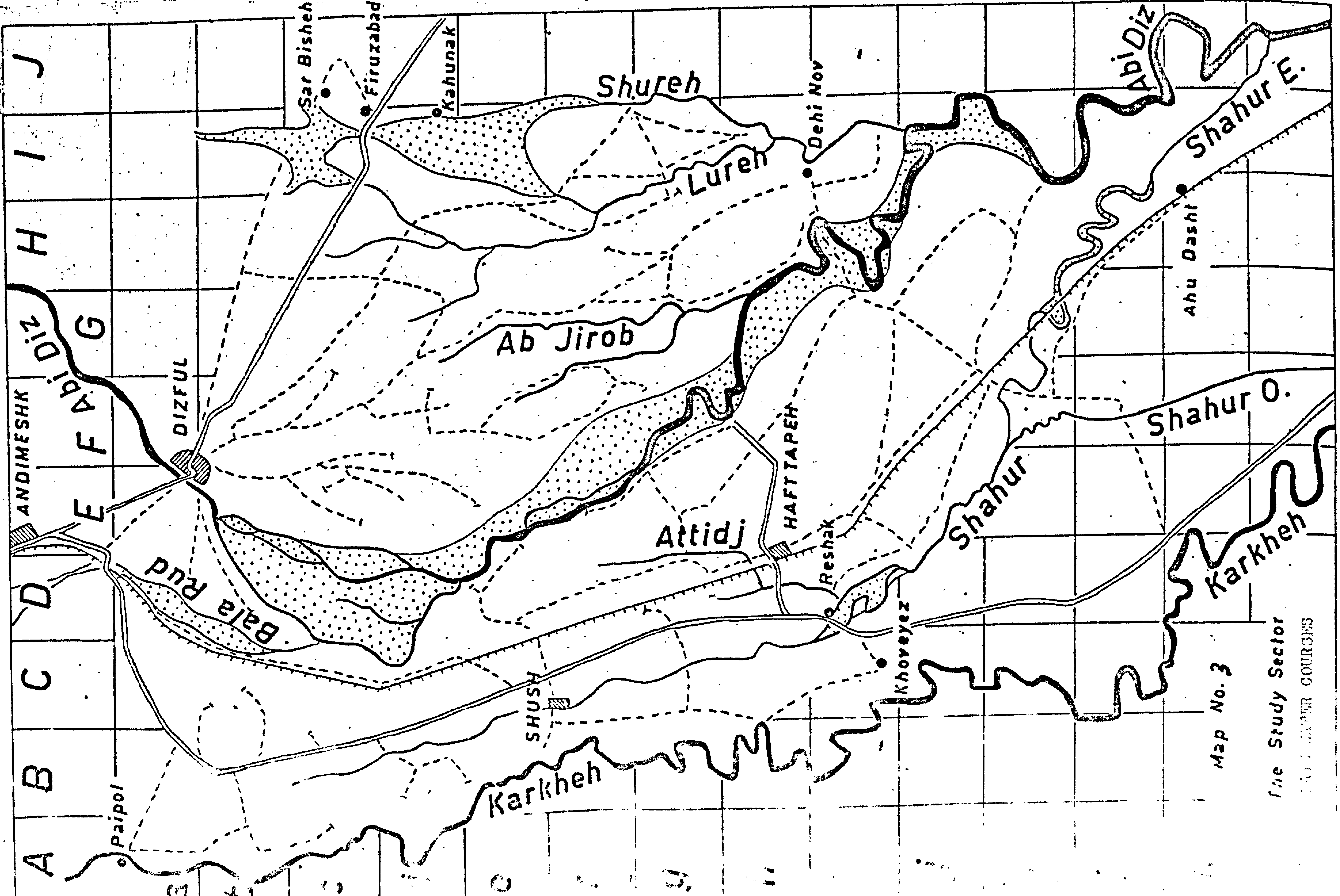
The area south-east of Ahwaz (Ramhormoz) is suitable for snail breeding, but only *L.gedrosiana* was found and no *B.truncatus*. In Shadegan area where the dry Jarahi river ends there is a shallow large saline swamp and no live snails. In the Khorramshahr area, dead shells of *B.truncatus* were found in the field channel of date farms but in the canals and ponds *L.gedrosiana* was found. The Dehloran area north of Dasht-Mishan is a vast and dry area.

CHAPTER 2

A GENERAL DESCRIPTION OF THE SPECIAL STUDY AREA

Studies on the seasonal population trends of L.gedrosiana and its relation to transmission of O.turkestanicum in animals were carried out from 1967-1970 in the special area (Pilot Project Area) located in the northern part of Khuzestan (Map, 3). This area lies mainly south of Andimeshk-Dezful, within an irregular quadrilateral of a somewhat rhomboidal shape. It is bordered in the west by the Karkheh river and crossed by the Teheran-Ahwaz highway and railroad, in its north-east part by the Dezful-Shushtar road, in the east by the Shureh stream. In the south it is bordered by the Sugar Cane Area (Haft-tapeh) and Ahudasht steppe. The great axis north-west from the Karkheh river down to the south-east (railway station of Ahudasht) measures 73 km. The small axis north-east to south west measures 45 km. from Sarbisheh village down to the intake of the great canal of the south Agricultural Company in the Karkheh river.

/ The Pilot....


A
B
C
D
E
F
G
H
I
J
Paipol
Karkheh
SHUSH
Khoveyez
Reshak
HAFTTAPEH
Attidj
Bala Rud
ANDIMESHK
DIZFUL
Abi Diz
Ab Jirob
Shahur
Shahur O.
Shahur E.
Karkheh
Ahu Dasht
Abi Diz
Dehi Nov
Lureh
Shureh
Kahunak
Firuzabad
Sar Bisheh
Map No. 3
The Study Sector

The Pilot Irrigation Area with its new system of irrigation canals is entirely included in the study sector. It almost covers the axial part of the area and represents approximately one sixth of the total study area. On the Dez river 25 km. north of the old city of Dezful, the first of a series of multiple-purpose dams, The Mohamad Reza Shah Pahlavi Dam (the highest dam in the Middle-East) was dedicated in March,1963. A pilot demonstration of approximately 50,000 acres (22,000 hectares) was selected to receive the first waters of the dam. A system of canals and laterals was constracted in this area which extends generally southward from the city of Dezful, which was to be known as the Dez Pilot Irrigation Area. The construction of the irrigation net works for the undeveloped balance of 250,000 acres known as the Greater Dez Irrigation Project, which is included in the Study Area is to be completed by the end of 1972.

The whole study area occupies 1,000 $km.^2$ the main water-course in this area is the Dez river which divides the area from north to south into equal parts and collects water from the west,

/ east of....

east of Bala Rud. The Bala Rud, a torrent which completely dries up in summer. The Karkheh river runs along the western edge of the area. From these rivers a great number of irrigation canals branch off. The excess irrigation water finds its way to the natural drains. These, which naturally flow north-southwards are: The Shahur and its tributary, the Attidj between Karkheh and Dez. The Abjirob, the Lureh and the Shureh, east of the Dez. The Shureh borders the eastern side of the study area. Down streams, before flowing into the Dez river these give rise to new irrigation network.

The flood plains have very different aspects: those of the northern and eastern part of the area (Bala Rud, upper Dez, Lureh and Shureh) are vast areas dry and covered with a thick layer of pebbles; those of the Karkheh and of the southern part of the Dez are mostly covered with a shrubby vegetation (Tamarix) in a relatively wet soil; The flood plains of the Shahur are much narrower and are swampy, with herbaceous vegetation.

/ Chapter,3.....

CHAPTER 3

FIELD OBSERVATIONS ON SEASONAL POPULATION TRENDS OF L.GEDROSIANA AND THEIR RELATION TO THE TRANSMISSION OF O.TURKESTANICUM IN VARIOUS HABITATS IN THE STUDY AREA

METHODS,

Selection of Sampling Areas,

Studies on the *L.gedrosiana* population dynamics and seasonal infections with *O.turkestanicum* were started in January 1967. Twenty different type of snail habitats (canals, swamps, ponds and drains) were selected for monthly study of natural infections of *L.gedrosiana* with *O.turkestanicum* cercariae (group A).

In October 1969 another series of observations were designed to study the population dynamics and bionomic of *L.gedrosiana* by fortnightly survey. Eight different type of habitats were selected (group B).

/Sampling....

Sampling of Snail Populations,

Snails were collected monthly at fixed sites in each habitat. The habitats sampled included 11 canals in different parts of the study area (6 canals in Abjirob area, 3 canals in Lureh area and 2 canals in Pilot Irrigation Area), 3 swamps, 4 ponds and 2 drains. In an attempt to detect seasonal fluctuations of snail populations, as well as cercarial infection rates, snails were collected in as uniform a manner as possible by using the methods of Oliver and Schneiderman (1956). The area selected for study was first measured and marked. If the body of water was a canal a portion 100 to 150 metres long was chosen, whereas if the habitat was a small pool the whole area was used. In large bodies of water, a segment of the margin was marked as the collecting area. For snail sampling one man carried out a dip-net in the marked area systematically and uniformly for 10 minutes.

Collected snails were brought to the laboratory, after separating the species, the size distribution of the collected snails

/was...

was determined by measuring the length of all snail shells(the distance from base to apex). Snails were divided into 3 size groups (0-6 mm., 6-10 mm. and 10-14 mm.).

The snails were crushed individually between 2 slides and examined under a dissecting microscope for cercarial infection. Proof of the identificatiin of _O.turkestanicum_ cercariae was obtained by exposing a local wild rodent (_Tatera indica_) which is susceptible to _O.turkestanicum_, to the cercariae obtained by crushing more than 2 infected snails. The exposed rodents were sacrifised 3 months later, the worms and the eggs deposited in the liver of the host were observed for identification of the species of parasites.

In addition, the water level of the habitats was recorded on each occasion. The highest water level was designated " 5 " and " 0 " was recorded when the habitat was near drying or dry. This study continued for 12 months from January to December 1967.

/ A. Standing....

A. Standing Waters,

Ponds,

A total of 4 ponds in different part of the Study Area was surveyed: the Bayatian pond, populated with a variety of snails, was 2 metres in depth and was overgrown by submerged vegetations. The Khanabad Ghotb pond was in the east side of the Study Area with shallow water. The Sardarabad pond was in the Pilot Irrigation Area and was a small pond surrounded with trees and a large amount of decayed tree leaves and vegetation. The Dehbar pond was small and shallow and was located against a garden wall and received water from the garden drainage. The snail data from monthly surveys from Jan-Dec. 1967 are given in Table, 57 and Fig. 2. During the observation period the peak of snail population occured mostly from April to June with a small peak also in autumn. Infected snails were most abundant in March and April and also in September, October and November.

/ Swamps....

Swamps,

Three swamps were selected in different parts of the Study Area for monthly observations: the Deylam Sofla swamp which was a large swamp located in the Pilot Irrigation Area with dense vegetation and varieties of snails. Animal and human contact with this swamp was abundant. The Khanabad Ghotb swamp in the east side of the Study Area, was very large and shallow, had numerous tributaries. The Boneh Jawas swamp in the Abjirob Area was very large and deep, *Typha* vegetation was grown all over the swamp making a dense bush in the central parts.

The snail data from the monthly observations are given in Table, 58 and Fig. 3. The peak density of snail populations differed in each swamp, *L.gedrosiana* was most abundant in April and May with low density in summer. The Khanabad Ghotb swamp dry in July and August. Infected snails were found from April to November 1967.

/ B. Running....

B. Running Water.

Drains.

Two newly made irrigation drains were selected in the Pilot Irrigation Area: the Sardarabad and the Boneh Rahimeh drains characterised by their long course, dense vegetation and low water velocity.

The data from the monthly snail collections from Jan.-Dec. 1967 are presented in Table, 59 and Fig. 4. L. gedrosiana densities were moderate with a peak in May and June. A few infected snails were found in the Sardarabad drain in May and September and in Boneh Rahimeh drain in March, April and August.

New Canals.

Boneh Charry and Boneh Yakub canals were located in the Pilot Irrigation Area, and were newly constracted irrigation canals.

/ The Boneh....

The Boneh Charry canal terminated in many ponds and water depressions which were mostly populated with *L.gedrosiana* and *B.truncatus* snails. Data from monthly collections are given in Table, 59. In the Boneh Charry drain the peak of the *L.gedrosiana* population density was in May and June but in the Boneh Yakub canal it was irregular during the observation period. Infected *L.gedrosiana* were collected in the Boneh Charry canal from July to October; in the Boneh Yakub canal from August to December.

Old Canals (Lureh Area)

Three old canals in the Lureh Area were surveyed monthly. The Farash-abad canal; this canal passes through the village creating many ponds and water depressions. The Shongor Sofla canal, with low velocity and dense submerged vegetations. The Shongor Olia canal, with tortuous course and a shallow spread side drain running along the canal.

/ The data....

The data from monthly observations are given in Table, 60. The L.gedrosiana densities were more or less constant throughout the observation period, only low density were found in June and July. The infected snails were found irregularly, in the Farash-abad canal from April to December, in the Shongor Sofla canal all over the year and in the Shongor Olia canal in winter and late autumn.

Old Canals (Abjirob Area)

Six canals in the Abjirob Area were surveyed monthly; the canals received water from the main Abjirob canal which branched off from the Abjirob stream above a dam built some 20 years ago. The flow of water in these canals was lower in winter than in summer. In summer the ricefields adjoining the canals used much of the water which continued to run into the tertiary canals.

The most prevalent snail was the L.gedrosiana. The results on Table, 61 show that the L.gedrosiana population was rather high with peak densities in most of the canals in April and May and low densities in summer and winter. The summer decline was due to very high water level in the canals and the winter decline presumably was due to cold temperatures.

Infected snails were found in most of the canals during the whole period except in the Boneh Jawaz canal where infected snail were found only from January to May 1967. Human and animal schistosomes were prevalent in the Abjirob Area and it is likely that infested canals played a major role in transmission of schistosomes in this area.

In general snail densities in the canals in different parts (Fig. 5) showed a major peak in spring and a minor peak in autumn with a decline in summer. The infection rate of L.gedrosiana was high in summer from June to October, and low in winter.

/ Conclusions....

CONCLUSIONS,

1. Seasonal transmission potential of various types of habitats,

Canals were permanent transmission sites of O.turkestanicum throughout the year. Infectivity of swamps also continued throughout the year with a peak in the summer season but a very low level in winter. In ponds the number of infected L.gedrosiana was very low in summer and winter with peaks in spring and autumn. Drains played a very minor role in transmission, with very low and irregular infectivity (Table, 62 and Fig. 6).

2. Size-frequency of infected snails,

The prevalence of infection in large snails was higher than small size snails, presumably due to their having more chance to contact miracidia during their longer life-span comparing with young snails. The Percentage of infected snails in different size groups was 0.1 %, 0.5 % and 1.1 % in $0\text{-}6^{mm.}$, $6\text{-}10^{mm.}$ and $10\text{-}14^{mm.}$ hight of

/ shell....

shell of L.gedrosiana respectively (Table, 63).

3. Infection rates in snails from different habitats,

The infection rates of L.gedrosiana in different type of habitats was o.68 % in canals, 0.38 % in swamps, 0.25 % in ponds and 0.15 % in drains. The highest rate of infection was observed in the canals (Table, 64).

4. Snail infection rate in different parts of Study Area,

The infection rate of L.gedrosiana with O.turkestanicum cercariae in different parts of the Study Area was 0.66 % in Pilot Irrigation Area, 0.54 % in Abjirob Area and 1.0 % in the Lureh Area (Table, 65).

The full pattern of seasonal fluctuation of the infection rate of L.gedrosiana with O.turkestanicum cercariae in the different type of habitats is shown in Table, 62 and Fig. 6 .

/ Addendum....

ADDENDUM:

Non-schistosome parasite larval stages found in *L.gedrosiana*,

1. *Fasciola gigantica*. *L.gedrosiana* is an intermediate host for *Fasciola gigantica* on the Khuzestan plain and in fact the infection rate of *L.gedrosiana* with *F.gigantica* cercariae was higher than with the *O.turkestanicum* cercariae. The prevalence of *F.gigantica* in cattle and sheep was also higher that of *O.turkestanicum*.

2. There was an unknown cercariae more or less like the *F.gigantica* cercariae but smaller in size and very active: this cercariae did not encyst on vegetation, the infection rate of this cercariae in *L.gedrosiana* was much higher than the other cercariae. It may be that this cercariae is the same species that Miriam Rothschild(1936) described from *Lymnaea tenera euphratica* in Iraq, as belonging to the Gymnocephalic group.

/ 3. There....

3. There was also an apharyngeal longifurcate cercariae much longer than the brevifurcate cercariae of _O.turkestanicum_. Laboratory animals exposed to these cercaraie developed no infections. The infection rates of this distome in _L.gedrosiana_ was very low and it was seen only occasionally in certain canals.

Table. 57

Numbers of *L. gedrosiana* collected monthly from 4 ponds in Khuzestan and numbers infected with *O. turkestanicum*.

Month	Beyatian	Khan-abad Ghutb	Sardar-abad	Dehbar
Jan.	0/ 52	0/ 34	0/ 69	1/ 67
Feb.	0/ 91	0/ 63	0	0/108
March	0/ 10	0/ 90	1/ 24	2/185
April	0/ 68	3/166	0/ 72	0/1111
May	0/191	0/175	0/745	0/1138
June	0/ 66	1/307	0/390	0/263
July	1/179	0/305	0/102	0/ 13
Aug.	0/110	0/ 5	1/554	0/2
Sept.	2/210	0/ 7	3/764	0/ 30
Oct.	0/ 44	2/152	0/227	0/ 50
Nov.	0/90	9/ 78	0/254	0/214
Dec.	0/250	0/ 12	0/106	0/136

Numerators- number of snails with *O. turkestanicum* infections.

Denominators-total number of snails collected.

Table, 58

Numbers of *L.gedrosiana* collected monthly from 3 swamps in Khuzestan and numbers infected with *O.turkestanicum*.

Month	Deylam Sofla	Khan-abad Ghutb	Boneh Jawaz
Jan	1/104	0/433	0/ 61
Feb.	0/131	1/343	0/ 69
March	1/148	0/ 250	0 41
April	3/300	0/237	0/205
May	1/ 41	1/1171	2/110
June	3/ 41	0/ 74	0/104
July	0/ 24	dry	1/124
Aug.	0/ 29	dry	1/ 40
Sept.	0/ 31	0/ 4	0/ 74
Oct.	1/ 52	0/ 42	1/101
Nov.	1/ 65	0/ 16	0/101
Dec.	0/ 46	0/ 53	0/162

Nemerators- number of snails with *O.turkestanicum* infection.

Denominators- number of snails collected.

Table, 59

Numbers of L.gedrosiana collected monthly from new irrigation system(2 canals and 2 drains) in Khuzestan and numbers infected with O.turkestanicum

Month	Boneh Charry Canal	Boneh Yakub Canal	Sardar-abad Drain	Boneh Rahimeh Drain
Jan.	0/417	3/120	0/ 25	0/ 32
Feb.	3/125	0/304	0/199	0/561
March	0/ 73	0/516	0/ 33	1/228
April	0/270	0/227	0/165	1/223
May	2/490	2/320	1/424	0/1067
June	0/447	0/320	0/ 38	0/437
July	4/217	2/271	0/ 19	0/182
Aug.	3/452	2/ 88	0/27	3/534
Sept.	9/195	4/ 78	2/ 66	0/570
Oct.	2/247	1/ 30	0/143	0/179
Nov.	0/133	0/118	0/ 73	0/ 66
Dec.	0/198	1/74	0/ 22	0/ 25

Nomerators- number of snails with O.turkestanicum infections.

Denominators- total number of snails collected.

Table, 60

Numbers of L.gedrosiana collected monthly from 3 canals in Khuzestan (Lureh Area) and numbers infected with O.turkestanicum.

Month	Shongor Olia	Shongor Sofla	Farash-abad
Jan.	3/145	2/358	0/ 59
Feb.	2/206	3/155	0/349
March	0/ 24	0/ 13	0/ 83
April	1/181	7/152	0/473
May	0/202	-	0/285
June	0/ 24	1/ 28	2/448
July	0/ 76	0/ 34	4/ 50
Aug.	0/260	3/ 36	3/122
Sept.	0/359	3/ 76	3/270
Oct.	0/268	8/283	0/ 27
Nov.	1/121	6/285	0/ 61
Dec.	1/138	2/265	1/ 42

Numerators- number of snails with O.turkestanicum infections.

Denominators- number of snails collected.

Table, 61

Numbers of *L. gedrosiana* collected monthly from 6 canals in Khuzestan(Abjirob Area) and numbers infected with *O. turkestanicum*.

Month	Seyed Nur	Seyed Majid	Seyed Jaafar	Boneh Ayesh	Boneh Hajat	Boneh Jawaz
Jan.	1/152	1/ 74	0/ 88	1/ 78	0/ 92	1/491
Feb.	2/224	1/179	1/200	0/244	0/100	1/184
March	0/ 54	3/193	0/ 66	0/107	0/ 70	2/367
April	3/601	1/391	0/336	4/481	0/ 87	5/188
May	3/381	5/581	3/331	1/437	1/271	1/451
June	7/131	0/305	1/207	1/121	0/ 10	0/ 52
July	0/74	1/269	0/ 5	0/ 2	0/ 22	0/334
Aug.	0/230	4/259	0/ 10	0/ 4	1/ 39	0/ 53
Sept.	0/196	3/149	1/ 57	0/ 54	3/ 96	0/111
Oct.	0/231	2/ 68	1/ 59	1/ 59	1/298	0/ 32
Nov.	3/294	3/ 74	0/59	0/51	0/140	0/51
Dec.	0/138	3/ 49	0/ 75	0/ 68	0/ 80	0/ 37

Numerators- number of snails with *O. turkestanicum* infections.

Denominators- total number of snails collected.

Table, 62

O.turkestanicum infection rate of Lymnaea gedrosiana in different habitats in Khuzestan.

1967

Month	11 canals		3 swamps		2 drains		4 ponds		total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Jan.	11/2084	0.5	1/598	0.1	0/ 56	0	1/222	0.4	13/2961	0.4
Feb.	13/2171	0.6	1/543	0.1	0/760	0	0/262	0	14/3736	0.3
March	5/1354	0.3	1/419	0.2	1/261	0.3	0/309	0	7/2343	0.3
April	28/3209	0.8	1/742	0.1	1/388	0.2	3/1417	0.2	33/5756	0.5
May	18/3937	0.4	4/1322	0.3	1/1491	0.06	0/2249	0	23/8999	0.2
June	12/2093	0.5	3/219	1.3	0/475	0	1/1026	0.1	16/3813	0.4
July	11/ 643	1.7	1/148	0.6	0/201	0	1/599	0.16	13/1591	0.8
Aug.	16/1553	1.0	1/ 69	1/5	3/561	0.5	1/671	0.14	21/2854	0.7
Sept.	26/1631	1.5	0/109	0	2/636	0.3	5/401	1.2	33/2777	1.1
Oct.	16/1559	1.0	2/195	1.0	0/322	0	2/548	0.3	20/2624	0.7
Nov.	13/1395	0.9	1/182	0.5	0/169	0	5/640	0.7	19/2386	0.8
Dec.	8/1164	0.7	0/261	0	0/ 47	0	0/504	0	8/1976	0.4

Numerators- number of snails infected with O.turkestanicum.

Denominators- total number of snails collected.

Table, 63

Size frequency of L.gedrosiana and infection rate of O.turkestanicum collected from 11 canals, 4 ponds, 3 swamps and 2 drains in Khuzestan.

	Size Distribution of Snails (mm.)			
	0 - 6	6 - 10	10 - 14	Total
Total no. of snails collected	18858	14660	10799	44317
No. of snails infected	26	74	125	225
Rate of infection %	0.14	0.5	1.1	0.5

Table, 64

Infection rates of L.gedrosiana with O.turkestanicum cercariae in different type of habitats in Study Area.

	Canals	Swamps	Ponds	Drains
No. of snails collected	25718	4687	8690	5224
No. of snails infected	177	18	22	8
Rate of infection	0.68 %	0.38 %	0.25 %	0.15 %

Table, 65

Infection rates of *L.gedrosiana* with *O.turkestanicum* cercariae in *canals* in different parts of Study Area.

	New Irrigation Area	Abjirob Area	Lureh Area
No. of snails collected	5718	13992	6008
No. of snails infected	38	76	63
Rate of infection	0.66 %	0.54 %	1.0 %

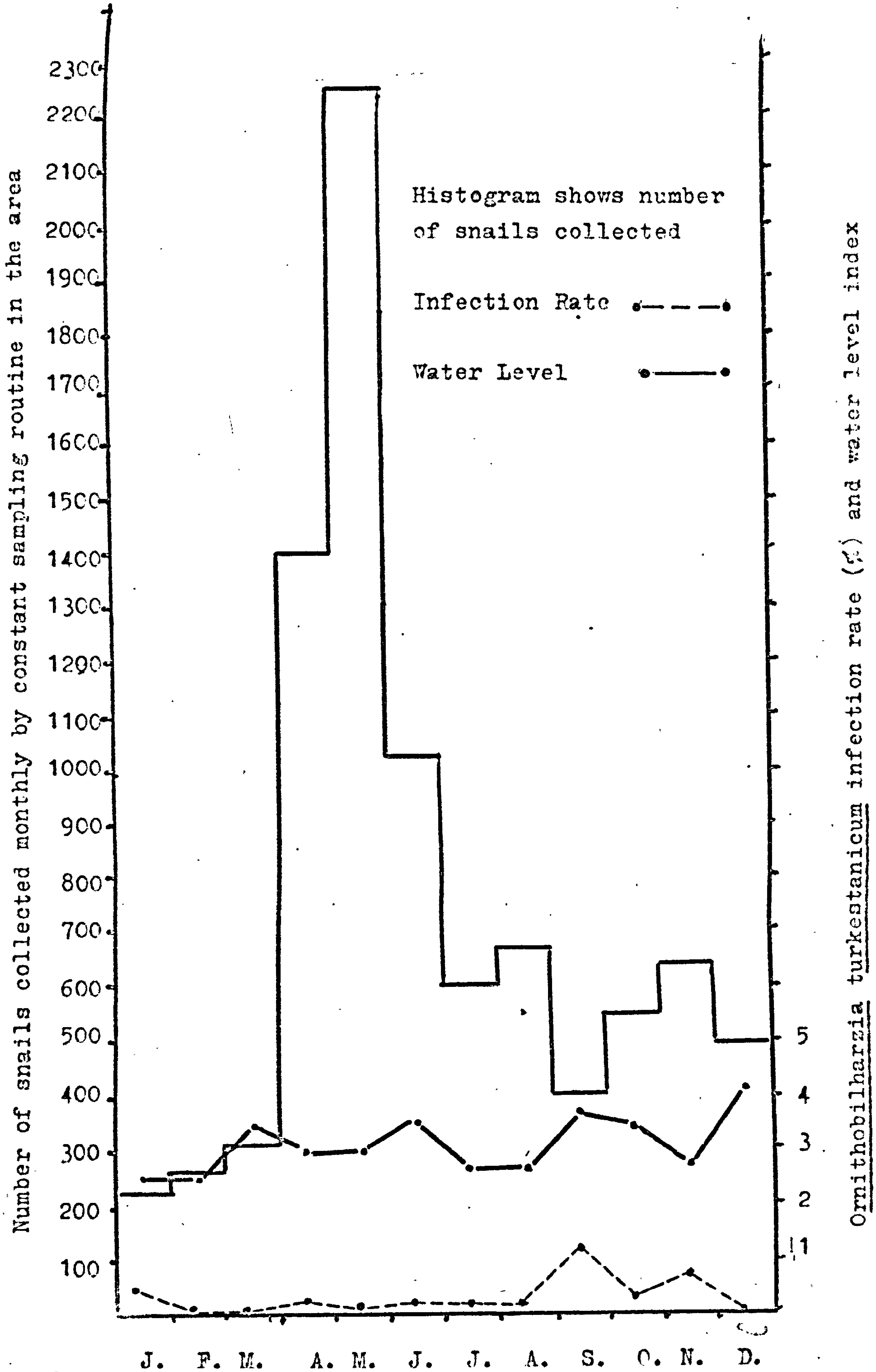


Fig. 2
Numbers of Lymnaea gedrosiana collected monthly under uniform conditions in observation area(4 ponds) and numbers infected with Ornithobilharzia turkestanicum and fluctuations of water level

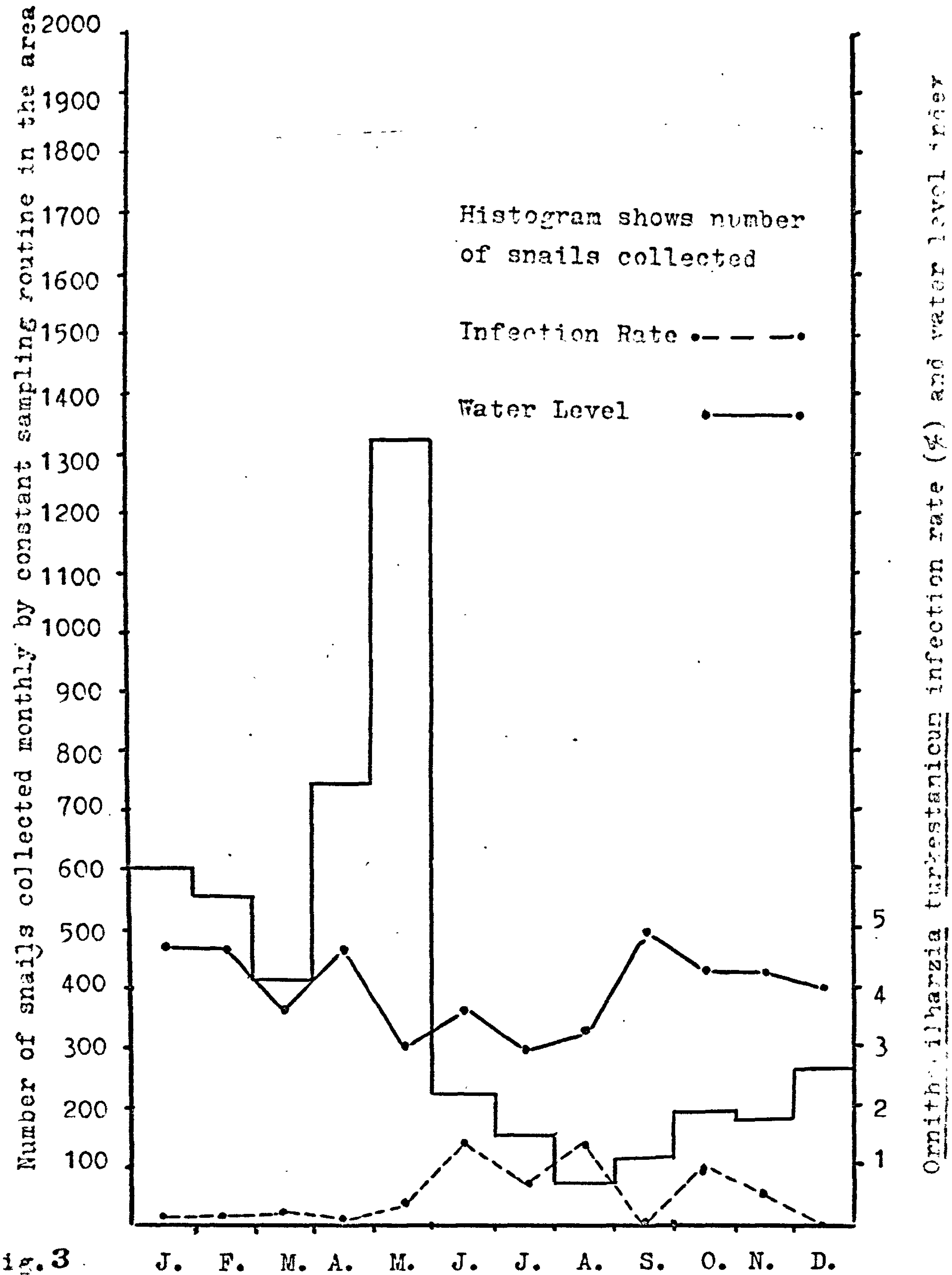


Fig. 3
Numbers of *Lymnaea gedrosiana* collected monthly under uniform conditions in observation area (3 swamps) and umbers infected with *Ornithobilharzia turkestanicum* and fluctuations of water level.

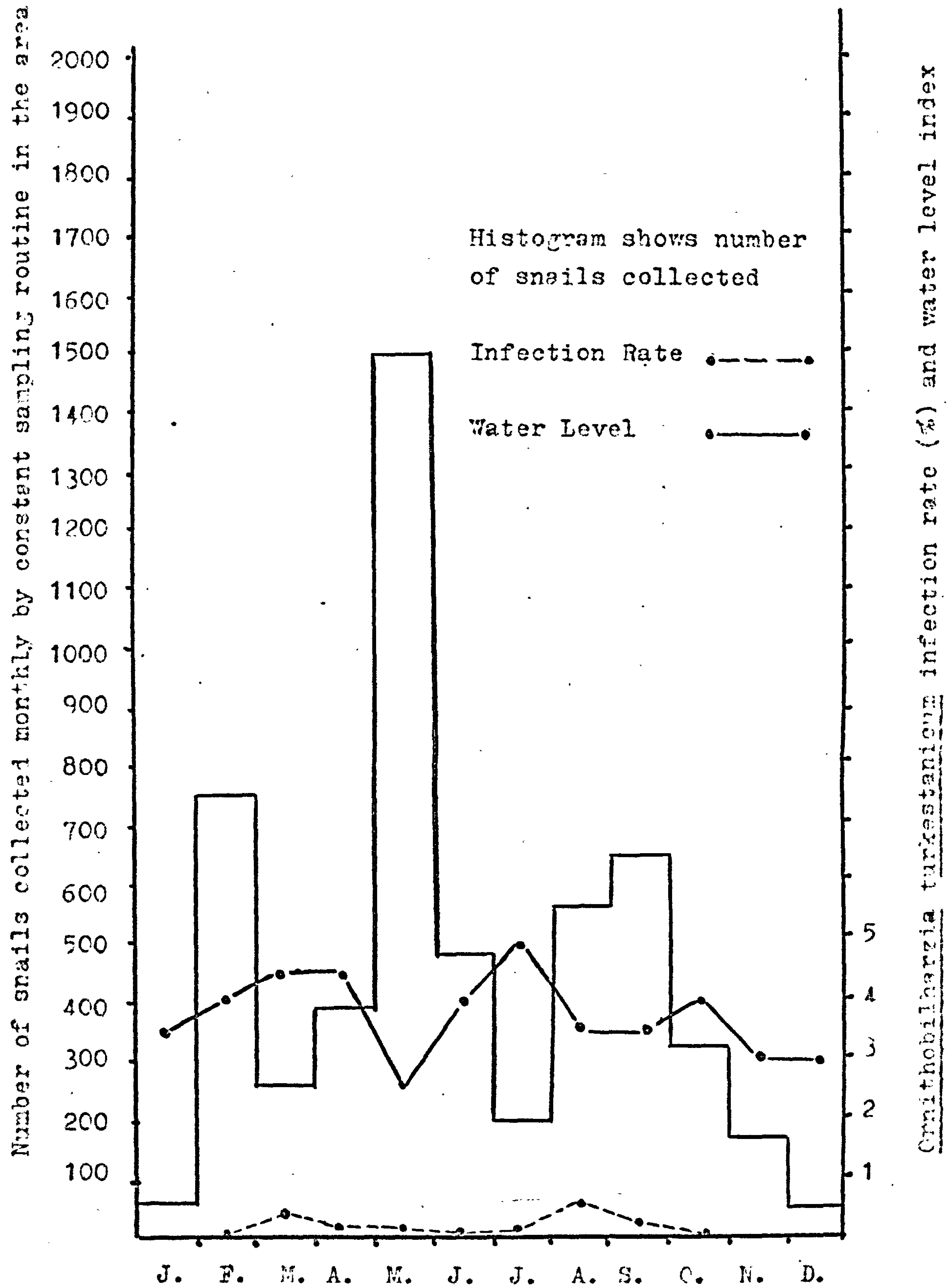


Fig. 4
Numbers of *Lymnaea gedrosiana* collected monthly under uniform condition in observation area (2 drains) and numbers infected with *Ornithobilharzia turkestanicum* and fluctuations of water level.

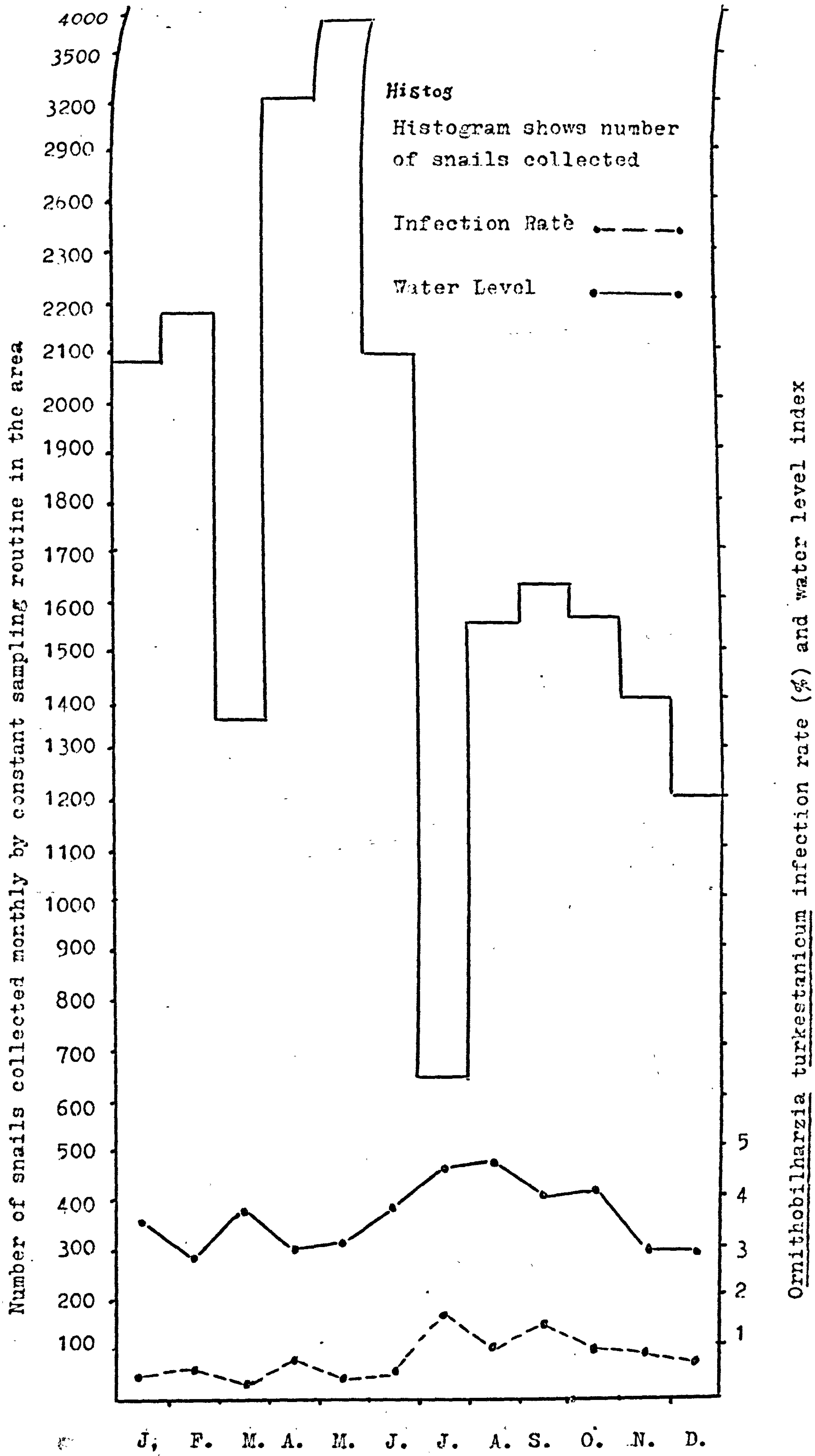
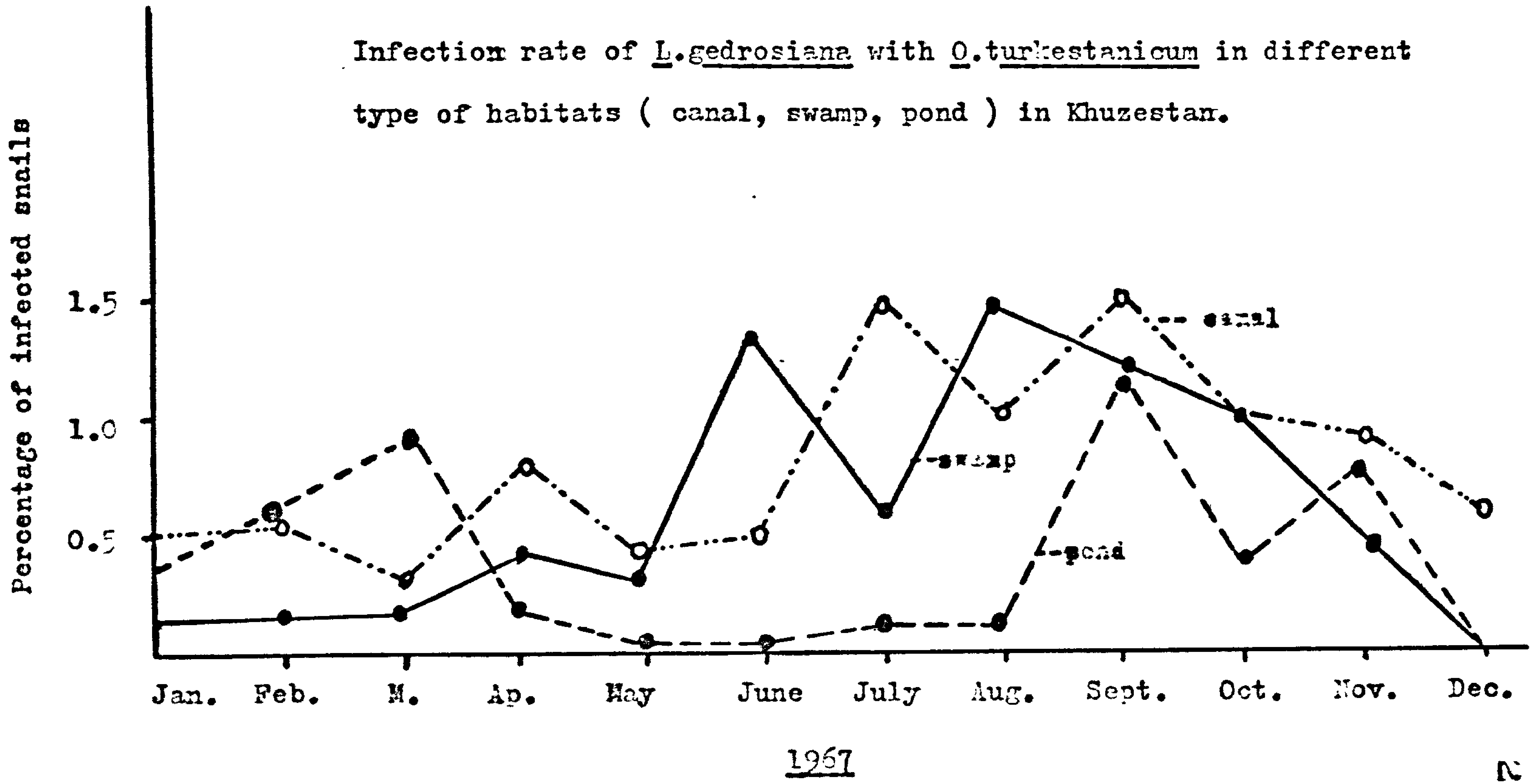


Fig.5
Numbers of _Lymnaea gedrosiana_ collected monthly under uniform conditions in observation area (11 canals) and numbers infected with _Ornithobilharzia turkestanicum_ and fluctuations of water level.

Fig.6

Infection rate of L.gedrosiana with O.turkestanicum in different type of habitats (canal, swamp, pond) in Khuzestan.



CHAPTER 4

DETAILED STUDIES ON THE POPULATION DYNAMICS OF L.GEDROSIANA IN SELECTED HABITATS.

METHODS,

Eight different type of habitats (2 canals, 2 swamps and 4 ponds) were selected for observation of the population dynamics of L.gedrosiana. The above habitats were highly populated with snails and for convenience in each habitats only 10 dip-nets were used on each occasion once fortnightly from October 1969 to September 1970.

The size of each snail was determined and allocated to one of the following groups: 0-4 mm., 4-6 mm., 6-8 mm., 8-10 mm., 10-12 mm., 12-14 mm. and over 14 mm..

The number of eggmasses was counted at each visit and all the snails were returned to their respective sites within 24 hours, to

/avoid....

avoid any disturbance of snail densities due to survey collections. Water level index, water temperature and other ecological information of the habitats was recorded occasionally.

RESULTS,

A. Snail density fluctuation in various habitats,

Habitat, 1. Balengun Pond,

This shallow pond was connected to an irrigation canal with moderate vegetation. The data from fortnightly observations are shown in Table, 66 and Fig. 7. Eggmasses were collected from September to November and April to August (Table, 67). Snail densities were high from October to January.

Habitat, 2. Sardarabad Pond,

This pond also was connected to a canal and shaded with trees and was all the time exchanging water with the adjoining canal.

/ The data....

The data from fortnightly observations are given in Table, 66 and Fig. 8. The reproduction of L.gedrosiana stopped only in the winter season (Table, 67). The peak of snail densities were observed in August and September.

Habitat, 3. Nahr-Khan Pond,

This was a pond with heavy vegetation and was elongated in shape and had a more constant water level. The data from fortnightly collection are given in Table, 66 and Fig, 9. The eggmasses were abundant from October to December 1969 and from June to September 1970. The first peak of snail densities occured from October to December and a second peak from April to July.

Habitat, 4. Shams-abad Pond,

This was a smaller pond permanently fed from the Market-gardening excess water, with dense vegetation. The data from fortnightly observations are given in Table, 66 and Fig. 10. indicating that

/ major....

major peaks of snail densities and egg production were in autumn and spring.

Habitat, 5. Ghaleh Sheikh Swamp,

This swamp was irregular with dense vegetation and a high water fluctuation rate, water come from a nearby garden. The data given in Table, 66 and Fig. 11 indicate that the first peak of snail densities was in October and November, the second peak occured in June and July.

Habitat, 6. Ghaleh Seyed Swamp,

This was a very large and rich swamp, suitable for snail colonies throughout the year. Vegetation particularly Typha was abundant. The data collected from fortnightly observations from October 1969 to September 1970 are given in Table, 66 and Fig. 12. The L. gedrosiana population and egg production showed 2 major peaks

/one in....

one in autumn with a sharp decline in winter, the second peak was from May to August 1970.

Habitat,7. Chagha Sabz Canal,

This was a newly built secondary irrigation canal in the Pilot Irrigation Area, with high submerged vegetation and slow water flow. The data collected fortnightly are shown in Table,66 and Fig. 13. The snail densities and egg production first peak was in autumn and the second peak was from May to July with a sharp decline in winter.

Habitat, 8. Main Canal No. 80,

This canal was a large and main new irrigation system with large amounts of water and high submerged vegetation. The velocity of water flow was high in middle part of the canal but low at the bank, with many cement barriers for water diversion to the secondary canals. The main peak of *L.gedrosiana* densities and egg production was from October to December 1969, declined in winter with second minor peak from April to June 1970 (Tables, 66, 67 and Fig. 14).

/ B. Reproduction

B. Reproductio^{n}Rate of L.gedrosiana,

In appearance the eggmasses of the L.gedrosiana were elongated and their length varied from 5-15$^{mm.}$. Eggmasses were transparent and contained some 7-108 egg sacs with an average of 60 egg sacs in 112 eggmasses collected in a snail habitat in April.

The number of eggs collected in a series of samples on any day represents the eggs laid by the adult snails present in the same samples. Table, 68 shows the reproduction rates of L.gedrosiana obtained by estimating the egg production per adult snail per day during successive time-intervals calculating by dividing the number of eggs collected from each sampling by the number of adult snails over 6$^{mm.}$ length. It was clear that the reproduction capacity of L.gedrosiana was highest in running water systems, possibly due to high aeration. The peak reproduction rate was observed in autumn and spring in all habitats and it was nil in the winter season in swamps and ponds, though some fresh eggmasses were obtained from the

/ canal ....

canal No. 80. In summer egg laying activity continued but at lower level, whereas the snail densities did not decline very much in summer compared with the marked decrease in winter(Tables, 66 and 67).

C. Size distribution of snail populations,

The size-frequency (Tables,69 and 70) show that most of the snails in different habitats were young snails less than 6-8 $^{mm.}$. L.gedrosiana is very sensitive to environmental changes and their tolerance to drought is very poor compared with other snails (see Chu et al, 1967). Because of this sensitivity and short life-span of L.gedrosiana it was difficult to draw a synchronized growth rate curve for this snail as has been done for Bulinid and Biomphalaria snails.

Table, 66

Number of L.gedrosiana collected fortnightly from 2 canals, 2 swamps, 4 ponds in Khuzestan for 12 months.

Months	No.80 canal	Chagha Sabz canal	Q.Seyed Swamp	Q.Sheikh Swamp	Shams-abad Pond	Nahr-Khan Pond	Sardar-abad Pond	Kalengun Pond
Oct.	352	209	3641	409	469	480	563	27
	1732	757	1707	260	312	391	699	105
Nov.	1100	1730	1494	469	426	368	-	211
	323	-	786	70	533	416	-	93
Dec.	422	-	1311	89	230	280	302	dry
	398	272	978	212	535	159	271	dry
Jan.	45	128	589	126	52	210	196	101
	140	52	393	95	3	104	180	43
Feb.	80	84	123	109	3	32	37	21
	47	1	58	20	0	35	23	3
March	30	2	38	130	0	34	48	3
	33	14	35	-	0	-	43	11
April	83	13	22	46	288	16	98	dry
	64	50	375	96	444	68	191	106
May	91	504	711	121	237	102	407	61
	81	236	619	139	383	58	540	22
June	35	127	826	57	156	162	431	19
	18	489	588	547	231	133	482	71
July	10	365	605	879	225	104	676	18
	17	214	880	125	222	51	365	13
Aug.	17	112	639	14	233	39	396	4
	29	148	567	6	123	117	610	2
Sept.	45	76	310	5	132	117	608	7
	20	76	140	6	41	82	800	7

Table, 67

Numbers of L.gedrosiana eggmasses collected fortnightly from 2 canals, 2 swamps and 4 ponds in Khuzestan for 12 months (10 deep nets)

Months	No.80 canal	Chagha Sabz canal	Q.Seyed Swamp	Q.Sheikh Swamp	Shams-abad Pond	Nahr-Khan Pond	Sardar-abad Pond	Balengun Pond
Oct.	37	68	51	18	55	131	5	8
	44	30	23	10	23	89	17	1
Nov.	8	20	22	21	22	28	0	2
	23	0	25	3	53	14	1	4
Dec.	14	0	10	1	28	8	3	0
	7	36	0	1	8	4	6	0
Jan.	4	8	0	0	0	3	4	0
	0	4	0	0	0	0	0	0
Feb.	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
March	3	0	3	0	0	1	0	0
	1	0	2	0	0	0	0	0
April	29	0	17	2	23	5	9	0
	15	0	49	6	11	2	7	1
May	5	6	23	4	7	4	3	3
	15	2	7	1	15	3	4	0
June	5	3	3	1	5	2	5	0
	0	24	4	6	3	6	13	2
July	0	5	12	5	7	12	6	0
	0	3	7	3	16	9	15	2
Aug.	0	3	8	3	14	4	12	2
	0	5	4	0	6	6	11	0
Sept.	0	3	1	0	2	3	3	0
	0	3	0	0	0	0	11	0

Table, 68

Reproductive rates(eggs per adult snail per day) of *L.gedrosiana* in different habitats at fortnightly intervals from Oct.1969 to Sept. 1970.

Month	interval (weeks)	Canals	Swamps	Ponds
Oct.	2	18.0	3.0	14.4
	4	16.8	3.0	12.0
Nov.	6	30.0	5.4	7.2
Dec.	8	49.2	11.4	9.0
Dec.	10	33.6	1.8	20.4
	12	31.8	0.4	10.8
Jan.	14	60.0	0	5.4
	16	180.0	0	1.8
Feb.	18	0	0	5.4
	20	16.2	0	0.
March	22	25.2	4.8	0
	24	5.4	6.6	1.8
April	262	108.6	87.6	52.8
	28	450.0	60.0	6.6
May	30	10.2	2.4	9.0
	32	16.2	0.3	3.6
June	34	12.6	0.6	1.8
	36	18.0	4.2	2.4
July	38	15.0	4.8	3.0
	40	5.4	4.2	5.4
Aug.	42	6.0	1.8	5.4
	44	30.0	0.6	4.2
Sept.	46	4.2	1.2	2.4
	48	7.8	0	3.0

Table, 69

Observations on the reproductive capacity of L.gedrosiana

in different habitats.

Name	Type of habitats	Total no. of mature snails over 6 mm.	Total no. of eggmasses	No. of egg-masses per snail	No. of eggs per snail
No. 80	Canal	677	213	0.3	18
Chagha Sabz	Canal	562	223	0.4	24
Ghaleh Seyed	Swamp	9375	271	0.02	1.2
Ghaleh Sheikh	Swamp	1320	85	0.06	3.6
Shams-abad	Pond	2626	298	0.1	6.0
Sardar-abad	Pond	2748	334	0.1	6
Nar-Khan	Pond	932	135	0.1	6
Balengun	Pond	242	25	0.1	6

Mean number of eggs in one eggmasses is 60.

Table, 70

Size-Frequency of *L.gedrosiana* collected fortnightly from the snail habitats during the period of 12 months.

Type of habitat	Size-Frequency of collected snails(mm.) shown:						
	0-4	4-6	6-8	8-00	10-12	12-14	14 over
Canal No.80	2944	1508	517	133	21	6	0
Cañal Chagha sabz	3673	1359	460	84	17	1	0
Swamp Ghaleh Seyed	8217	5321	8443	838	84	10	0
Swamp Ghaleh sheikh	1558	1075	866	422	30	2	0
Pond Shams abad	1911	920	1182	920	473	51	0
Pond Sardar abad	2923	2873	1976	669	95	8	0
Pond Nahr-Khan	1461	1255	714	106	1	1	0
Pond Balengun	424	310	100	37	29	26	50

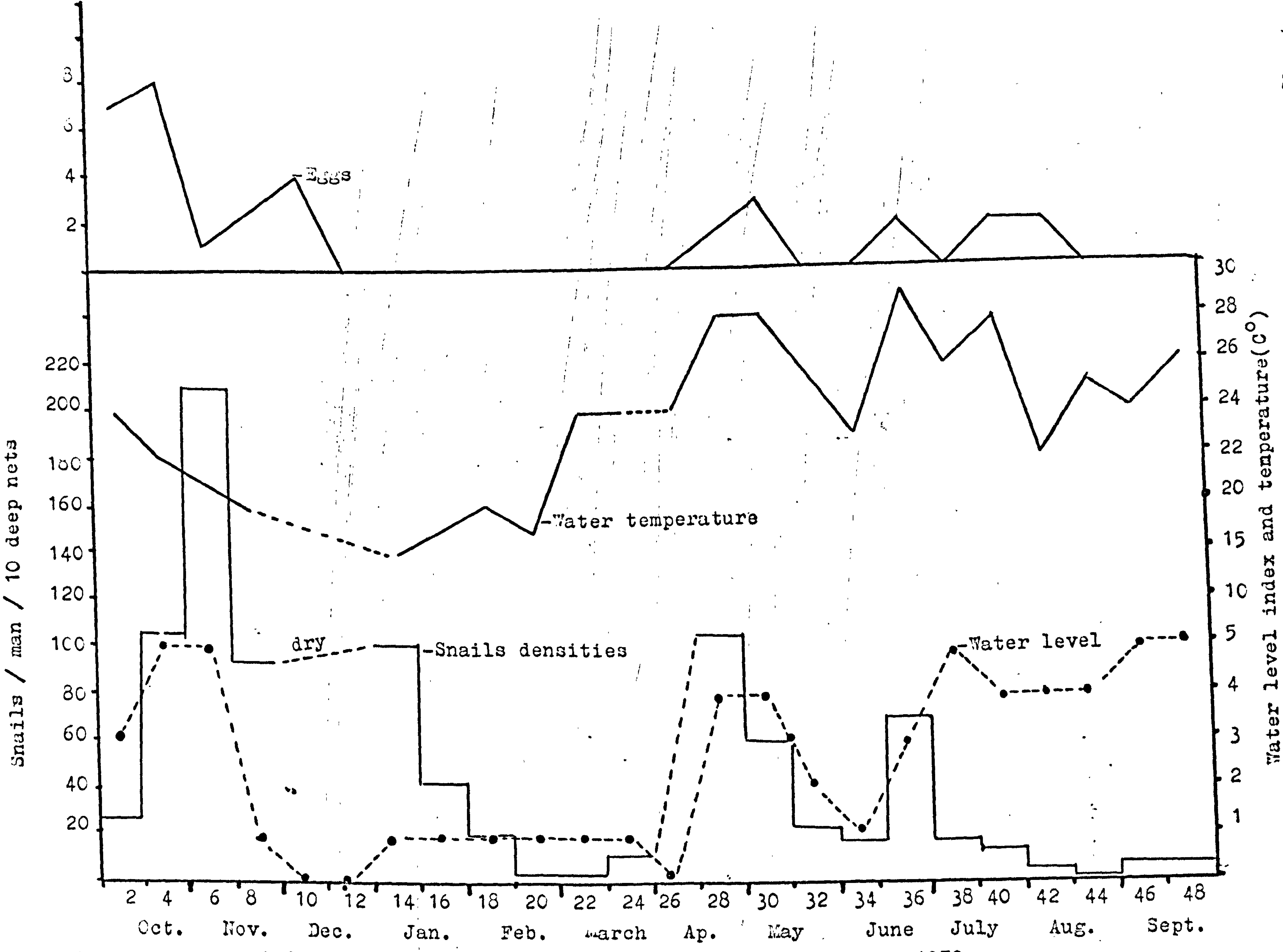


Fig. 7

Fortnightly densities of *Lymnaea gedrosiana* adults, eggs, water temperature and fluctuations of water level in a Balengun Swamp

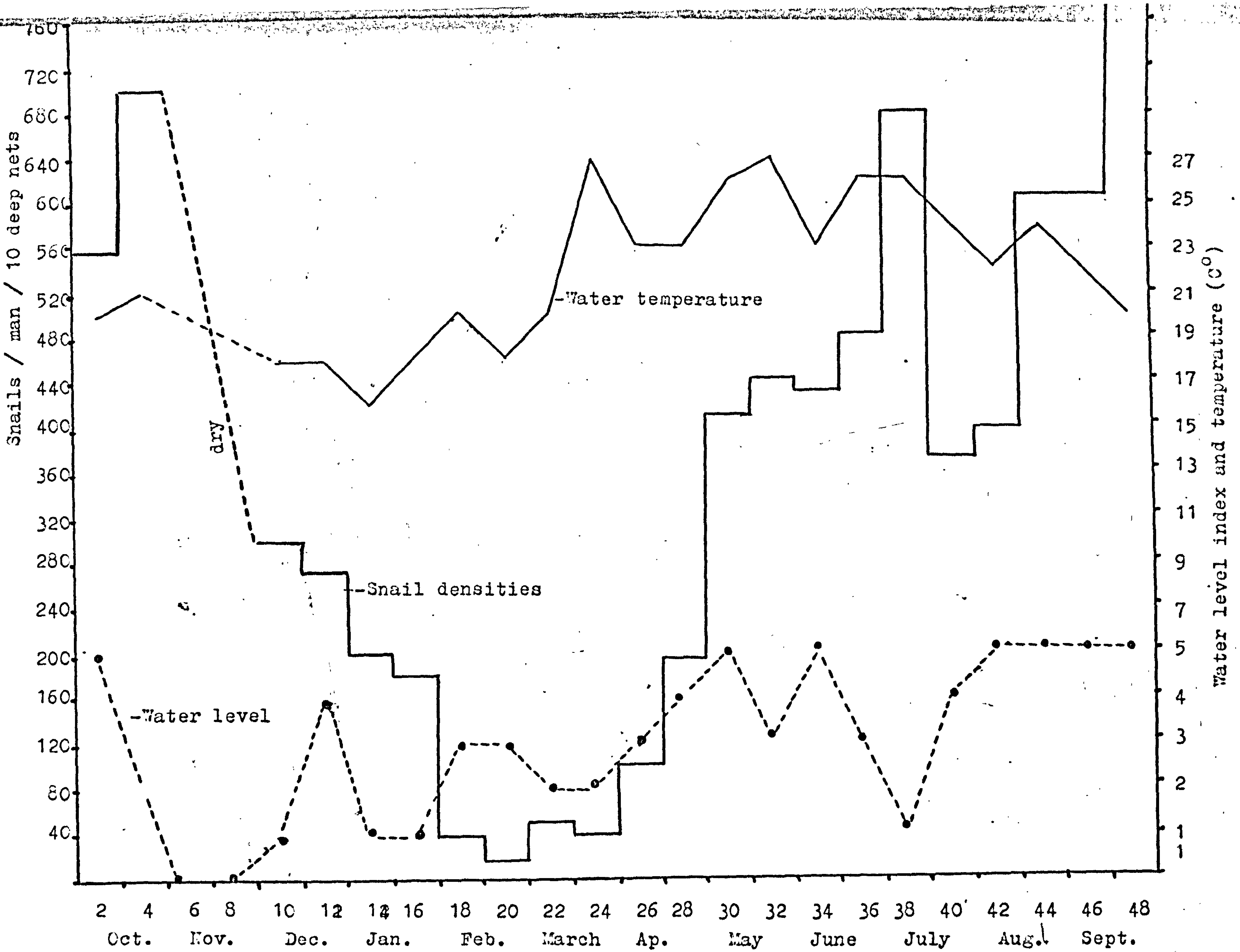


Fig. 8
Fig. Fortnightly densities of Lymnaea gedrosiana, adults and eggs, fluctuation of water level and water temperature in a Sardar Abad pond

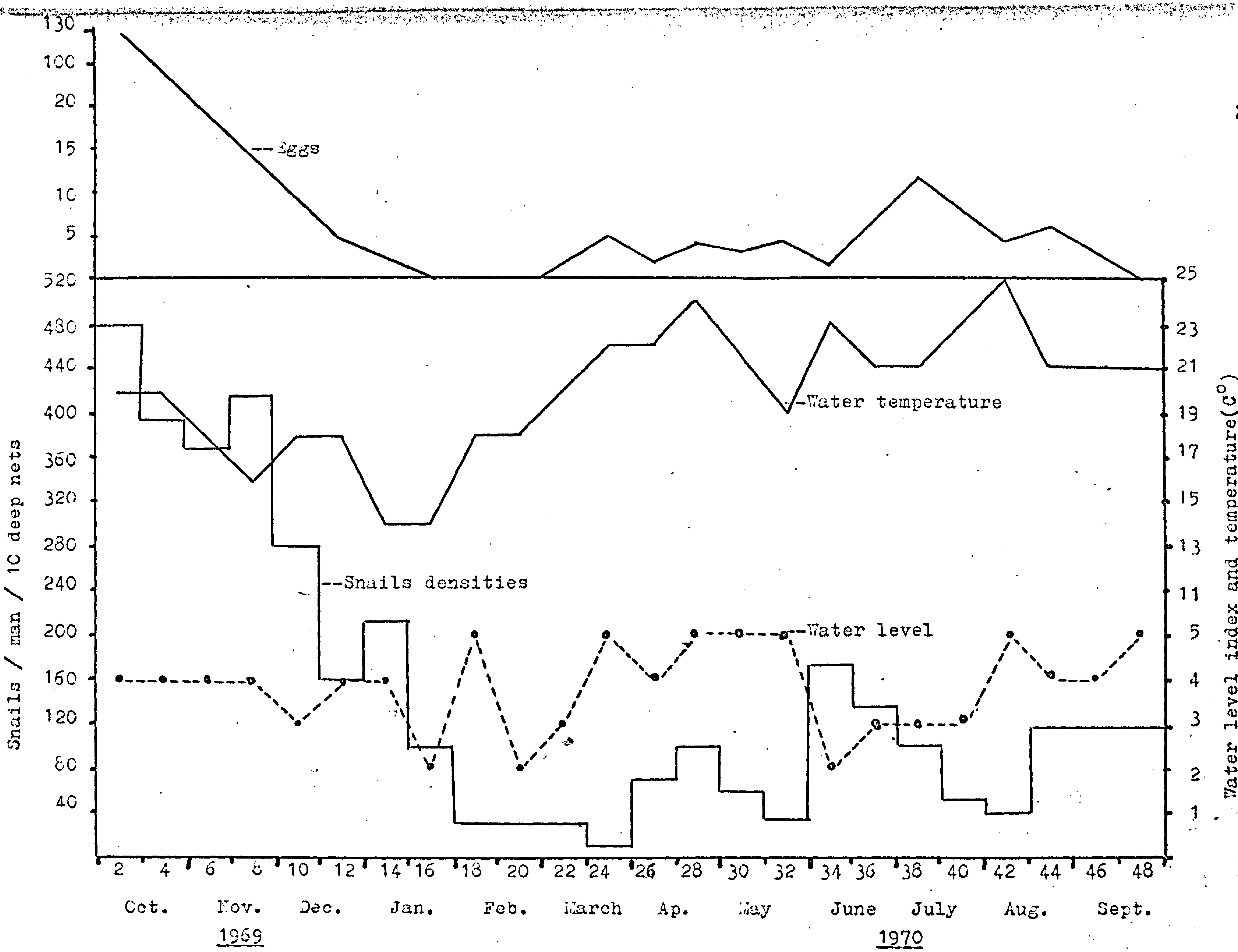


Fig. **9**
Fortnightly densities of *Lymnaea gedrosiana*, adults, eggs, water temperature and fluctuations of water level in a Nahr-Khan pond

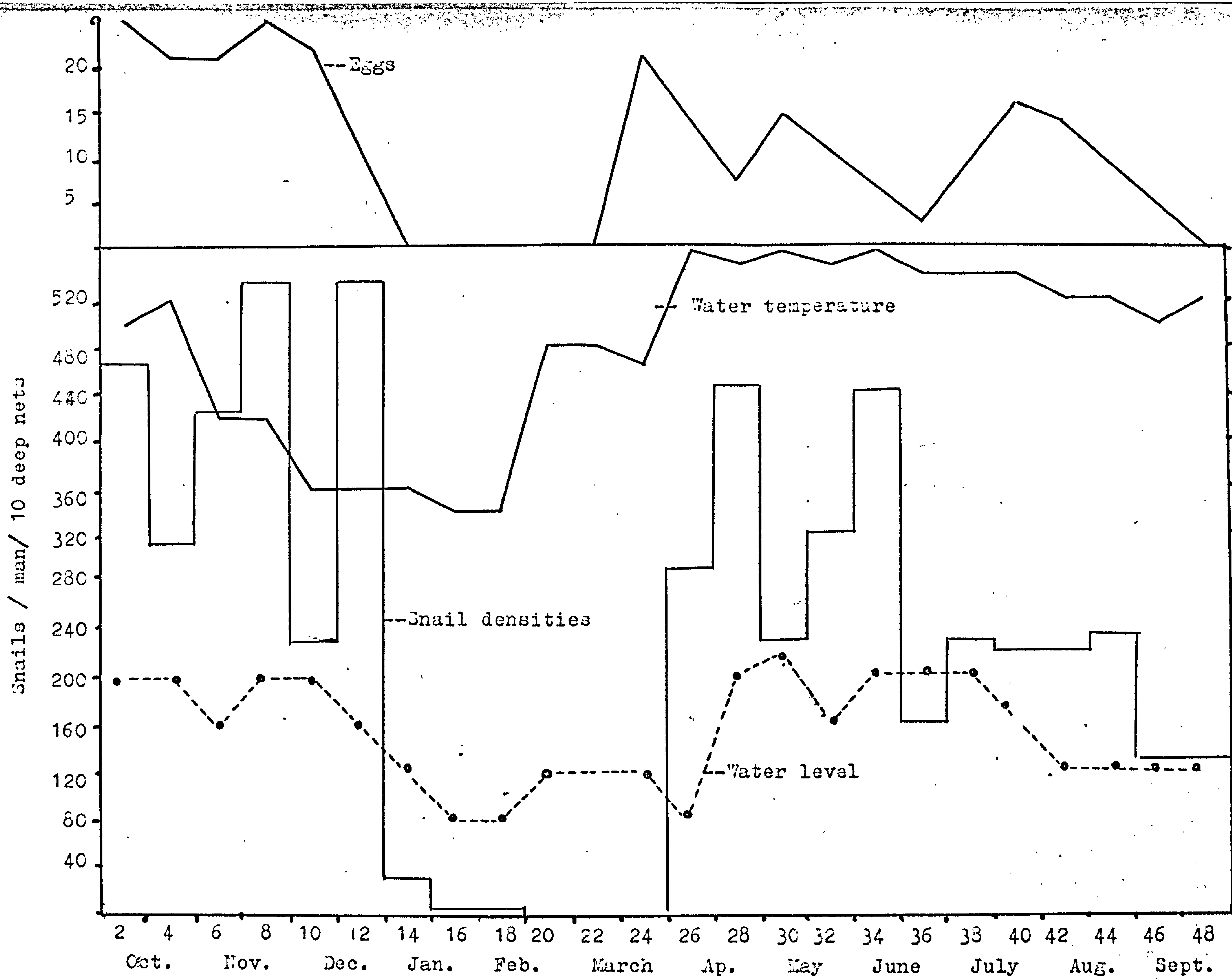


Fig.10

Fortnightly densities of *Lymnaea gedrosiana*, adults , eggs, Water temperature and fluctuations of water level in a Shams-Abad pond

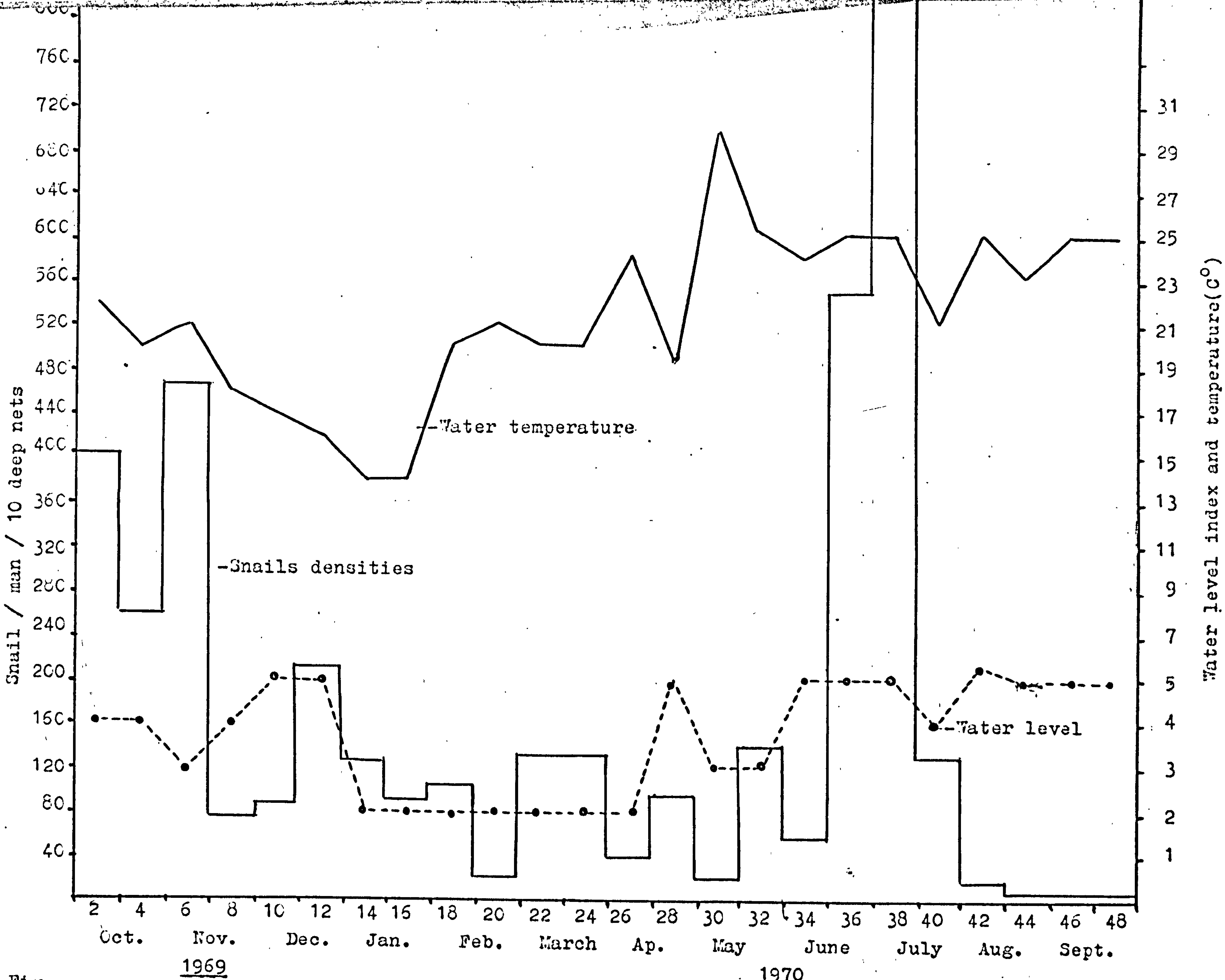


Fig. 11

Fortnightly densities of Lymnaea gedrosiana adults, eggs, water temperature and fluctuations of water level in a Ghaleh Sheikh Swamp

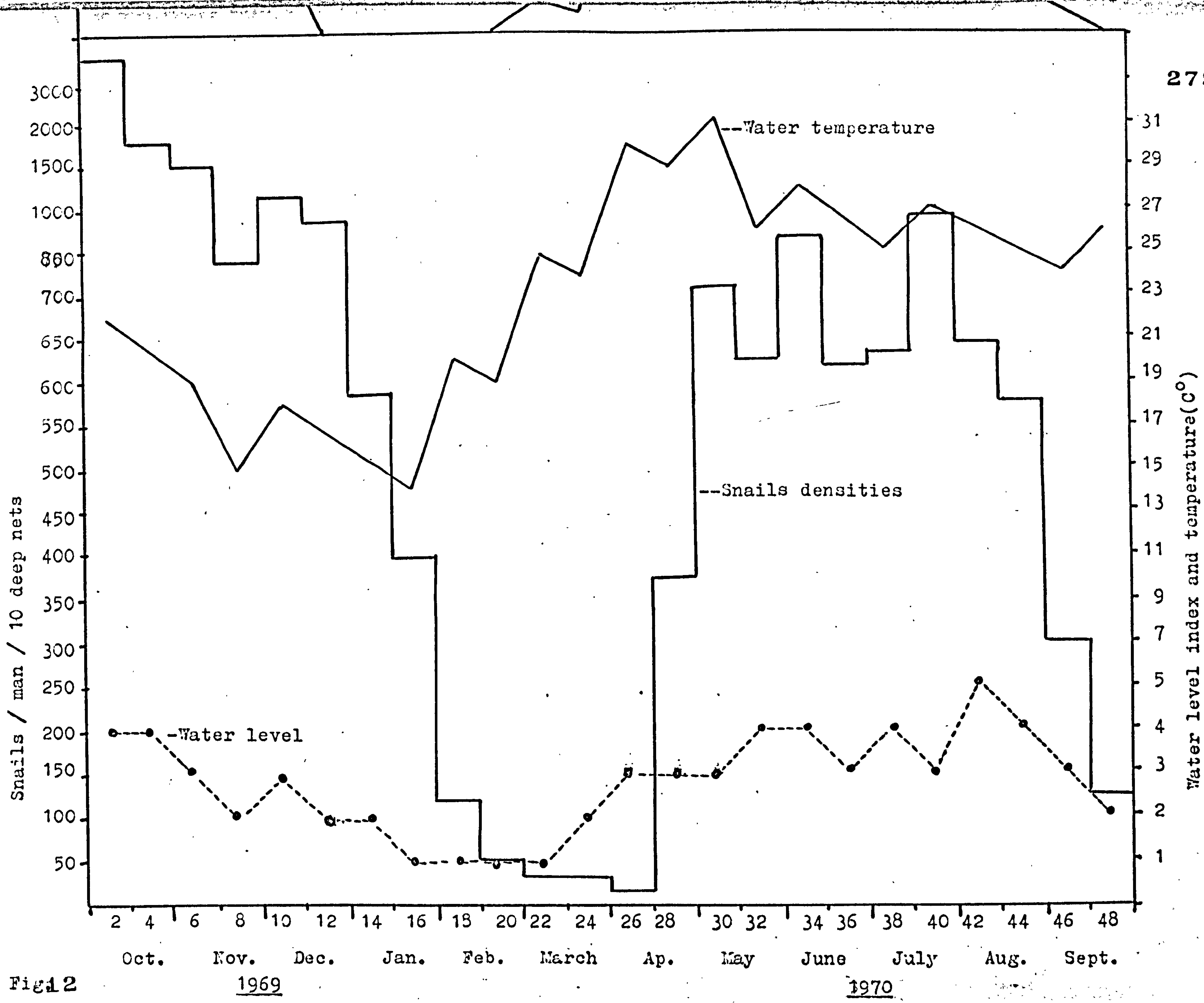


Fig.12

Fortnightly densities of Lymnaea gedrosiana adults, eggs, water temperature and fluctuation of water level in a Ghaleh Seyed Swamp

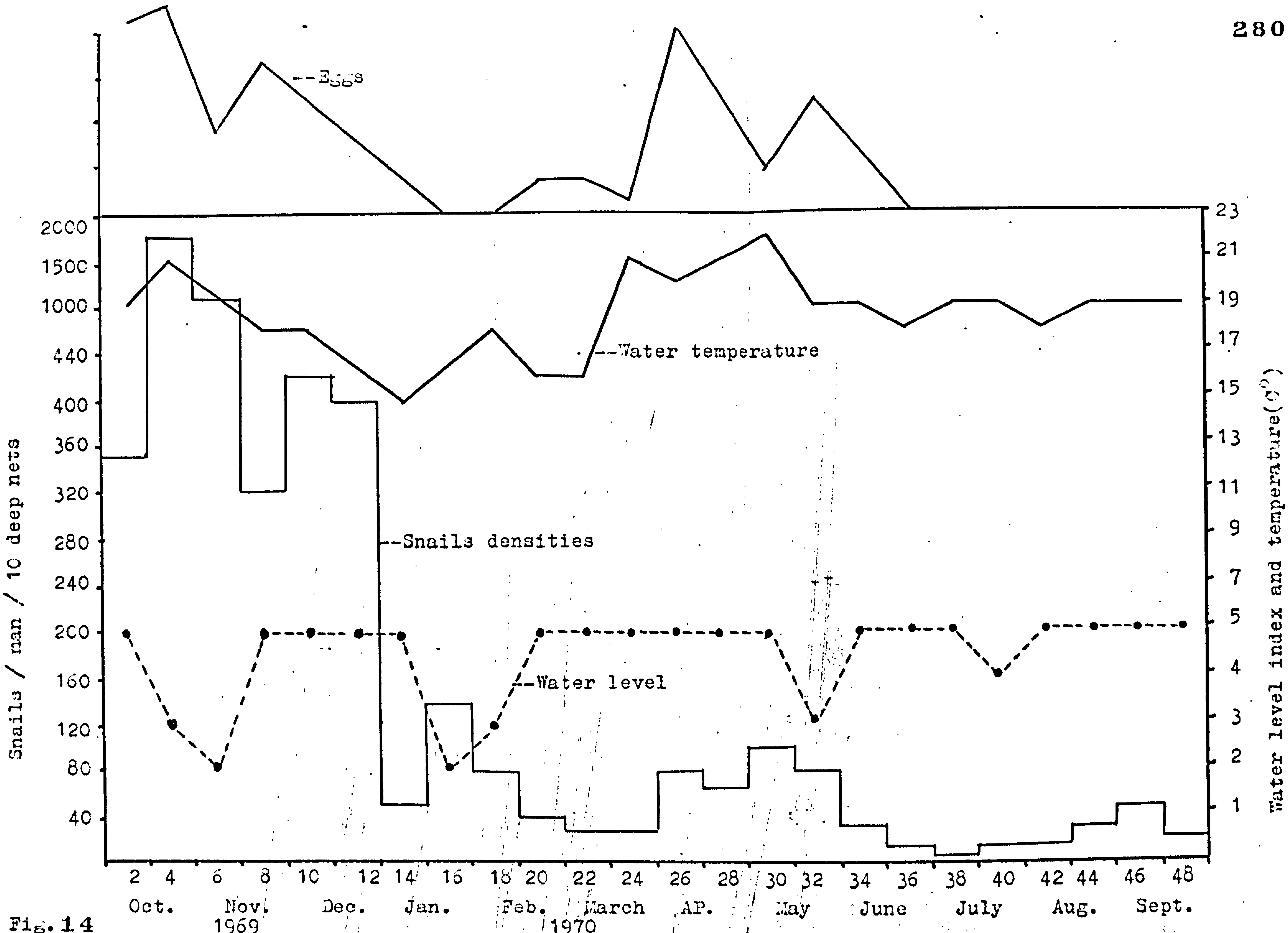


Fig. 14

Fortnightly densities of Lymnaea gedrosiana, adults, eggs, water temperature and fluctuations of water level in a new irrigation system canal no.80

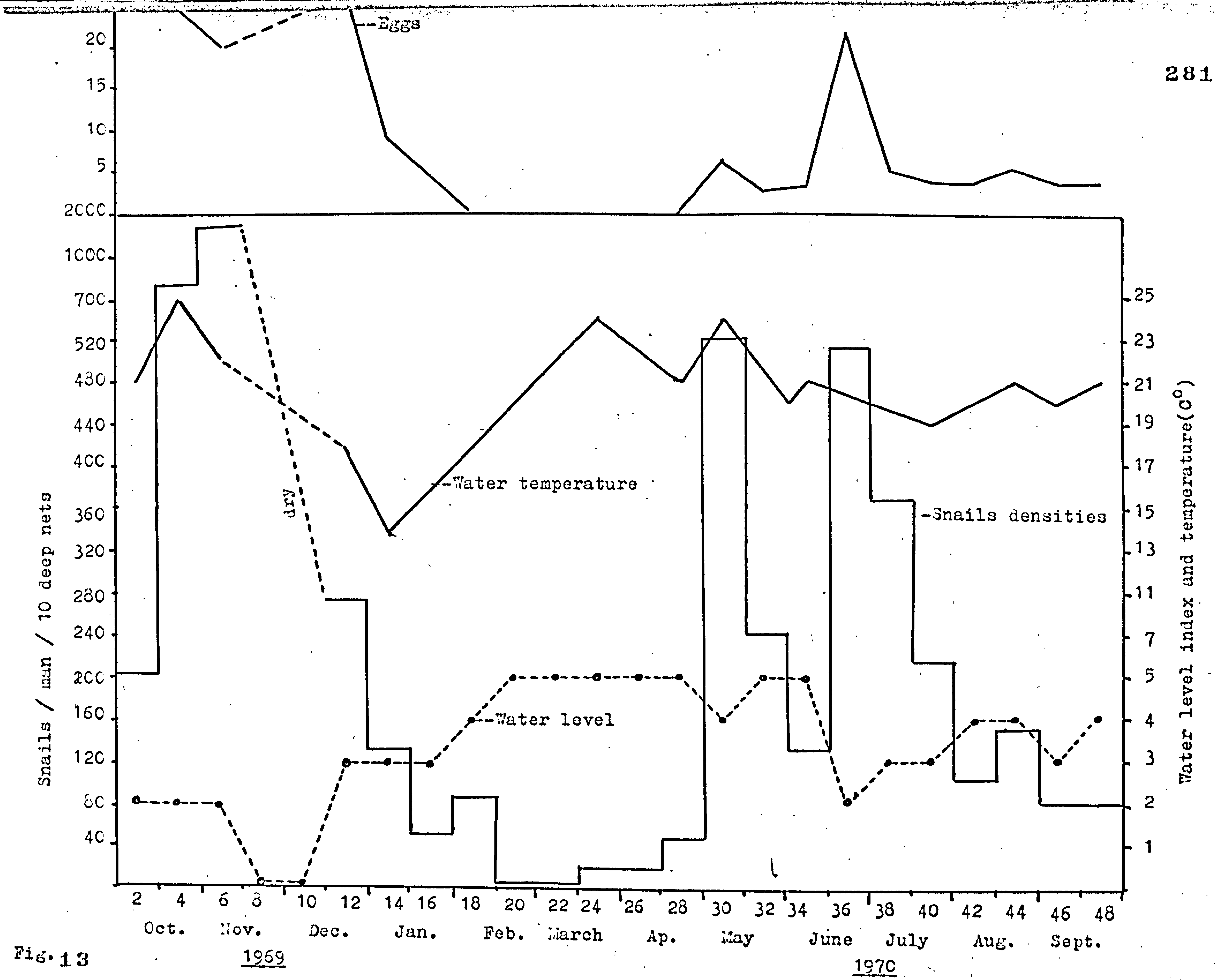


Fig. 13

Fortnightly densities of Lymnaea gedrosiana adults, eggs, water temperature and fluctuations of water level in a new irrigation system canal No. 40

CHAPTER 6

DISCUSSION,

Seasonal Fluctuation in Population Dynamics,

L. gedrosiana was found only in permanent water bodies. According to Gaud *et al* (1962) drought and flooding are the two main factors limiting snail populations in Khuzestan. The size of a snail population is controlled by the physical, chemical and biological factors in the habitat, especially temperature and competition with the other species of snails as well as the activities of natural predators. It has been noticed many times in Khuzestan that following molluscicieiding that when snails have reappeared the population has built-up very rapidly and after some while the snail densities have reached a peak, the controlling factors having produced a natural balance again.

/ Effect of.....

Effect of Drought,

Drought may occur in every season in Khuzestan but the summer drought is very severe and most of the L.gedrosiana population in dry habitats are eleminated. Previous studies concerning the adaptation of B.truncatus, B.alexandrina and L. gedrosiana to drought in the laboratory have been presented by Chu, Massoud and Arfaa(1967). We found that B.truncatus was able to burrow into mud in response to the disappearance of water, while L.gedrosiana was unable to do so and had only a slight tolerance for dessication, becausd of the wider aperture of its shell and inability of withdrawing the body far into the shell. Cawston(1929) in South Africa also concluded that Lymnaea natalensis was less able than allied species to survive in drying conditions. McCullough(1965) observed that L.natalensis the intermediate host of Fasciola gigantica in Ghana shows the least resistance to drought of all the local species of fresh water gastropods. Other factors, such as a wider range of distribution in water bodies and a high rate of reproduction,

/ ensure....

ensure that this species outnumbers B.truncatus in most of the snail habitats of Khuzestan. Only in habitats where drought is frequent and prolonged are larger B.truncatus population found.

Effect of Rainfall,

Rainfall has a significant effect on the ecological changes of the habitats and provides suitable condition for snail breeding. As shown in Fig. 1, the rainfall from October to December 1969 in the study area was high and the L.gedrosiana population densities were also high, but in the following months in 1970 there was low rainfall and the snail densities decreased in all habitats in the area from February to September 1970. Mozley(1939) working in Tanzania recorded that B.(p). globosus breed rapidly during and after the rainfall. Webbe (1962) showed that major seasonal fluctuation in B.(p).nasutus productus density in Tanganika mainly depends upon rainfall, temperature and the ecological changes brought about by them.

/ Effect of temperature.

Effect of Temperature,

In the present observations it appears that temperature has some influence on the population dynamics of L.gedrosiana. The low water temperature (14^{o}C.- 15^{o}C.) in winter stopped egg production and the population density declined to the minimum level; high temperatures above 30^{o}C. in July and August also decreased the egg production and snail densities in shallow ponds.

The snail population densities varied in different type of habitats. In standing waters, the peaks of snail populations occur in 2 periods one from May to July and the other from October to December. In flowing waters, snail colonies were collected mostly in autumn and spring and to some extents in summer when the water temperature is suitable for snail breeding. The winter was the most unfavourable season for L.gedrosiana breeding in the different types of habitats, when snail densities fell to a very low level in cold temperatures. Our previous observations (Chu, Massoud & Arfaa, 1968) on the B.truncatus population curve in the same area showed

/ that the....

that the yearly peak of *B.truncatus* population may occur twice or only once a year.

Transmission Potential of Different Type of Habitats,

The present results suggest that in Khuzestan, canals are permanent transmission sites of *O.turkestanicum* throughout the year, ponds play a rather minor role in transmission, but swamps also are important sites and transmission can occur in late spring, summer and autumn. Drains in general are unlikely to be of importance as transmission sites for *O.turkestanicum*. Chu, Massoud & Arfaa(1969) reported the importance of canals and ponds as the main transmission sites of *S.haematobium* in the same area of Khuzestan.

The prevalence of infection in large snails was much higher than in those of smaller size. *L.gedrosiana* serves as the molluscan intermediate hosts for *O.turkestanicum*, *Fasciola gigantica* and other unknown flukes in the Khuzestan area, but there was no evidence of mixed cercarial infection.

/In our....

In our previous observations (Chu et al, 1968) we showed that the optimum transmission seasons of S.haematobium and S.bovis were mainly spring and autumn. In the present observations we concluded that transmission of O.turkestanicum in Khuzestan was likely throughout the year particularly from June to November, when there are more infected snails and more animals in contact with infected water. The differences in transmission of O.turkestanicum compared with S.haematobium and S.bovis were mostly due to different patterns of distribution and dynamics of the molluscan intermediate hosts.

Implication[s] for the Planning of Control Compaigns.

A sound knowledge of the bionomics of L.gedrosiana is essential for the precise understanding of the epidemiology and control of O.turkestanicum and Fasciolasis. In particular, if snail control measures are to be undertaken, the seasonal population fluctuations must be understood. Our observations show that the population densities increase in autumn and spring and decline towards cold winter months. The observations suggest that the application

/of moll....

of molluscicides would be most effective if first applied in April to June and repeated at the early autumn (October to December).

The control measures against L.gedrosiana are rather different from the compaign against B.truncatus in Khuzestan, because B.truncatus has a patchy and discontinuous pattern of distribution, while L.gedrosiana has a permanent and continuous type of distribution. Molluscicliding which seems to be the only feasible way of control measures against this snail host should be applied in each water body in the area. Focal application has no value, because as was observed in mollusciciding against B.truncatus in Khuzestan (Massoud et al, 1969) the L.gedrosiana population is re-established in treated habitats in a very short period due to the rapid introduction of the snails from up-stream.

CHAPTER 6

LABORATORY STUDIES ON THE HOST-PARASITE RELATIONSHIP: THE EFFECTS OF VARIATIONS IN MIRACIDIAL EXPOSURE DOSAGE

INTRODUCTION.

At present our knolwdge concerning the host-parasite relationship of *L.gedrosiana* and *O.turkestanicum* is very meagre. Because this knowledge may be necessary for effective control measures, experimental studies on various aspects of the problem are of importance. The effect of the exposure dosage of miracidia on the biology of the snail host and on the subsequent development of the larval stages of the parasite is interesting problem involving the host-parasite relationship of *L.gedrosiana* and *O.turkestanicum*. The similar topic of study made by Pesigan *et al* (1958) on *S.japonicum*, Najarian (1961), and Chu, Massoud and Sabbaghian (1966) on *Bulinus truncatus* and *S.haematobium*.

/ The present....

The present study describes some research on the host parasite relationship and larval development of O.turkestanicum in L.gedrosiana under laboratory condition.

MATERIAL AND METHODS,

Laboratory bred L.gedrosiana 5-7 mm. long were used. The snails were divided into six groups, each group consisting of 35 to 50 snails. Snails in group I were used as non-infected controls. Each individual snails in group II to VI was exposed to 1, 2, 5, 10 and 20 miracidia respectively. The miracidia were counted carefully under a dissecting microscope and transferred with a fine pipptte to a watch glass. One snail was added to each glass and left there for at least 4 hours, after which the snails in each group were maintained in separate plastic tanks at 25°C.- 27°C.

The mortality of snails was recorded every day and from day 15 after exposure snails were examined for cercaria-shedding in

/separate.....

separate test-tubes. The snails in each group were examined every other day for cercariae shedding after 3 hours exposure to light in a $50^{ml.}$ beaker. After 3 hours the snails were removed from beaker and the number of cercariae were counted with the ninhydrin staining technique (Mc Clelland,1961). The total number of cercariae divided by the number of snails in each group gave the average number of cercariae shed per snail per day. The cercarial counting was continued during the whole cercarial shedding period which was usually the life-span of the infected snails.

RESULTS,

Prepatent periods (Table, 71),

On day 17 after exposure 4 snails in group III and 3 snails from group VI were found to be shedding cercariae. The last snail from group IV started to shed cercariae on the 24th day after exposure. All the negative snails at 30 days after exposure were crushed between

/two slides...

two slides and searched for cercariae under a dissecting microscope; they were all uninfected. The average length of the cercarial prepatent period from the snails in groups II-VI was 21, 18, 20, 21, and 18 days respectively. Thus, was observed no marked differences between the different groups.

Infection rates (Table, 72)

The infection rates of snails in groups II-VI were 54.5 %, 57.1 %, 62.5 %, 84.0 % and 100 % respectively. This result indicate that the infection rate increased with the exposure dosage of miracidia.

Number of cercariae shed by the infected snails, (Table, 73)

The mean daily number of cercariae shed by the snail from groups II-VI during their entire infection life-span was: 62; 225; 799; 172 and 565 respectively. Thus the snails in group II exposed

/to one ....

to one miracidium and group III exposed to 2 miracidia shed fewer cercariae than those in the other groups. The difference between the number of cercariae shed in group II-III and the other groups was statistically significant ($P < 0.02$ and $P < 0.05$).

Cercaria-shedding curve,

Beginning from the day of the first shedding of the cercariae, the average number of cercariae shed per snail per day was at first low, it then gradually increased. In group II and III the peak of cercaria-shedding was reached at 25 days after the first shedding, then the snails died; in group IV the peak was at about 27 days after first shedding remaining high until the snails died; in group V the peak was recorded at about 22 days; in group VI peak was at 32-34 days after the first shedding.

Survival rates of the exposed snails, (Table, 74)

At the time of exposure to miracidia there were 50 snails in group II, 40 snails in groups III, IV, VI and 35 snails in groups

/ I and V...

I and V. At the end of the shortest cercarial prepatent period (17 days) the number of the surviving snails in the control group was 35, and the number of surviving snails in groups II, III, IV, V and VI were 44, 35, 24, 25 and 12 respectively. The survival rates of snails in group IV, V and VI which were exposed to the larger numbers of miracidia were thus considerably lower than the other groups.

The mean survival periods of the cercaria-shedding snails were 39, 45, 36, 40 and 34 days from the time of exposure to miracidia for groups II to VI respectively, and the maximum survival periods were 50, 45, 60, 40 and 55 days after exposure respectively. Comparision with the maximum survival period of non-infected control group I, where 28 live snails out of 35 with 80 % survival rate in 60 days observation period showed that the differences between the mean life-span of the infected snails and non-infected snails in the control group were statistically highly significant (P 0.001).

DISCUSSION,

Cercarial output rates,

Cercarial output varied greatly, both from snail to snail and from day to day. In general, few cercariae were produced at the beginning of the shedding period, then the number increased over a period of days or weeks until it reachs a more or less constant level, which was maintained until a few days before the termination of shedding by death or self-cure.

Some snail species produced only small numbers of cercariae and remain infected for a short period. For example cercarial output figures for S.haematobium are reported to be low up to 1500 from a single B.(p). africanus (Porter 1920) and 50-400 from B.(p). globosus (Gordon et al 1934) and up to 790 from B.truncatus (Chu et al 1966) whereas with S.mansoni in B.glabrata as many as 1000 to 3000 cercariae per snail per day is not unusual and individual snail may shed for many weeks. L.gedrosiana with cercariae production of only 62 to

/799...

799 maximum in 4-5 weeks is therefor a poor shedder.

The daily pattern of output of the cercariae shedding corresponds with the temperature and intensity of illumination, particularly with animal schistosome cercariae which are more susceptible to temperature and light. Thus, S.bovis and O.turkestanicum cercariae are very susceptible to sun light and cercaria-shedding usually takes place in the morning, particularly in the case of O.turkestanicum.

With regard to the effect of the exposure dosage of miracidia on the number of cercariae shed by the infected snails Pesigan et al (1958) reported that Oncomelania quadrasi exposed to one miracidium of S.japonicum shed twice as many cercariae per day as the snails exposed to 2-5 miracidia. Conversely Chu et al (1966 a) found B.truncatus exposed to 2 or more miracidia of S.haematobium shed more cercariae than those snails exposed to one miracidium.

/ Our present....

Our present studies coincide with those of Chu et al(1966 a). L.gedrosiana infected with one or two miracidia shed markedly less cercariae than those snails infected with 5 tO 20 miracidia each.

Cercarial prepatent periods,

Chu et al (1966 a) found that the cercarial prepatent periods in B.truncatus exposed to various numbers of miracidia of S.haematobium were inversely proportional to the exposure dosage. Conversely Pesigan et al (1958) studied the prepatent period of S.japonicum in O.quadrasi and found that there was no difference in the length of the cercariae-prepatent period between the snails exposed to one miracidium and those to 2-5 miracidia. The results of our studies on the cercarial-prepatent period in L.gedrosiana exposed to various numbers of miracidia of O.turkestanicum were at variance in this respect with those obtained by Chu et al (1966 a) and coincide those obtained by Pesigan et al (1958). The mean prepatent period in L.gedrosiana in our experiments was 18-21 days in different groups at water temperature of 25°C., but Azimov et al

/1968.....

(1968) reported that development of *O. turkestanicum* cercariae in *Radix auricularia obliquata* took 22-25 days in Uzbekstan(SSR).

Infection rate.

Investigating the infection rate of snails to miracidia, Shraiber and Shubert(1949 b) found an increasing percentage of snails shedding cercariae as a result of exposure to increasing numbers of miracidia. Similarly Etges (1963) found that *B. glabrata* was 100 % susceptible to *S. mansoni* infection if snails were exposed to a sufficiently large number of miracidia and the failure to achieve 100 % infection of the snails is attributable to deficient infectivity of the miracidia rather than to innate resistance of the snails. Chu *et al*(1966 a) achieved 100 % infection rates in *B. truncatus* exposed to 20 miracidia of *S. haematobium*. Similarly we obtained a 100 % infection rate in *L. gedrosiana* with 20 miracidia of *O. turkestanicum*.

/ Lengy....

Lengy (1962 a) found that summer-winter variations in temperature have little effect on the success of infection and that *B.truncatus* become infected with *S.bovis* miracidia equally well at 14°C. and 30°C. However, Chu *et al* (1966 b) found that the infection rate of *B.truncatus* exposed to *S.haematobium* miracidia at temperature of 10°C.-20°C. was low, although the longevity of miracidia in cold water was increased. In the present study we found *L.gedrosiana* infected with *O.turkestanicum* in field conditions all around the year which average water temperature varied from 14°C. in winter to 30°C. or more in summer. From our studies on the life-span of *L.gedrosiana* infected in the laboratory which show a maximum of 65 days after exposure to miracidia it seems unlikely that under natural condition snails infected during the summer would survive into the winter period.

Snail mortality rates,

Snail mortality subsequent to infection was high, all the snails in different groups were dead 65 days after exposure to

/ miracidia...

miracidia. Similarly Lengy (1962 a) observed high mortality rates (50 %) in *B.truncatus* infected with *S.bovis* over a period of 2 months. After 2 months the infections in the surviving snails had died out and the snails ceased shedding cercariae. In our experiments infected *L.gedrosiana* always died and no self-cures were ever observed.

Table, 71

Effect of dosage of miracidia on the cercarial-incubation period of

<u>O.turkestanicum</u> in <u>L.gedrosiana</u>.

Group	Number of miracidia used to infect each snail	Total number of cercaria-shedding snails	Number of snails shedding cercariae for the first time on the following days after exposure								Length of cercarial-incubation period in days (mean)
			17	18	19	20	21	22	23	24	
II	1	24	0	0	0	15	4	0	5	0	20-23(21)
III	2	20	4	10	0	6	0	0	0	0	17-20(18)
IV	5	15	0	0	0	5	10	0	0	0	20-21(20)
V	10	21	0	0	4	7	5	0	0	5	19-24(21)
VI	20	12	3	7	2	0	0	0	0	0	17-19(18)

Table, 72

Effect of exposure dosage of O.*turkestanicum* miracidia on infection rate of L.*gedrosiana*

Group	Number of miracidia used to infect each snail	Number of snails exposed to infection	Number of snails surviving at the time of first shedding of cercariae	Number of surviving snails cefcaria-positive	Percentage of survivng snails cercaria-positive %
II	1	50	44	24	54.5
III	2	40	35	20	57.1
IV	5	40	24	15	62.5
V	10	35	25	21	84.0
VI	20	40	12	12	100.0

Table, 73

Cerearial production of L.gedrosiana, infected with different number of O.turkestanicum miracidium in the laboratory at temperature 25°C.-27°C.

Number of days after first shedding of cercariae	Group II (1 miracidium)		Group III (2 miracidia)		Group IV (5 miracidia)		Group V (10 miracidia)		Group VI (20 miracidia)	
	No. of cercaria-shedding snails (mean)	No. of cercariae per snail per day (mean)	No. of cercaria-shedding snails	No. of cercariae per snail per day (mean)	No. of cercariae shedding snails	No. of cercariae per snail per day (mean)	No. of cercaria-shedding snails	No. of cercariae per snail per day (mean)	No. of cercariae shedding snails	No. of cercariae per snail per day (mean)
1	14	14	17	7	4	3	12	74	8	17
4	15	165	16	25	3	48	12	20	8	22
6	18	25	16	282	3	430	13	20	5	230
8	16	12	16	155	3	200	11	206	5	320
10	16	11	16	218	1	120	8	215	5	415
13	15	5	16	100	1	198	8	344	4	310
15	12	17	14	160-	1	196	8	175	4	450
18	10	9	14	136	1	126	7	405	4	218
20	5	50	12	693	1	1740	1	90	4	1565
22	5	195	10	123	1	998	1	252	4	713
25	1	180	8	560	1	2460			3	804
27					1	357			3	300
29					1	2115			2	135
32					1	1554			2	2095
34					1	1440			2	962
									2	495
Mean		62		223		799		180		565

Table, 74

Longevity of non-infected and cercaria-shedding *L.gedrosiana*.

Time since exposure (day)	Surviving snails group I (non-infected)		Surviving snails group II 1 miracidium		Surviving snails group III 2 miracidia		Surviving snails group IV 15 miracidia		Surviving snails group V 10 miracidia		Surviving snails group VI 20 miracidia	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
20	35[a]	100.0	18[a]	100.0	17[a]	100.0	15[a]	100.0	13[a]	100.0	12[a]	100.0
25	35	100.0	18	100.0	16	94.1	15	100.0	13	100.0	6	50.0
30	35	100.0	16	88.8	16	94.1	12	80.0	8	61.5	5	41.6
35	35	100.0	15	83.3	14	82.3	6	40.0	8	61.5	4	33.3
40	33	94.2	10	55.5	12	70.5	4	26.6	7	53.8	2	16.6
45	30	82.9	1	5.5	10	58.8	1	6.6	0	0	2	16.6
50	30	82.9	1	5.5	0	0	1	6.6	0	0	2	16.6
55	28	80.0	0	0	0	0	1	6.6	0	0	2	16.6
60	28	80.0	0	0	0	0	1	6.6	0	0	0	0
65	28	80.0	0	0	0	0	0	0	0	0	0	0

a– number of snails used when experiment was started.

SUMMARY AND CONCLUSIONS

Geography and freshwater ecology of Khuzestan.

1. Khuzestan is a semi-arid plain extending over 157,000 square kilometres in the south-west of Iran. The maximum temperatures are very high in the summer (over 50°C.) and minimal winter temperatures are around 0°C.

2. Five major water courses drain the south-west slopes of the Zagros Range and transverse the plain of Khuzestan. These are the Hindijan, the Jarahi, the Karum, the Karkheh and the Dez rivers. The northern and central parts of Khuzestan are important endemic areas for the human and animal schistosomiasis.

3. The aquatic molluscan fauna is limited in species and B.truncatus and Lymnaea snails are the only medically and veterinary important snails.

4. The human activities: agriculture, rice fields market gardening, cattle breeding and clay pits are more or less directly connected with the ecology of the snails.

/ 5. S.haematobium....

5. S.haematobium is the only ' human ' schistosome and S.bovis and O.turkestanicum are the only ' animal ' schistosomes occuring in Khuzestan. The molluscan host of the S.haematobium and S.bovis is B.truncatus whereas O.turkestanicum is transmitted by L.gedrosiana. The high prevalence of animal schistosomiasis in Khuzestan and the economic importance of the parasites in ruminants prompted the present detailed studies on different aspects of two bovine parasites in various laboratory and domestic animals and the studies on L.gedrosiana the snail intermediate host of O.turkestanicum.

Studies on O.turkestanicum,

Morphology,

Most of the species in the genus Ornithobilharzia are parasites of birds but a few occur in large animals. O.turkestanicum is restricted to mammals and is a parasite of ruminants in which it is common. The morphological characteristics of O.turkestanicum which distinguish this species from the genus Schistosoma are the

/smaller....

smaller size of the adult worms ($21\text{-}10^{mm.}$), the larger number of testes in the male worm (50-90) and the spiral shape of ovary in the female worms. The eggs are much smaller than eggs of bovine schistosomes ($76^{\mu} \times 26^{\mu}$) and are oval with a blunt spine at one pole and a nipple-shaped appendage at the other end.

Observations on naturally infected domestic animals,

1. The wide distribution of L.gedrosiana in the irrigation system in Khuzestan has provided an excellent apportunity for the dissemination of this parasite. O.turkestanicum infection is uniformly distributed in the northern and central parts of Khuzestan, but in the southern part the infection rate is very low. Other workers have found this parasite in Isfahan and Babolsar in the central and northern parts of Iran.

2. The prevalence of O.turkestanicum in cattle (30.3 %) was higher than sheep (15.6 %), and goats (6.7 %) or in buffaloes (2.1 %). In other types of animals the infection rate of O.turkestanicum was very low.

3. The intensity of infection (worm burden and tissue egg loads)

/ in naturally....

in naturally infected cattle declined with increasing age. In contrast in sheep the intensity apparently increased with age. The intensity of O.turkestanicum infection in nature was much higher in cattle than in sheep and goats.

Laboratory experiments in domestic animals,

1. Eight calves, 5 sheep, 2 goats, one buffalo-calf and one young wild pig were exposed to O.turkestanicum in the laboratory; autopsies were performed either 9 or 10 weeks after exposure to cercariae. A new technique was devised for exposing large animals to cercariae by leg immersion in a cercarial suspension in a polythene bag and good infection rates were obtained by this method.

2. The prepatent period in O.turkestanicum infection in different type of animals varied from 43-46 days.

3. The adult worms recovery rates were 37.6 % in calves, 33.9 % in sheep, 22.5 % in goats and 9.6 % in a buffalo.

4. The distribution pattern of adult worms was characteristic. Very few worms were recovered from the hepatic veins (3.1 % in calves, 8.3 %

/ in sheep....

in sheep and 5.2 % in goats) compared with the large numbers of adult worms recovered from the mesenteric veins, particularly the veins of the duodenum (96.9 % in calves, 91.7 % in sheep and 94.8 % in goats). No worms were found in the inferior mesenteric veins of the large intestine.

5. The distribution of the eggs in different organs was similar to the distribution of the adult worms. The duodenum was most intensely involved in O.turkestanicum infections. The density of eggs declined gradually in the jejunum and few eggs were found in the ileum. The large intestine was entirely free from eggs. The liver showed very low egg counts (1-2.3 % in calves, 9-11% in sheep and 20.6% in goats). Other organs (lung, spleen, rumen, reticulum, omasum and abomasum) were free from any eggs. In the buffaloes only a few non-viable eggs were detected.

6. The mean daily egg output per gram of faeces was higher in calves than in sheep or goats being 179, 37, and 59 respectively. The daily egg output in faeces per individual female worm was also higher in calves than in sheep or goats, being 283, 52 and 82 respectively when estimated 9 weeks after exposure to cercariae.

/Susceptibility...

Susceptibility of small mammals and birds,

1. *O.turkestanicum* showed very little infectivity to small mammals: *Tatera indica* (a wild local rodent) produced a few viable eggs after 62 days, but other rodents including laboratory rodents produced only a few immature worms with non-viable eggs. The susceptibility of *Tatera indica* to *O.turkestanicum* was rather low and laboratory maintenance of this parasite in *Tatera indica* was dificult as this wild rodent will not breed easily in the laboratory and stocks had to be provided by trapping wild animals. It is more practical to maintain this parasite in calves or sheep.

2. The dog, mongoose, domestic duck and chicken were insusceptible to infection and no worms were recovered at autopsy.

3. Surveys of mammals dn Khuzestan and the above laboratory studies show that *O.turkestanicum* has very little infectivity to the small mammals and that birds are probably completely resistant to the parasite. The main natural hosts of *O.turkestanicum* are the large domestic animals, particularly ruminants.

/ Studies....

Studies on S.bovis,

Prevalence,

1. Obsevations on the prevalence of S.bovis infection in Khuzestan shgwed that only 0.8 % of the eattle were infected wdth S.bovis and among sheep the infection rate was nil.

2. The dramatic reduction in the S.bovis infection rate in ruminants in Khuzestan from 20.8 % to 0.8 % in cattle and from 14.0% to nil in sheep from 1964 to 1970 is due to the successful snail control measures against B.truncatus, the snail intermediate host of S.bovis and S.haematobium during this period.

3. This observations shows that prevalence studies on bovine schistosomiasis before and after snail control measures may be used as a criteria for the evaluation of the effectiveness of control measures directed against human schistosomiasis where the snail hosts of the human and animal schistosomes are the same.

Laboratory experiments on S.bovis in large animals,

1. Seven calves, 5 sheep, 2 goats and one buffalo-calf were exposed to S.bovis cercariae in the laboratory; autopsies were performed 9 or /18 weeks....

18 weeks after exposure to cercariae.

2. The prepatent periods varied from 44-45 days in calves, 47-50 days in sheep and 47-48 days in goats. The differences between the prepatent periods in calves and sheep or goats were statistically significant.

3. The recovery rates of adult worms were 62.1 % in calves, 41.4 % in sheep and 67.3 % in goats. These infection rate were much higher than those obtained by previous workers, possibly because of the improved infection technique in the course of the present studies.

4. A buffalo-calf exposed to S.bovis cercariae showed no signs of infection. Studies in slaughter houses also showed no infections in buffaloes in nature suggests that they may be insusceptible to the Iranian strain of S.bovis.

5. The distribution of adult worms in the liver was 14 % in calves, 7.6 % in sheep and 24 % in goats. The adult worms in the mesenteric veins were evenly distributed in superior and inferior mesenteric veins. This was a quite different picture from O.turkestanicum infection where the parasites mainly were concentrated in the veins of duodenum.

/ 6. The.....

6. The pattern of egg distribution also differed from O.turkestanicum as the eggs were uniformly distributed between the small and large intestine. A few eggs were also found in the abomasum.

7. The mean daily egg output per gram of faeces was 75 in calves, 60 in sheep and 75 in goats. The daily egg output in faeces per individual female was 106 in calves, 52 in sheep and 48 in goats 9 weeks after exposure to cercariae. The number of eggs passed in the faeces decreased in calves and increased in sheep as the duration of infection was prolonged.

8. Similarly the egg counts expressed as eggs per gram of tissue increased as the duration of infection was prolonged in sheep, whereas in calves these counts declined considerably with the duration of infection. The pathological and clinical manifestations in sheep also become more serious with longer infection, and in some cases S.bovis produced a fatal infection in sheep. It thus seem that there is some tendency in calves to self-limit infections with the parasite but no such effect was observed in sheep.

/ Comparative....

<u>Comparative pathology of S.bovis and O.turkestanicum in ruminants,</u>

1. Severe pathology was observed in one cow with a chronic natural infection of <u>S.bovis</u>; there was blockage and thrombus formation in the mesenteric veins with numerous dead worms and acute superficial haemorrhagic ulcerations in the small intestine.

2. In the experimental infections sheep and goats were more severely affected with <u>S.bovis</u> than calves. The gross appearance of the liver showed numerous minute greyish nodules and some large lymphoid nodules.

3. The different ruminants showed different histopathological responses to <u>S.bovis</u>. Calves had pronounced medial hypertrophy of portal veins; sheep had proliferative endophlebitis and thrombophlebitis with abundant eosinophil infiltration; goats had a large number of eggs surrounded by stellate-shaped accumulation of eosinophilic antigen-antibody material (the Hoeppli phenomenon).

4. <u>O.turkestanicum</u> produced a milder pathological response in ruminants than <u>S.bovis</u>. The liver showed mild or moderate pathological changes with minute greyish nodules all over the surface and deep in tissue substances in sheep and goats but not in cattle.

/5. In the ....

5. In the intestine O.turkestanicum eggs were found chiefly in the mucosa and lumen of duodenum.

6. In sheep with natural heavy infection of O.turkestanicum inflamatory reactions and eosinophils infiltration in the mucosa and villi of duodenum were striking.

7. Infection of O.turkestanicum and S.bovis in ruminants in Khuzestan is probably of considerable economic importance. The damage to the intestine produces inadequate nutrition and fattening of such animals becomes difficult. Furthermore, damage in the intestines makes them useless for processing as sausage skins and causes economic loss, and porous intestines or ' sprinklers ' are discardable. Control measures to combat the economic loss caused by schistosomes of domestic animals should concentrate on snail control programs and the provision of a safe water supply.

/ Immunity....

Immunity experiments,

1. Immunity experiments on bovine and human schistosome species were carried out in mice, calves and sheep using heterologous and homologous systems. Heterologous immunity experiments in albino mice immunizing with 100, 150 and 200 cercariae of O.turkestanicum produced the same level of partial protection against S.bovis and S.haematobium. Immunisation with 200 cercariae of O.turkestanicum produced lower protection against challenge infection of S.mansoni and 50 cercariae of O.turkestanicum produced very little protection against S.mansoni. Homologous immunity experiment in albino mice with S.bovis produced a moderately high protection against the subsequent challenge infection with the same parasite.

2. In albino mice immunized with O.turkestanicum there was a minimum threshhold value for the immunizing exposure which was less than 100 cercariae.

3. A single exposure of albino mice to O.turkestanicum cercariae produced as effective an immunity as repeated exposure.

/ 4. A high....

4. A high degree of reciprocal heterologous and homologous immunity was produced with *O.turkestanicum*, *S.bovis* and *S.haematobium* in calves but the effect was poor in sheep. Calves could be partially protected against bovine schistosomiasis by previous exposure to *S.haematobium* cercariae. Cattle have advantages in experimental and field studies on schistosome immunity as one is observing the effect on their natural parasites.

5. Homologous immunity and heterologous immunity between bovine and human schistosomes could be a great importance in protecting animals in Khuzestan from the severe effects of subsequent reinfections.

6. It was shown that calves can develop *S.haematobium* infection but only immature worms are produced. In nature in the endemic areas this must occur since cattle are in very close contact with *S.haematobium* infested waters. This may help to protect them against subsequent infection with the bovine schistosomes. The reciprocal effects of immunisation of man against *S.haematobium* by *S.bovis* and *O.turkestanicum* may also occur.

/ Field studies....

Field studies on L.gedrosiana the molluscan host of O.turkestanicum,

1. A study on the distribution and population dynamics of *L.gedrosiana* in relation to the transmission of *O.turkestanicum* in various habitats was carried out in Khuzestan. *L.gedrosoana* is the most common aquatic snail in Khuzestan and occurs throughout the year in different types of water courses. It is the intermediate host of *Fasciola gigantica* as well as *O.turkestanicum*.

2. Populations of *L.gedrosiana* were higher in canals and swamps than in ponds, with peaks in spring and autumn, and low densities in summer and winter. The winter decline was more marked than in summer. *L.gedrosiana* is soon killed by exposure to drought and other environmental changes. In summer the populations of *L.gedrosiana* were larger in canals and deep swamps than in small and shallow habitats.

3. The reproductive capavity of *L.gedrosiana* was higher in running water than in stagnant water. The peak reproduction rate was in spring and autumn and continued at low levels in summer but it was nil in winter.

/ 4. The size.....

4. The size-frequency studies of L.gedrosiana showed that most of the snails in different habitats were young snails less than 6-8 mm. in height.

5. The prevalence of infection in large size L.gedrosiana with O.turkestanicum was higher than in small snails, being 0.1 %, 0.5 % and 1.1 % in snails measures 0-6 mm., 6-10 mm. and 10-14 mm. respectively.

6. Transmission of O.turkestanicum occurred through the year in canals with a peak in late summer. Infectivity of swamps also continued in spring, summer and autumn with a peak in early summer. In ponds most of the transmission probably occurs in spring and autumn. Drains played a very minor role in the transmission of O.turkestanicum in Khuzestan.

7. The infection rates of L.gedrosiana in canals was higher than in other habitats, being 0.68 % in canals, 0.38 % in swamps, o.25 % in ponds and 0.15 % in drains.

8. Non-schistosome parasite larval stages found in L.gedrosiana were:

a, Fasciola gigantica cercariae.

b, Cercariae of the heterogeneous Gymnocephalic group.

c, An unkown apharyngeal longifurcate cercariae.

/ Laboratory...

Laboratory experiments on L.gedrosoiana,

1. In the laboratory infection of L.gedrosiana with 1, 2, 5, 10 and 20 miracidia of O.turkestanicum the following results were obtained:

2. The infection rates in different groups increased with the dosage of miracidia, being 54.5 %, 57.1%, 62.5%, 84.0% and 100% from 1, 2, 5, 10 and 20 miracidia respectively.

3. The mean prepatent period varied from 18-21 days in the different groups but the differences between the groups were not significant.

4. The mean numbers of cercariae shed daily by single snails exposed to one miracidium or 2 were significantly fewer than those exposed to 5, 10 or 20 miracidia.

5. Few cercariae were shed per snail during the early patent period but the number increased with the duration of infection. All the infected snails died at the peak of cercarial shedding, and no infected snails survived more than 60 days after exposure to miracidia. The differences between the mean life-span of the infected snails and non-infected snails in the control group were statistically significant.

/6. Mollusciciding...

6. Mollusciciding seems to be the only practical way of controlling L.gedrosiana. Our previous works on mollusciciding in the Khuzestan showed that L.gedrosiana is more susceptible to the molluscicides than Bulinus snail. Since L.gedrosiana is wide-spread and has a permanent and continuous type of distribution, molluscicides should be applied throughout the water bodies of the area.

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