STUDIES ON
THE EARLY STAGES OF
Gigantocotyle explanatum (CREPLIN, 1847)
IN
THE DEFINITIVE HOST

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INTRODUCTION

Domestic ruminants are indispensable in building up our national economy, which is to a large extent dependent on agriculture. Since cattle, buffalo, sheep and goat have been reared from times immemorial for milk, meat, fleece and work and now that there is an increase in the requirements of aforesaid products, it becomes patent that the improvement in our livestock is imperative. In India, the estimated populations of cattle, buffalo, sheep and goat are 188,800, 56,539, 67,200 and 44,410 thousand respectively (Animal Health Year book, 1966) but their productive ability is of a very low order when compared with those from other countries. The productivity of the animals can be improved in several ways. It is accepted that in addition to better nutrition and breeding, the prevention of various diseases caused by bacteria, viruses and metazoan parasites among our domesticated livestock is equally important.

Of the various parasitic diseases of domesticated ruminants, amphistome infestations show a very high incidence rate in India, and the young stock is severely affected. A single animal may harbour one or several species of amphistomes in large numbers, which may be both mature and immature. One characteristic feature of amphistomiasis is that, in the adult stage, the parasites are non-pathogenic or only
slightly pathogenic but the immature forms are more destructive and produce clinical symptoms of amphistomiasis. The intensity of pathogenicity produced is generally correlated with the number of immature parasites present in an individual host at one time.

A variety of fresh-water snails act as the intermediate hosts for these amphistomes. The snails abound in swampy areas, ponds and slow moving streams from where they spread onto the fields and pastures through irrigation channels and by the overflowing of the tanks in the monsoon. Thus the snails are distributed over a wide area and are therefore readily available to the miracidia of the parasites. These infected snails very soon start shedding the cercariae which encyst on the lush vegetation. In consequence, a large number of metacercariae are available to the domesticated animals, which when ingested produce the infection.

Among the amphistome parasites of domesticated animals in India, Gigantocotyle explanatum (Creplin, 1847) Nasmark, 1937 is a very common parasite and is important in as much as it affects the liver and causes considerable damage to this organ and obstruction of the bile-ducts. The details of the early stages in the life of this parasite in the definitive host are not known fully though a number of
workers (Srivastava, 1944; Singh, 1958; Mukherjee, 1960) have studied the details of the life-cycle in the snail host. Kulasiri & Seneviratne (1956) while describing the histopathology of infected liver gave a short but incomplete account of the early migration of G. explanatum in the definitive host.

This thesis embodies the results of the investigation on (a) the early phase in the life of the parasite; (b) the route of migration of the young fluke in the definitive host commencing with the ingestion of metacercariae till they reach the sites of predilection i.e., the bile-ducts; (c) the host tissue reaction with reference to the migrating early stages; (d) the migratory behavior of the immature flukes as revealed by suitable histochemical techniques, and (e) the growth pattern of the immature flukes during the course of migration.
REVIEW OF LITERATURE

(I) Amphistomiasis

Several outbreaks of amphistomiasis have been reported from various parts of the world resulting in heavy losses to domestic ruminants. The amphistomiasis is produced by different species of Paramphistomum Fischoeder, 1901, Gigantocotyle Nasmark, 1937, Calicophoron Nasmark, 1937, Cotylophoron Stiles and Goldberger, 1910, Gastrothylax Poirier, 1883, Gastrodiscus Leuckart, 1877, Fischoederius Stiles and Goldberger, 1910, Ceylonocotyle Nasmark, 1937, Pseudodiscus Sonsino, 1895, Homalogaster Poirier, 1883, and Oliveria Thapar and Sinha, 1945.

India

In India, amphistomiasis was first recorded by Baldrey (1906) and Walker (1906) in sheep and goat in Punjab. According to Baldrey, it resembled fascioliasis and the chief pathogenic lesions were seen in the small and large intestines. Walker stated that this disease in ovines was confined to the low lying swampy areas and it reached its worst during the months of December, January and February. In Assam, Pande (1935) carried out a survey on acute amphistomiasis in the villages of Kamrup district and recorded 30 to 40 per cent mortality in cattle.
The affected animals were emaciated and suffered diarrhoea because of the presence of immature amphistomes in the small intestine. Haji (1935) reported a disease known as "Phet" or "Pitto" in the animals in Sind which broke out after severe floods. The causal agent was identified as immature forms of *Paramphistomum cervi* (Zeder, 1790).

Outbreaks among sheep and goat, caused by *Cotylrophoros* cotylophorum (Fischoeder, 1901) were investigated by Srivastava (1938) who produced the clinical signs in experimentally infected animals which were characterised by dullness, weakness, anaemia, general unthriftness with persistent foetid diarrhoea. The faeces of the infected animals contained a large number of immature amphistomes. In Sind, Bawa (1939) described an outbreak in sheep and reported 80 to 90 per cent mortality. The disease appeared after rains and the affected sheep showed signs of general unthriftness, diarrhoea and swelling of the sub-maxillary area. The post-mortem lesions were essentially catarrhal chronic verminous enteritis associated with immature amphistomes.

Maqsood (1944) reported acute amphistomiasis in a cow in Northern India, which showed characteristic signs of amphistomiasis with decreased milk production, oedematous swelling in the region of breast and passed dark and offensive diarrhoeetic faeces. The post-mortem examination revealed the
presence of numerous immature amphistomes in the duodenum, pyloric region, abomasum and also in the other compartments of the stomach. The parasite was identified as \textit{P. cervi}. Bhulerao (1944) carried out a survey in the States of United Provinces (now Uttar Pradesh), Bihar, Assam, Madras and Sind (now in Pakistan), and concluded that "immature amphistomiasis" occurred throughout the country affecting the cattle, sheep and goat and the disease was caused by different species of \textit{Cotylophoron}.

Fatal enteritis in goat was reported by Mudaliar (1945) in Madras, which he attributed to immature stages of \textit{C. cotylophorum}. He also noticed all the typical signs of immature amphistomiasis in the animals. Moghe (1945) reported a high percentage of infection in cattle with \textit{C. cotylophorum} and \textit{Gastrothylax crumenifer} (Creplin, 1847) in Central Provinces (now Madhya Pradesh) and Bihar. Srivastava (1948) produced acute amphistomiasis experimentally in a goat with 20,000 metacercariae of \textit{G. crumenifer} and he observed general unthriftiness, marked emaciation and submaxillary oedema and persistent foetid diarrhoea often tinged with blood and containing large number of immature parasites.

Kuppuswamy (1948) reported heavy losses among sheep and goat in Bihar where the disease due to immature amphistomes was known as "Pitto" or "Gillar". He found typical
signs of immature amphistomiasis besides discharge of mucus from nostrils and death occurred in a day or so after the onset of diarrhoea. A severe outbreak of amphistomiasis with typical clinical signs due to immature stages of Paramphistomum was reported in a flock of sheep at Hosur Cattle Farm, Madras by Iyer (1949). Outbreaks of fatal enteritis among sheep, cattle and buffalo were also reported by D'Souza (1949) from Madras and the causal agent was identified as immature stages of C. cotylophorum and G. crumenifer.

Alwar (1949) reviewed the subject of amphistomiasis in Indian livestock and said that the percentage of infection in sheep and goat, cattle and buffalo was 90, 80 and 50 per cent respectively and the mortality rate was 90 per cent in sheep and goat and 40 to 80 per cent in cattle and buffalo. An outbreak of acute amphistomiasis among cattle was reported by Ramakrishnan (1951) in the Nellore district of Madras, where 30 per cent of the cattle showed severe symptoms and in these the mortality was as high as 72 per cent among untreated and 16 per cent among the treated animals. The parasites were identified as G. crumenifer and F. cobboldi (Poirier, 1883).

Varma (1957, 1961) recorded the incidence of amphistome infection in sheep, goat, cattle and buffalo in Bihar
and produced clinical signs in experimentally infected lambs with the metacercariae of *C. cotylophorum*. Katiyar and Varshney (1963) examined 18,438 sheep and 2,974 goats in Uttar Pradesh and found 5,183 and 1,541 deaths due to amphistomiasis in sheep and goat respectively. In order of frequency, the following species were involved: *G. crumenifer, C. cotylophorum, P. cervi, F. elongatus* (Poirier, 1883) and *P. explanatum (=G. explanatum)*. According to Katiyar and Varshney (1963) the outbreaks occurred from September to January following the rains.

**Countries other than India**

In Australia, outbreaks of intestinal amphistomiasis among cattle were recorded by Wooldridge (1923). Later, Ross and Gordon (1936) also reported cases of amphistomiasis. As high as 30 per cent mortality among young calves was observed in South Coast region of New South Wales by Edgar (1938) who described "a syndrome of anaemia, debility, persistent diarrhoea" in calves and concluded that fatal effects may occur in young cattle due to migrating young *P. cervi* and particularly when a concomitant infestation with *Monodontus phlebotomus* (= *Bunostomum phlebotomum*) was present. Roberts (1939) reported a large number of immature parasites from two heads of cattle. In the Casino district of New South Wales,
a severe outbreak of intestinal amphistomiasis with 20 per cent mortality of young calves was reported (Anon., 1949).

In the report of the Commonwealth Scientific and Industrial Research Organisation, Australia (1950), a severe outbreak of amphistomiasis was recorded in sheep at Guyra and in the Casino district of New South Wales. Harding (1950) reported the death of 15 out of 45 adult cattle and 8 out of 23 young animals due to massive infection with immature amphistomes. Bray (1954) reported death of a Jersey cow caused by a heavy infestation of the reticulum with *P. cervi*.

In New Zealand, an outbreak of amphistomiasis was reported by Whitten (1955) who found 14 per cent mortality in ewes showing symptoms of diarrhoea and anaemia with a heavy infestation of the duodenum with immature amphistomes.

In North America, Price and McIntosh (1944) reported 30 to 40 per cent mortality in cattle due to *P. microbothrioides*. Olsen (1949) reported 90 per cent mortality in sheep and 30–40 per cent mortality in cattle due to *Paramphistomum* sp.

In Africa, Simson (1926) reported mortality amongst cattle and sheep due to amphistome parasites and in one flock of Merino sheep, 10 per cent mortality was observed. Two serious outbreaks of amphistomiasis occurred in
Orange-Free State of South Africa in which 30 and 50 per cent of the flock had died (LeRoux, 1930) due to the immature stages of *C. cotylophorum*. Outbreaks of acute intestinal amphistomiasis were also recorded in the Zebu cows of Tanganyika (Butler and Yeoman, 1962). Roach and Lopes (1966) reported nearly 50 per cent mortality and this was attributed to a mixed infection of liver-fluke and *P. daubneyi* and *P. microbothrium*.

In Egypt, outbreaks of amphistomiasis, caused by *P. cervi*, were reported in sable antelope (Sharaf, 1955). Several reports of amphistomiasis in the Middle East have appeared particularly from Israel (Aharoni, 1955; Witenberg, 1955, and Nobel, 1956). Lengy (1962) produced the disease experimentally in lambs infecting them with *P. microbothrium*.

In Europe, outbreaks of amphistomiasis were reported from France (Guilhon and Priouzeau, 1945, and Priouzeau, 1947), Hungary (Boray, 1959), Sardinia (Deiana, Lei and Arru, 1962), Poland (Anczykowski and Chowaniec, 1955), Bulgaria (Visnjakov and Ivanov, 1964), U.S.S.R. (Podberezski, 1951; Orlova, 1953; Gusev, 1954; Densov, 1955; Trach, 1956; Podlesni, 1959; Karabaev and Amangaliev, 1964, and Vorobev, Korolev and Korolev, 1965) and United Kingdom (Craig and Davies, 1937).

In Ceylon, Crusz (1952) reported the incidence and
geographical distribution of amphistome infestation in cattle, buffalo and goat and the flukes belonged to four genera: *Gastrothylax*, *Gigantocotyle*, *Paramphistomum* and *Calicophoron*. In Malaya, amphistome infection amongst cattle and buffalo was reported to be high (Lee, 1967).

(2) **Life-history study**

*Gigantocotyle explanatum* was first described by Creplin (1847) as *Amphistomum explanatum* from the gall-bladder and bile-duct of a Zebu cattle. Later, Fischoeder (1901) doubted the systematic position of this species and considered it as "species inquirendae". Stiles and Goldberger (1910) included it in the genus *Paramphistomum*. Finally in 1937, Nasmærk created a new genus, *Gigantocotyle*, to include *A. explanatum* Creplin, 1847 and other related species. Another species *G. bathycotyle* has been considered to be a valid species by some while others regard it as a synonym of *G. explanatum* (Willmott, 1950; Gupta, 1951; Singh, 1958, and Mukherjee, 1960).

*G. explanatum* has been reported from *Bos taurus indicus* and *Bubalis bubalis* from India, Burma, Ceylon, Indo-China, Philippines and Celebes Islands (Nasmærk, 1937; Willmott, 1950; Crusz, 1952, and Yamaguti, 1954).
Intermediate host

In India, Srivastava (1944) first reported in an abstract form the life-history of *G. explanatum* and incriminated the snail *Indoplanorbis exustus* as the intermediate host. Singh (1958), while studying the life-history of *G. explanatum*, tried a large number of snails of the following species: *Lymnaea acuminata*, *Indoplanorbis exustus*, *Melanoides tuberculata*, *Vivipara bengalensis*, species of *Bulinus* and *Gyraulus convexiusculus* for the experimental infection and found that only *G. convexiusculus* acted as the intermediate host and not *I. exustus*. Singh (1958) studied in detail, the morphology of egg, miracidium, sporocyst, redia and cercaria of *G. explanatum* and succeeded in experimentally infecting a goat with metacercariae. The mature and immature parasites of *G. explanatum* were recovered from the bile-duct of goat. Mukherjee (1960) also reported *G. convexiusculus* as the intermediate host and described the life-history of this parasite.

Route of migration of *G. explanatum* in the definitive host

Apparently only one paper has described the route of migration adopted by the immature *G. explanatum*. Kulasiri and Seneviratne (1956), described the histopathology of liver of buffalo, infected with *G. explanatum*
and collected at an abattoir. The lesions in the material studied suggested a transperitoneal infection of the liver and subsequent migration to the bile-ducts similar to *Fasciola hepatica* (Linnaeus, 1758). They found sexually mature adult parasites in the cystic spaces or cylindrical dilatations of numerous bile-ducts which were uniformly distributed in all parts of the liver though the gall-bladder never contained any parasites. The dilated cystic duct was packed with parasites right up to the neck of the gall-bladder. Though they examined many sections only on two occasions flukes were encountered within the veins of the liver. In one case the fluke was enclosed in granulation tissue and in the other it was calcified, indicating that they do not commonly travel in veins or survive in them for long.

**Route of migration in other amphistomes**

A majority of the amphistomes in ruminants, have an unvarying route of migration as followed by the immature parasite. The encysted metacercariae when ingested by the susceptible final host reach the small intestine where the excystment takes place. The young amphistomes attach firmly to the intestinal mucosa with the help of the suckers and gradually migrate anteriorly and in due course of time reach the rumen to mature and oviposit (LeRoux,
1930; Srivastava, 1938; Dinnik and Dinnik, 1962 and Horak, 1967).

**Route of migration in other flukes of liver**

The classical researches of Thomas (1881) and of Leuckart (1881) in respect of the life-history of *Fasciola hepatica*, did not indicate the exact migratory route by which the immature forms reached the seat of predilection. It was Lutz (1892, 1893) who first reported that after excystment the young flukes enter the intestinal wall and are carried to the liver by the blood stream. His findings were later supported by Railliet, Moussu and Henry (1913), Compes (1923), Noller (1925), Marek (1927), Noller and Schmid (1928) and Bugge (1935). Another possible route of migration was suggested by Sinitsin (1914) who observed in rabbit that, after the infection, the young flukes pass through the wall of the intestine, creep over the abdominal viscera and finally penetrate the liver from the outside.

Shirai (1927) conducted some experiments in guinea-pig and recovered the flukes from the abdominal cavity and the surface of liver and supported Sinitsin's theory. This was also confirmed by Susuki (1931) who reported that when cysts of *F. hepatica* were given through mouth, they left the intestine and moved into the abdominal cavity.
Shaw (1932) fed cysts to guinea-pigs and lambs and three days later recovered the living flukes from the abdominal cavity. Further, he carried out transplantation of young flukes in guinea-pigs and lambs and confirmed that the young flukes travel to the liver via the abdominal cavity.

Later, several other workers recorded intra-uterine infection in young calves (Curtze, 1932; Dizier, 1933 and Enigk and Duwel, 1959) and accordingly held the view that the flukes arrived in the liver via the blood stream. Vogel (1934), however, rejected their views and supported Sinitsin's theory.

The researches of Schumacher (1938), Krull and Jackson (1943) and Ono and Isoda (1952) showed that the young flukes gain entry into the abdominal cavity after piercing the intestine, penetrate the liver capsule from outside and begin to wander in the liver substance till they get into the bile-ducts, where they mature. Sogoyan (1955), however, contradicted their views and believed that the young flukes when boring into the wall of the intestine fell into the lymph and blood vessels and were thus carried to the liver. With the help of detailed histological studies Schumacher (1956) and Dawes (1961a, 1961b, 1963a) traced out the migratory route commencing with the ingestion of metacercarial cysts and confirmed Sinitsin's theory of migration by *F. hepatica*. 
Srivastava (1962) reported upon the early migration of *F. gigantica* into the liver thus: "the presence of a clear migratory tract in the upper part of the duodenum and similar tracts beneath the Glisson's capsule, together with the presence of juvenile flukes in the liver parenchyma, reveal that this parasite migrated directly from the duodenal wall through the peritoneal cavity and into the liver though the possibility of lymphatics and blood vessels serving as a secondary migratory pathway cannot be ruled out on the basis of the present study".

Dawes (1963a) described the excysted flukes in the intestine of mice moving over the mucosa and coming to lie in the folds between villi. Further, he observed that the flukes enter the villi through minute penetrations, move through the submucosa and aggregate beneath the muscle layers before they reach the serosa. After penetration of the intestinal wall, the young flukes browse over the visceral peritoneum and move towards the liver.

**Clonorchis**

According to the observations made by Mukoyama (1921), Faust and Khaw (1927) and Hsu and Wang (1938), the young flukes of *C. sinensis*, after emerging from the cysts in the intestine, migrate through the choledochus duct in the definitive host. Wykoff and Lepes (1957),
however, observed that the young flukes could reach the liver even though the bile-ducts had been ligatured before giving cysts, and after injecting the excysted individuals directly into the mesenteric vein. There is, thus, a possibility of migration through the portal circulation.

**Opisthorchis**

While dealing with the life-history of *O. felineus*, Vogel (1934) described that the young flukes reach the liver by way of the common bile-duct.

**Dicrocoelium**

The migratory route of the metacercariae of *D. dendriticum* was first studied by Neuhaus (1938) who reported that the young flukes migrate from the intestine to the bile-ducts via the portal blood system. Later, Krull (1958) showed that the metacercariae of *D. dendriticum* may reach the biliary system of the definitive host by migrating from the intestine through the opening of the common bile duct.

**Development of G. explanatum in other vertebrate and laboratory animals**

Very little information is available about the experimental infection and development of *G. explanatum* in buffalo. So far only kids and lambs have been used
for the recovery of adult parasites in life-history studies (Singh, 1958; Mukherjee, 1960).

(3) Pathology of amphistomiasis

The first study of the pathology of *G. explanatum* infection of buffalo liver based on material from abattoirs, was made by Kulasiri and Seneviratne (1956). According to them, the parasite was encountered in three different sites - the liver substance, portal veins, and the bile-ducts and the changes comprised widespread blocking of the bile-ducts by the parasites resulting in monolobular type of cirrhosis, extraordinary muscular hypertrophy and subendothelial proliferation of vessels, particularly the hepatic arteries. They did not encounter the parasites in the gall-bladder.

Patnaik (1964) described the histopathology of the duodenum and jejunum of buffalo-calves infested with *G. explanatum* (=Paramphistomum e.) and found that the young flukes attached to the mucosa with the help of the acetabulum and this resulted in strangulation and necrosis of these areas which consequently became thickened, oedematous and haemorrhagic. The early stages were also found in and below the muscularis mucosae and in the plica muscularis accompanied by lymphocytic infiltration.

*G. explanatum* infection of buffalo was also studied in the liver collected from Bareilly abattoir (Sharma
Deorani, 1965; Sen Gupta, 1966). Sharma Deorani (1965) reported linear hypertrophy of the bile-duct epithelium with much cellular infiltration. The ducts showed large number of raised papillae-like elevations to which parasites were attached. Periportal and perilobular cirrhosis were observed along with retrogressive changes in the hepatic cells. Sen Gupta (1966) observed haemorrhagic tracts in the parenchyma of the liver infected with G. explanatum and the chief lesions consisted of hypertrophy and hyperplasia of the bile-duct epithelium. The common bile-duct showed masses of polypoid hyperplastic epithelium filling the acetabulum of the parasite. Besides, monolobular cirrhosis was also observed. Singh and Kuppuswamy (1969) described the gross and histopathological changes encountered in the liver of goat associated with G. explanatum infection.

The pathological studies on amphistomiasis in ruminants have been carried out mostly on naturally infected cases and the changes were encountered chiefly in the intestine and stomach, being caused by immature stages of different species of amphistomes.

In the majority of cases reported in literature the gross lesions consisted of moderate to marked thickening of the infected parts of the intestine in the form of corrugations, especially of the duodenum. The mucosa of
the small intestine was congested and haemorrhagic and it was covered by a thick catarrhal exudate, giving an overall picture of acute catarrhal enteritis (Simson, 1926; LeRoux, 1930; Pande, 1935; Srivastava, 1938; Maqsood, 1944; Guilhon and Priouzeau, 1945; Mudaliar, 1945; Iyer, 1949; Nobel, 1956; Boray, 1959; Ahluwalia, 1960; Varma, 1961; Butler and Yeoman, 1962; Horak and Clark, 1963; Sharma Deorani and Katiyar, 1967, and Horak, 1967).

The rumen mucosa did not reveal any significant gross lesions except that the mature flukes were attached to the papillae of the ruminal pillar and caused atrophy, and the tips of the papillae got sloughed off due to pressure necrosis (Seyfarth, 1938; Mukherjee and Sharma Deorani, 1962; Cankovic and Batistic, 1963, and Horak, 1967).

Pande (1935) found that the lesions caused by young stages of *Paramphistomum* sp. were confined to the duodenum and ileum. The flukes were embedded in the mucosa and sub-mucosa producing varying degree of hyperaemia. Besides, there was destruction and proliferation of the mucous cells and catarrhal enteritis. Several other workers have also described the histopathological changes in the intestine (Srivastava, 1938; Mudaliar, 1945; Nobel, 1956; Boray, 1959; Ahluwalia, 1960; Varma, 1961; Butler and Yeoman, 1962, and Horak, 1967). The
changes usually encountered in the intestine followed the attachment of immature flukes on the surface of mucosa, and their being embedded deeper in submucosa and also in the muscularis mucosae, thus resulting in necrosis and strangulation of the mucosa and inflammatory reaction in the submucosa. Sometimes the flukes were also seen penetrating the intestine and causing hæmorrhage on the serosa.

Sharma Deorani and Katiyar (1967) in a detailed histopathological study of 24 specimens of intestine of sheep and goat heavily infested with immature amphistomes found three phases of the infection. In the mucosal phase, focal hæmorrhages, congestion, linear hypertrophy of the mucosa, increase in number of the goblet cells with marked mononuclear infiltration were seen. In the submucosal phase, there was congestion, oedema, heavy mononuclear infiltration of the mucosa and submucosa with the appearance of eosinophils in the latter, manifold hypertrophy of Brunner's glands making up most of the intestinal thickness and superficial desquamation of the mucosa. The post-migration phase was the stage of re-organisation of the submucosa and regeneration of the mucosa.

Seyfarth (1938), Mukherjee and Sharma Deorani (1962), Cankovic and Batistic (1963) and Horak (1967) described the histopathological changes of the infested rumen and this comprised oedema of the epithelial layer,
lymphocytic infiltration in the propria and submucous layer, necrosis and sloughing off of the mucosa near the attachment sites, hypertrophy of the stratum corneum and degeneration of the tips of the papillae.

Since a number of workers have described the histopathology of fascioliasis, our knowledge of the changes produced by the species of *Fasciola* is much more complete than for other flukes inhabiting the liver.

The first account of the pathological changes in the liver infected with *F. hepatica* was given by Svitil (1934). Microscopically, the lesions were described as inflammation of the bile-duct epithelium infiltrated with leucocytes and accompanied by hypertrophy of the connective tissue, regressive and dystrophic changes with frequent calcification of the bile-ducts and dilatation of the lumen. More or less similar changes in fascioliasis were reported by other workers (Finzi, 1941; Sinclair, 1949; Stemmermann, 1953; Isoda, 1954; Sugiura and Fujio, 1954; Gordon, 1955).

Morill and Shaw (1942) described focal subcapsular lesions in the liver and suggested that the flukes gained entry into the liver through the capsule. Besides degenerative and necrotic changes, Bonciu, Heitmanek, Rasmerita, Lescinsky and Margineanu (1954) reported cholangitis and pericholangitis along with hyperplastic changes in the
blood and lymph vessels and the bile-ducts.

Sogoyan (1955) described acute traumatic hepatitis caused by the migrating flukes which reached the liver by passing through the intestinal wall and the abdominal cavity. Sogoyan (1956) compared the pathology of *F. hepatica* and *F. gigantica* infections in sheep and concluded that *F. gigantica* caused profuse haemorrhages and severe damage to the liver parenchyma.

Lapage (1956) described haemorrhages in the liver in the early stages due to the migrating flukes and severe inflammation and fibrosis resulting from the irritation caused by the spines of the young flukes. Some of the bile-ducts were found calcified as a result of obstruction by the parasites. Urquhart (1956) studied in detail the pathology of experimental fascioliasis in rabbit and described the early lesions as necrotic tracts on the liver surface which were caused by the migration of the young flukes through the parenchyma. He observed that the space left in the wake of the migrating fluke was filled with cell debris, neutrophil leucocytes, lymphocytes and red cells. At later stages, a series of changes leading to the healing of these migratory tracts by heavily collagenized non-vascular granulation tissue, granulocytes and mononuclears were found. The bile-duct epithelium showed hyperplasia and in some cases it was completely desquamated. Gresham and Jennings (1962) described
haemorrhagic tracts through the liver surface and focal areas of fibrinous peritonitis produced by the migrating young flukes. In addition, chronic cholangitis and biliary obstruction were also noted. According to Dawes and Hughes (1964), peritonitis was caused by the older flukes which left the liver and re-entered the abdominal cavity.

More recently the pathology of experimental fascioliasis has been studied in great detail by a number of workers (Dawes, 1961a, b, c, 1963a, b, c; Srivastava, 1962; Dawes and Hughes, 1964; Thorpe, 1965; Ross, Todd and Dow, 1966; and Sen Gupta, 1966).

Human clonorchiasis has been mainly reported from China (Hoeppli, 1933; Helwig and Brown, 1946; Ling and Taur, 1949, and Wang, 1949) and the histopathological changes encountered in the liver consisted of dilatation, thickening, and sometimes obstruction of the bile-ducts. While studying the pathology of _C. sinensis_, Chin, Lei and Wang (1955), Hou (1955; 1956, 1965a, b) and Lingard, Huestis and McLean (1958) stated that _C. sinensis_ was the aetiological factor for the development of primary carcinoma in the liver.

Essex and Bollman (1930) described biliary cirrhosis due to infection with _Opisthorchis pseudofelineus_. Several other workers reported tumorous growth in the liver provoked
by *O. felineus* (Hoogland, 1932; Otto, 1937; Botti, 1954, and Salutini, 1954). The important pathological changes associated with the infections of *O. pseudofelineus* (Levine, Beamer and Maksie, 1956), *Paropisthorchis caninus* (Bhatia, Sood and Pande, 1959), *Amphimerus pseudofelineus* (Rothenbacher and Lindquist, 1963), *O. caninus* (Gupta and Pande, 1963) consisted of desquamation and proliferation of the bile-ducts and cirrhosis of varying degrees. The pathology of experimental opisthorchiasis was studied in detail by Koskukov (1963), Sahai (1967) and Ansari (1968) and the chief lesions were described to be degenerative changes in the parenchyma, cholangitis, biliary and portal cirrhosis, hyperplasia of the biliary epithelium, and hypertrophy of the von Kupffer's cells.

Earlier work on *dicrocoeliasis* (Lavier, LeRoux and Callot, 1938; Mapes, 1951) suggested that the parasites were responsible for carcinomatous growth in the liver. Sogoyan (1960), while studying the pathological changes in sheep noticed that the migrating young flukes caused desquamation of the bile-duct epithelium and also dilatation of the ducts.

The changes encountered in the early stage of *dicrocoeliasis* were characterized by catarrhal inflammation of the bile-ducts with parasites and masses of desquamated epithelial cells, mucus and inflammatory cells in the lumen
and in later stages there was biliary, pyetal and peri-
lobular cirrhosis (Dhar and Singh, 1964; Soulsby, 1965).

(4) Histochemical studies

There has been no work done on the histochemistry
of tissues affected by amphistomiasis. The literature on
the histochemical studies in other helminthic infections
is, however, reviewed here for the purpose of comparison.

Mercado and von Brand (1954) and von Brand and
Mercado (1956, 1958) studied the histochemistry of Plasmodium
berghei infection and showed that in the liver of the infec-
ted host there was reduction in glycogen because of dys-
function and an increase in the distribution of lipid
contents of the hepatic cells which was centrilocular.

Lewert and Lee (1954) studied the changes in the extracellular
glycoproteins of the dermis when traversed by skin-penetrating
infective helminth juveniles and found that the basement
membrane was altered with the production of a collagenase-
like enzyme. In Taenia taeniaeformis infection, Lewert
and Lee (1955) observed depletion of glycogen in the liver
and depolymerisation of the acellular glycoprotein. Silver-
man and Maneley (1955) observed cytolytic properties in
the secreting glands of taeniid hexacanth embryos which
helped them to penetrate the connective tissue core of
the villi in the intestine.
Sawada, Hara, Tagaki, Nagazawa and Oak (1956) carried out cytochemical studies of the liver infected with *Schistosoma japonicum* and observed an increase in glycogen and fat, decrease in the ribonucleic acid (RNA) of the hepatic cells and presence of increased acid mucopolysaccharides in the schistosomal granulomata. In addition, haemosiderin-like pigment was also seen in the Kupffer cells.

In *C. sinensis* infected livers, Kuwamura (1958) reported reduction in the content of nucleic acid, proteins and polysaccharides but there was increased activity of alkaline phosphatase.

Munnich (1958) while studying the histochemical changes in the liver of mouse produced by the juveniles of *Ascaris lumbricoides* observed decrease of glycogen, RNA and fat and an increase in glycoproteins. At some places slight increase of the activity of alkaline phosphatase was also seen. Later, Munnich (1960) studied the migration of the juveniles of *A. lumbricoides* in the intestine, liver and lungs of mouse and reported that the migration through the tissues was without the action of histolytic enzymes.

Andrade and Barka (1962) found increased activity of acid phosphatase in the reticulo-endothelial cells of the liver and spleen in *Schistosoma mansoni* infection. In liver-fluke cirrhosis, Kadziolka (1962) observed a marked
reduction in liver glycogen while Kublitskene (1962) found, in addition, an increase in DNA.

Dhar and Singh (1963) conducted detailed histochemical studies on the liver of cattle naturally infected with *D. dendriticum* and found almost complete depletion of glycogen, a higher lipid content and lipofuchsin deposition in the liver cells. There was increased mucin production in the bile-duct and according to Dhar and Singh, this was suggestive of a chemical irritant produced by the parasite. They also reported an unidentified pigment in the endothelium and lumen of the portal blood vessels.

In *Toxocara cati* infection, Roneus (1963) reported depletion of glycogen from the hepatic cells and the cytoplasm was positive to Gomori's silver stain. Chatterjee (1963) noticed an increase in mucopolysaccharides in the goblet cells as well as in the parasitic tracts of the small intestine in ascaridiasis. There was an increase in the activity of alkaline phosphatase in the damaged epithelium and also on the brush border of the villi. Srivastava and Singh (1964) studied the histochemical nature of the nodule in *Oesophagostomum* infection and reported that it contained glycoprotein but no glycogen while its fibrous capsule was mainly composed of collagen. Besides, the infected intestine showed increased mucin production and an unidentified pigment in the submucosa.
Chari (1967), while studying the mode of infection of *T. canis*, observed Periodic Acid–Schiff (PAS) negative reaction and also negative results for acid mucopolysaccharides at the sites of invasion and penetration of the juveniles in the host tissue.

Histochemical observations made by Kumar (1967) in *A. lumbricoides* infection revealed depletion of glycogen, a reduction of RNA level in the hepatic cells, and an increased activity of alkaline phosphatase in the liver. Bannerjee (1967) reported increased activity of alkaline phosphatase in the small intestine of rat, during penetration by oncospheres of *Taenia taeniaeformis*.

Recently, Ansari (1968) reported, in opisthorchiasis, marked depletion of glycogen in the hepatic cells, increased mucin production of the glandular epithelium of the bile-duct, enhanced activity of alkaline and acid phosphatase and reduction of RNA in the liver tissue. He also noticed deposition of bile and haemosiderin pigments in the hepatic and Kupffer cells.
MATERIAL AND METHODS

(1) Collection of adult parasites from buffalo

Adult specimens of G. explanatum were collected, when required, from the bile-ducts of the buffaloes destroyed at Bareilly abattoir and from the animals sacrificed at the Indian Veterinary Research Institute, Izatnagar. Some of the parasites were flattened and made into permanent stained preparations for the specific identification. The other specimens were washed well in water by shaking to remove debris adhering to the body and then rinsed in normal saline a number of times. The washed parasites were then dissected in a small quantity of distilled water to squeeze out the eggs from the terminal part of the uterus. The eggs thus collected were mixed with the tissues of the parasite. The larger pieces of tissues were picked up with a pair of forceps and the smaller particles were removed by repeated sedimentation. The eggs were then allowed to settle in thin layers in petri dishes of 10 cm diameter and covered by another dish. The petri dishes, prepared thus, invariably had the eggs in a single layer. The cultures were incubated at room temperature. After 24 hours, the water was gently siphoned off and replaced with tap water from a deep well which was free of chlorine. The water was subsequently
changed daily and during the summer months, twice every
day. The eggs stuck to the bottom of the petri dish
and the water of the dish could be changed without any
appreciable loss of eggs. There was no need to add any
bacteriostat or fungicide.

(2) Collection and examination of snails

A large number of specimens of the fresh-water
snail, Gyraulus convexiusculus (Hutton) were collected
throughout the year from various ponds in the neighbour-
hood of Bareilly and kept in large enamel trays and
earthenware containers in the laboratory. The snails
were placed singly in tubes containing water and provi-
ded with leaves of aquatic plants and boiled algae as
food. The snails were then exposed to bright light
every day and the morphology of the cercariae that were
shed was studied in detail in order to identify them.
Those snails that discharged cercariae that were iden-
tified as belonging to G. explanatum according to the
description of the cercaria given by Singh (1958) and
Peter and Srivastava (1960) for C. gyraulusi which
is considered to be a synonym (see under discussion),
and Mukherjee (1960), were placed together in an enamel
bowl. For the study of the genital rudiments the cer-
cariae were stained with Schneider's acetocarmine
(Singh, 1955). Only the metacercariae resulting from them were used for further infection and studies.

(3) Breeding of snails in semi-natural conditions (in laboratory)

The specimens of G. convexiusculus which did not shed any cercariae for many days were maintained in the laboratory in large enamel containers provided with aquatic plants and dead and dried leaves of the jackfruit tree. These fleshy leaves were a good source of food for the snails and the mortality among them when kept as above was negligible. When the snails deposited the eggs in masses, the same were removed from the enamel containers daily and kept in separate containers with fresh water. As the young snails hatched out they were removed daily and snails of a particular age were kept together in small enamel cups. At first, these were provided with boiled algae for food. A large number of snails which were known to be free of all trematode infections were thus available in the laboratory.

(4) Infection of the intermediate host

A large number of laboratory-bred snails of different ages (below 4 weeks, 4-6 weeks, 7-9 weeks, 10-12 weeks, 12-13 weeks) were maintained in groups according to their age. The snails were placed singly in tubes or
in batches of fifty in small beakers. A petri dish having fully developed eggs of the parasite was exposed to bright light and within 10 minutes a large number of free swimming miracidia were hatched. These were collected using a pipette and placed in small cavity blocks. The snails of different ages were then exposed, either individually by introducing 2-3 miracidia to each tube or a large number of miracidia were placed in a small beaker which contained the snails. In the latter case, the number of miracidia was approximately three times the number of the snails. All the snails exposed to infection were kept undisturbed in the tubes till the evening and then transferred to larger vessels and maintained in the laboratory as described before. About three weeks following infection, the individual snails were placed in tubes and exposed for about two hours to bright sunlight daily. When the snails commenced discharging cercariae, the latter were allowed to encyst on leaves as described below.

The cercariae were studied in detail and compared with the cercariae obtained from naturally infected snails. Throughout the year it was possible to have infected snails on hand.

(5) Collection and storage of cercariae

The snails which began to shed cercariae were removed to an enamel cup containing fresh water and lettuce
leaves and exposed daily to bright sunlight. The cercariae were shed generally in the morning and a few also in the afternoon and these readily encysted on the sides of the cup and also on the lettuce leaves. The metacercariae were removed afterwards with the help of a blunt needle and transferred into small tubes with water and plugged with cottonwool and stored in the refrigerator at 4°C till needed for setting up infection. The lettuce leaves containing the cysts were removed and also kept in the refrigerator in a separate container. Water of these containers was changed frequently to prevent fungal and bacterial growth.

(6) Experimental infection of vertebrate hosts

Buffalo-calves

Ten male buffalo-calves, age 6-8 months, procured from a local contractor were maintained in the sheds of the Parasitology Division. Their faeces were examined daily for 15-20 days before being accepted free of infection. A known number of metacercariae of *G. explanatum* still attached to the lettuce leaves were fed to two calves which had been starved overnight. Immediately thereafter the animals were given a drink of water. The other buffalo-calves were also infected orally, with a known number of cysts contained in gelatin capsules or
suspended in water which was directly pipetted into the
interior of the mouth and followed by a drenching with
water. The infected animals were maintained in the
stalls and provided with a balanced ration and were
never allowed to graze outside. Strict hygienic precau-
tions were taken to prevent them from picking up other
extraneous infections. After about 8-10 weeks of infec-
tion, the daily faecal examination of these animals was
commenced and continued till the faeces became positive
for the eggs of *G. explanatum*.

**Goat-kids**

A number of 1-2 months old goat-kids of either
sex were procured and kept in the sheds. Their faeces
were examined daily for three weeks to detect any helmin-
thic infections. When found negative to trematode infection,
they were infected orally by feeding them a counted number
of metacercariae in gelatin capsules. These experimental
animals were also maintained under such conditions that
precluded all possibilities of picking up any other helmin-
thic infections. They were provided with fresh tree leaves
and clean water and were not allowed to graze outside.

**Laboratory animals**

A number of young guinea-pigs, rabbits and rats
available from the Institute small animal house were used
for the infection experiments. For infecting them, several small balls of wheat flour were prepared and with each ball a counted number of metacercariae was mixed. The animals were starved overnight and the infected flour balls were left in the feeding cup of each animal individually. This method gave good results as the starved animals ate the food with the metacercariae within a short time and no food was left over. Their faeces were examined after 6 hours and onwards for the presence of unexcysted metacercariae.

(7) Excystment of metacercariae

In order to study the excystment of the metacercariae of *G. explanatum* in the intestine of mouse, "sausage" technique described by Silverman and Maneely (1955) was employed. The mouse was anaesthetized in a jar by ether and then secured to a wooden board. For maintaining anaesthesia, a funnel containing a cotton swab soaked in ether was used to cover the head of the mouse when needed. Laparotomy was then performed under aseptic precautions and the portion of the duodenum at its junction with the stomach was exposed and ligatured, both at the junction of duodenum and stomach and about two inches below it. A known number of metacercariae in normal saline solution was then taken and introduced into the lumen of the gastric end of the "intestinal sausage" using a blunt needle on a syringe. The "sausage"
was then left undisturbed and a cotton swab soaked in Ringer's solution was put over the exposed parts to keep it from drying out. The anaesthesia was maintained as required. After varying intervals of time, ranging from 30 to 300 minutes in different mice, the infected animal was destroyed and the 'sausage' removed. A portion of the sausage was fixed in buffered neutral formalin while the rest was thoroughly teased and blended in a homogeniser. The homogenate was then examined in small quantities under a binocular stereoscopic widefield microscope. The "sausage" technique was employed using different parts of the small intestine.

(8) Necropsy of vertebrate hosts and recovery of parasites

The experimentally infected animals were autopsied after varying intervals of time to recover the developmental stages of the parasite along with the affected tissue. The abdominal cavity was opened carefully and the liver along with its choledochus duct, which was tied off, was removed. The liver was separated from the gall-bladder and the choledochus duct and washed two to three times with normal saline solution. All the washings were collected for the examination of immature stages of the parasite. After clamping the posterior part of the oesophagus and the terminal part of the rectum the whole
of the gastrointestinal tract was removed to a large container. Immediately thereafter the abdominal cavity and the peritoneum were thoroughly irrigated with normal saline solution and the washings were collected for the examination of immature parasites.

In buffalo-calves and goat-kids each of the four stomachs were examined separately. The rest of the intestine was transferred to another container and the various organs were isolated and kept separately in normal saline solution for further examination. The small intestine was separated and divided into three equal parts and each part was examined separately along with its contents.

The liver was divided into two portions and one portion was fixed in 10% neutral buffered formalin for subsequent microtomy while the other portion was teased, blended and examined for parasites. The choledochus duct and gall-bladder were opened separately and washed with saline solution. The washings were then examined for the parasites. In a similar manner the various organs such as spleen, kidneys, pancreas and adrenal glands, were also collected and examined separately for the presence of parasites. The tissues showing gross lesions were isolated and fixed in different fixatives as demanded for the various histochemical tests.
(9) Examination of immature stages

During the necropsy of the experimentally infected animals, several developmental stages were recovered and a few of each of them were flattened dorso-ventrally under gentle pressure and fixed in 90 per cent alcohol. They were then stained with acetic alum carmine and mounted in Canada balsam. The morphology of these specimens was studied and all the measurements were made with an eyepiece micrometer and the average measurements of 6-8 specimens were taken. The diagrams of stained toto mounts of all the stages were drawn with the aid of a camera lucida.

(10) Histopathological and histochemical methods

For histopathological studies the tissues were generally fixed in 10 per cent neutral buffered formalin and the usual procedure of dehydration, clearing and embedding in the alcohol/xylene-paraffin sequence was followed. The serial sections were cut at 6-8 micron thickness and were stained with Lillie-Mayer's haematoxylin and alcoholic eosin. The sections were then dehydrated, cleared and mounted in Canada balsam or DPX.

For histochemical studies, the tissues were fixed in various fixatives (10 per cent neutral buffered formalin, acetone at 4°C, absolute alcohol, alcoholic Bouin's fixative). Besides the
usual paraffin sections, frozen sections were also obtained with the help of a freezing microtome.

The following histochemical staining tests were performed as described by Lillie (1954), Culling (1957) and Pearse (1960).

(A) Carbohydrates and carbohydrate-containing tissue elements

(i) Periodic acid-Schiff technique (PAS),
(ii) Best's Carmine stain for glycogen,
(iii) Hale's dialysed iron for acid mucopolysaccharides,
(iv) Alcian blue method for acid mucopolysaccharides,
(v) Mucicarmine stain for mucopolysaccharide, and
(vi) Thionin method for metachromasia.

(B) Proteins

(i) Millon reaction (Baker modification),
(ii) Mercury-bromophenol blue method,
(iii) Ninhydrin-Schiff method,
(iv) DMAB-nitrite method, and
(v) Coupled Tetrazonium reaction.

(C) Lipids

(i) Sudan Black B method, and
(ii) Acetone-Sudan Black method.
(D) **Nucleic acids (DNA and RNA)**  
(i) Feulgen reaction,  
(ii) Galloxyanin-chromalum method, and  
(iii) Methyl Green-pyronin Y method.

(E) **Enzymes**  
(i) The calcium-cobalt method for alkaline phosphatase (after Gomori), and  
(ii) Modified lead nitrate method for acid phosphatase (after Tokeuchi and Tanoue).

(F) **Connective tissue**  
(i) Mallory's phosphotungstic acid haematoxylin method (MPTAH) for fibrin,  
(ii) Eosin-Gram Weigert method,  
(iii) Van Gieson method, and  
(iv) Masson's trichrome stain.

(G) **Amyloid**  
(i) Dahlia method (Lendrum, 1951), and  
(ii) Modified Congo red method.

(H) **Calcium**  
(i) Von Kossa method.

(I) **Pigments**  
(i) Gmelin reaction for bilirubin and haematoidin,  
(ii) The Iodine method for bile pigments (Stein, 1935), and  
(iii) Ferric Iron method for bilirubin (Kutlik, 1957).
OBSERVATIONS

(1) Incidence of *G. explanatum* in vertebrate hosts

During a period of 19 months (1966-68), the livers of 1,758 buffalo, 337 sheep and 77 goats were examined for natural infection with *G. explanatum* at Bareilly abattoirs and also at Indian Veterinary Research Institute. The table I shows the number of animals examined and found infected with this parasite throughout this period. During the survey, *G. explanatum* was never found in the blood vessels or parenchyma of the liver while concomitant infections with *Fasciola gigantica* and hydatid cysts were encountered frequently in a number of livers examined. In buffalo, the parasites were commonly found in the ductus choledochus, ductus cysticus and ductus hepatocysticus, though on rare occasions parasites were also seen attached to the mucosa of the rumen and in the gall bladder.

The table shows that of the three species of animals examined, buffalo is the only important host for the incidence was never lower than 73.78 per cent and it climbed up to 92.06 per cent. Also, there appears to be no seasonal variation in the rate of incidence since the top three figures refer to the months of December, January and March, and the three lowest figures are for the months of October.
(twice) and November. Also the difference between the maximum and the minimum figures is only a little over 18 per cent which is not considered to be significant. Though comparatively a small number of sheep and goat was examined, but the data indicate that the incidence of infection in these two animals is very low and the over all incidence can be called almost negligible. Even though the number of parasites present in each of the infected animal was not recorded, yet it was observed that the number of parasites found in these two hosts was always much lower than in buffalo.
Table I

Showing data of natural infection of *G. explanatum* in vertebrate hosts during 1966-68

<table>
<thead>
<tr>
<th>Serial No. of examination</th>
<th>Month</th>
<th>Buffalo</th>
<th></th>
<th>Sheep</th>
<th></th>
<th>Goat</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Number of livers examined</td>
<td>Percentage of livers containing bile ducts</td>
<td>Number of livers examined</td>
<td>Percentage of livers containing common bile ducts</td>
<td>Number of livers examined</td>
<td>Percentage of livers containing bile ducts</td>
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<td>1. October, 1966</td>
<td>49</td>
<td>49</td>
<td>81.63</td>
<td>28</td>
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<td>-</td>
</tr>
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<td>106</td>
<td>106</td>
<td>86.79</td>
<td>43</td>
<td>0</td>
<td>7</td>
<td>0</td>
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<td>3. December</td>
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<td>90.52</td>
<td>12</td>
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<td>-</td>
<td>-</td>
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<td>105</td>
<td>90.47</td>
<td>34</td>
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<td>83.58</td>
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<td>-</td>
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<td>63</td>
<td>92.06</td>
<td>-</td>
<td>-</td>
<td>21</td>
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<td>93.90</td>
<td>48</td>
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<td>88.29</td>
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<td>82.17</td>
<td>-</td>
<td>-</td>
<td>16</td>
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<td>12. September</td>
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<td>88.78</td>
<td>16</td>
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<td>-</td>
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<td>13. October</td>
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<td>105</td>
<td>79.04</td>
<td>21</td>
<td>9.52</td>
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<td>73.78</td>
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<td>15. December</td>
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<td>82.89</td>
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<td>-</td>
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<tr>
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<td>81.74</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>19. April</td>
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<td>93</td>
<td>83.11</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1758</strong></td>
<td><strong>84.21</strong></td>
<td><strong>337</strong></td>
<td><strong>1.24</strong></td>
<td><strong>77</strong></td>
<td><strong>0.79</strong></td>
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</tbody>
</table>
(2) Incidence of natural infection of G. explanatum in snail

During the period 1966-68, a large number of G. con-
 vexiusculus were collected from various places in the vicinity
of the campus of the Indian Veterinary Research Institute,
villages Nekpur, Bhojeeupura, and Rithora, river Nakatia, and
ditches on Bareilly-Pilibhit, Bareilly-Rampur, Bareilly-
Budaun and Bareilly-Nainital roads. The relevant data are
summarised in table II. The identification of the snails
was confirmed by the Director, Zoological Survey of India,
Calcutta.

In the course of screening the snails for cercarial
infection, in addition to the cercariae of the Pigmentata
group, other types of cercariae were also found. These
belonged to pleurolophocercus, diplocotylea, echinostome,
xiphidiocercariae and furcocercous groups of cercariae. The
percentage of snails naturally infected with the cercariae
of the Pigmentata group and morphologically conforming to
the descriptions given by Peter and Srivastava (1960) for
Cercaria gyraulusi and by Singh (1958) for the cercaria of
G. explanatum showed seasonal variations (Table II).

During the two winter seasons (November to February)
that the survey was carried out, 2009 snails were examined
from the various localities during the first season and
1062 snails during the second season. During the first
season the incidence varied from 0.24 to 1.3% on the four
occasions that the infection was encountered in 12 collections made. The overall average for this period was about 0.25%. Correspondingly, during the second season the incidence of infection was higher as during the four months the infection was encountered on four different occasions out of 14 collections made. The incidence varied from 1 to 4% on these four occasions with an overall average of about 0.56%.

During the two summer seasons (March–June), 855 snails were examined in the first season and 323 snails were examined during the months of March and April only of the second season. In all, the snails were collected on 15 different occasions from the various localities but in all cases, the snails were consistently found negative for *G. explanatum* infection though cercariae of other trematodes were found a few times. During the summer season, however, collection of the snails was made easy because the ponds, ditches and the river had comparatively little water.

The month of July sees the onset of heavy monsoon rains which taper off by October, and there is no sharp autumn in the plains of India. During this period the population of snails greatly increased. In the month of October 1966 when the survey was started, 725 snails collected on two occasions were examined and the percentage
of infection was 1.85% and 0.53%, with an overall average of about 1.5%. During the next rainy season a total of 2107 snails was examined. They were collected on 14 different occasions and the cercariae were found in 8 of these, the incidence on these occasions ranging from 1.19% to 5.88%. The overall average during this period was about 1.6%.

During the survey when collections were made over a continuous period of 19 months, 7081 snails were examined of which 58 were found infected with the cercariae of *G. explanatum*, thus giving an average of 0.81%.
### Table II

Showing data of examination of *G. convexiusculus* collected from the field for cercariae of *G. explanatum*

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Month of collection</th>
<th>Locality</th>
<th>Number examined</th>
<th>Number positive</th>
<th>Percentage of infection</th>
</tr>
</thead>
<tbody>
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<td>1.</td>
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<td>Number positive</td>
<td>Percentage of infection</td>
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<td><strong>58</strong></td>
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</table>
(3) Life-history study

The eggs of *G. explanatum* when incubated in the laboratory at a temperature range of 15.6°C – 22.8°C during October, 1966 to February, 1967 hatched out into miracidia in 18-22 days. During March, 1967 to June, 1967, the hatching took place in 7-10 days when the room temperature varied from 21.1°C to 37.7°C. But it took 9-12 days during July, 1967 to September, 1967, when the room temperature varied from 26.7°C to 36.6°C. The sunlight or strong artificial light had a triggering off effect on the hatching of the miracidia. The cultures of eggs were, as a routine, maintained in subdued light and when exposed to strong light produced a large number of miracidia in 15 to 30 minutes provided the miracidia were ready to hatch.

**Intermediate snail host**

Since the earlier work on the parasite had shown that *Gyraulus convexusculus* (Hutton) was the only known intermediate host for *G. explanatum*, the present study was confined to this particular species of snail. Of all the snails found in the fresh waters of Northern India, this snail is one of the smallest as most of the adult specimens measure 6 to 8 mm in diameter. It is a small spirally round aquatic snail. The shell is small and strongly depressed and discoidal. The whorls number
four to five and are well rounded. The aperture is oblique and ovately lunate with a thin outer lip. The shell is pale horny brown in colour. The animal is blackish or dusky brown in colour. From the field, specimens of *G. convexiusculus* of all age groups were collected throughout the year. It appears that the snails breed through most of the year in and around Bareilly where the winters can be severe. However, fluctuations in the numbers of snails were observed over the year but could not be correlated with any ecological factor.

During the morning and evening hours throughout the year the snails were mostly found attached to water plants or the decaying leaves in shallow waters, usually near the banks of the ponds and the ditches. In winter season, however, when there was bright sunshine, the snails were also seen floating free on the surface of the water. During winter months there was marked decrease in snail population and the maximum number of snails was found in the rainy season when the ecological conditions were optimum for the breeding and growth of the snails.

Some studies on the biology of *G. convexiusculus*, like the egg laying capacity, nature of egg masses, development of eggs and the process of hatching and growth of snails were carried out under laboratory conditions.
The egg laying capacity was determined for five snails individually which were maintained in the laboratory under similar conditions. The egg masses deposited by each snail were removed daily by means of a scalpel and measured in order to determine the possible differences in their size and number of eggs in relation to sizes of the snails themselves. The results are summarised in tables III and IV. During this period the temperature inside the room varied from 32.2°C to 37.7°C, while the water temperature ranged from 29°C to 34°C.

The snails laid the egg masses on the sides of the containers and on the leaves. The egg masses were pale yellowish in colour, and were oval or pear-shaped, sometimes elongated, one side being flat and the other convex. The number of eggs varied from 1-18 per egg mass. The whole egg mass was enclosed by a delicate transparent case. The eggs when laid were sub-spherical and single-celled and measured, on an average, 0.9 mm in diameter. Following deposition, it took 4-5 hours for the newly laid eggs to reach the 16-cell stage. The embryo measured 0.096 mm in diameter at 24 hours. The movement of the developing embryos was first observed on the second day when it measured 0.14 mm in diameter. On the 3rd day the embryo measured 0.25 mm in diameter and began to turn more than in the previous stage and the head-foot region
and pseudopodia could be made out. By the 4th day the embryo attained a size of 0.32 mm in diameter and occupied nearly half the space of the egg cell. At this stage, the turning of the embryo became very frequent and the pulsating heart, snail shell, two stubby tentacles and two clear eye-spots were also observed for the first time. However, by the 5th day the embryo occupied about 3/4th the space of the egg cell and in addition, the shell was better developed, when the embryo measured 0.38 mm in diameter. The young snails inside the egg cell were quite active and the hatching began as early as the 6th day when the young snails hatched out and became discernible to the naked eye. All the snails in one egg mass did not hatch out at one and the same time. The snail could be seen actually breaking out of the membrane when it measured 0.42 x 0.38 mm in size with a well developed pulsating heart, head-foot region, eye-spots and one whorl of shell.

Under the laboratory conditions the newly hatched out snails began to lay eggs on 26th day, when their shell diameter ranged from 4.5 to 4.8 mm.
**Table III**

Size of egg masses of *G. convexiusculus* of various sizes

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<th>Eggs per mass</th>
<th>8 mm Length</th>
<th>6.5-7 mm Width</th>
<th>5.5-6 mm Width</th>
<th>Length x Width of egg mass (mm)</th>
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<td>23-6-69</td>
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</table>

**Total**  | 45  | 310  | 50  | 372  | 109  | 832  | 93  | 868  | 55  | 459  |
Experimental infection of G. convexiusculus with 
with G. explanatum

For setting up of the infection of G. explanatum in the laboratory, specimens of G. convexiusculus which had been raised from egg masses in the laboratory as well as those that were collected in the field but were found negative for trematode infection were used. For obvious reasons, where it was pertinent to know the age of the snails being used, only laboratory raised snails were used.

During experimental infection it was observed that a large number of miracidia gathered around the snail and started the process of getting attached to it. Apparently this irritated the snail for it reacted by either retracting its body inside the shell or it moved away from that place. Occasionally, the snail suddenly contracted its body which brought about the expulsion of the miracidia from its mantle cavity.

Five different groups of snails bred in the laboratory, grouped age-wise, were used for infecting them with the miracidia of G. explanatum and the results are summarised in table V. Those snails that died following infection, were regarded as negative for the purpose of this experiment. A few such specimens that were dissected were found to be negative but it is quite possible that some of them may have been infected but the infection was too early to be apparent, specially if the snail tissue had become dis-organised to any extent.
<table>
<thead>
<tr>
<th>No. of snails</th>
<th>Period (1966-67)</th>
<th>Range of temp. inside room (°C)</th>
<th>Individual infection</th>
<th>Mass infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Number of miracidia exposed</td>
<td>Number of snails surviving positive of the infection</td>
</tr>
<tr>
<td>1. Below 4 weeks</td>
<td>Dec., 66–Jan., 67</td>
<td>15.6–24.4</td>
<td>30</td>
<td>2-3</td>
</tr>
<tr>
<td>2. –do–</td>
<td>Apr., 67–May, 67</td>
<td>30.0–37.7</td>
<td>30</td>
<td>2-3</td>
</tr>
<tr>
<td>3. –do–</td>
<td>Jul., 67–Aug., 67</td>
<td>27.8–39.9</td>
<td>30</td>
<td>2-3</td>
</tr>
<tr>
<td>4. 4–6 weeks old</td>
<td>Dec., 66–Jan., 67</td>
<td>15.6–24.4</td>
<td>30</td>
<td>2-3</td>
</tr>
<tr>
<td>5. –do–</td>
<td>Apr., 67–May, 67</td>
<td>30.0–37.7</td>
<td>30</td>
<td>2-3</td>
</tr>
<tr>
<td>7. 7–9 weeks old</td>
<td>Jan., 67–Feb., 67</td>
<td>15.6–25.6</td>
<td>30</td>
<td>2-3</td>
</tr>
<tr>
<td>8. –do–</td>
<td>May, 67–Jun., 67</td>
<td>29.4–37.7</td>
<td>30</td>
<td>2-3</td>
</tr>
<tr>
<td>10. 10–12 weeks old</td>
<td>Jan., 67–Feb., 67</td>
<td>15.6–25.6</td>
<td>30</td>
<td>2-3</td>
</tr>
<tr>
<td>11. –do–</td>
<td>May, 67–Jun., 67</td>
<td>29.4–37.7</td>
<td>30</td>
<td>2-3</td>
</tr>
<tr>
<td>15. –do–</td>
<td>Sept., 67–Oct., 67</td>
<td>19.4–32.2</td>
<td>30</td>
<td>2-3</td>
</tr>
</tbody>
</table>
(i) **Below 4 weeks old**

From December, 1966 to January, 1967, a total number of 30 snails were individually exposed to 2-3 miracidia each at room temperature ranging between 15.6° and 24.4°C. After the third week of infection the snails were examined regularly for the presence of cercariae. By the end of January and within 40 days postinfection, 73.3 per cent of the snails had died. Of the eight snails which survived the prepatent period only three snails began to shed cercariae and accordingly only 10.0 per cent of the total number of snails became positive for the cercariae. During the same period, when 62 snails were exposed to 200 miracidia in a mass infection, only 3.22 per cent of the snails became positive for the infection and 70.9 per cent of the snails exposed to infection died during 40 days postinfection.

The snails of the same age were exposed to the miracidia during April, 1967 and May, 1967 at room temperature, the range being 30.0° to 37.7°C. Out of 30 snails, that were individually exposed to 2-3 miracidia per snail, 93.3 per cent died within 38 days of being exposed to infection. The two snails that survived this period, began to shed cercariae. When a mass infection of 32 snails was attempted with 107 miracidia, none of the snails developed the infection to maturity and there was heavy mortality amongst these snails and by the 38th day postinfection, 87.5 per cent of the snails exposed to infection had died.
When 30 snails were individually exposed to 2-3 miracidia each during July, 1967 and August, 1967 at room temperature (27.8°C to 39.9°C), 60 per cent of them died during the next 37 days and only 10 per cent of the snails exposed to infection became positive and were shedding the cercariae. When 47 snails were exposed to 75 miracidia in a mass infection, the mortality within the next 37 days was 80.8 per cent and only 4.25 per cent of the snails became positive for the infection.

(ii) 4-6 weeks old

During the period December, 1966 to January, 1967 a total of 30 and 110 snails were exposed individually and in mass infections respectively. The number of miracidia varied from 2-3 for each snail in the individually infected experiment, whereas 110 snails were exposed to 250 miracidia in the mass infection. During the next 40 days, 80 per cent of the snails exposed individually and 83.6 per cent of those that were given mass infection died. Only 3.33 per cent of the individually infected snails and 1.81 per cent of snails exposed to mass infection became positive and were shedding the cercariae.

The experiment was repeated using two groups of 30 and 39 snails of the same age during April and May, 1967. Out of 30 snails which were exposed to 2-3 miracidia per snail, 86.6 per cent died during the prepatent period
and all the four snails that survived this period, did not shed any cercariae. When 39 snails were collectively exposed to 98 miracidia, only 2.56 per cent of the snails became positive for the cercariae and as many as 93 per cent of the snails exposed to infection died during the course of the prepatent period.

Similarly, when 30 snails were individually exposed to 2-3 miracidia each during the months of July, 1967 and August, 1967, 66.6 per cent of them died during the prepatent period and only four snails (13.3 per cent) began to shed the cercariae. In the mass infection, out of 58 snails which were exposed to 160 miracidia, only 5.17 per cent of the snails became positive and were shedding the cercariae, while 92.8 per cent of the snails died during the prepatent period.

(iii) 7-9 weeks old

During the months of January, 1967 and February, 1967 when the room temperature ranged between 15.6° and 25.6°C, 30 snails were individually exposed to 2-3 miracidia each. Of these, 93.3 per cent snails died during the course of the prepatent period and neither of the two surviving snails showed cercarial infection. When 71 snails were collectively exposed to 150 miracidia, 81.6 per cent of the snails exposed to infection died and out
of the 13 snails surviving the prepatent period, only
2.81 per cent of all the snails exposed became positive
for the cercariae.

When 30 snails were individually exposed to 2-3
miracidia each during the months of May, 1967 and June,
1967 at room temperature (29.4°-37.7°C), 90 per cent
snails died and only three snails survived the period of
patency of infection but none of them showed the cercariae.
When a mass infection of 26 snails was carried out with
84 miracidia, 92.3 per cent of the snails died during the
course of prepatent period and the two snails which sur-
vived did not shed the cercariae.

Another group of snails of the same age was exposed
to the miracidia during August, 1967 and September, 1967
at room temperature ranging between 26.7° and 34.4°C.
When 30 snails were individually exposed to 2-3 miracidia
each, 13 snails survived the prepatent period, while 56.6
per cent mortality was recorded during this period. Of
all the snails exposed to infection, 6.6 per cent became
positive for the cercariae. When a mass infection of 61
snails was carried out with 172 miracidia, 68.8 per cent
of the snails died during the prepatent period and only
6.55 per cent of the exposed snails became positive for
the infection.
(iv) 10-12 weeks old

During January, 1967 and February, 1967, 30 snails of this age group were individually exposed to 2-3 miracidia each. By the end of February and during the 40 days postinfection, 90 per cent of the snails died. Out of the three snails which survived this period, only one snail (3.3 per cent) began to shed the cercariae. During the same period 57 snails were exposed to 130 miracidia in a mass infection and 87.7 per cent mortality of the snails was observed and only seven snails (3.5 per cent) survived the prepatent period.

During May, 1967 and June, 1967, 30 snails were exposed individually to 2-3 miracidia per snail. Of these, 86.6 per cent of the snails died and none of the four surviving snails became positive. When 13 snails were exposed to 64 miracidia in a mass infection, all of them died during the patency of the infection.

Once again during August, 1967 and September, 1967, 30 snails were individually exposed to 2-3 miracidia per snail. Of these 46.6 per cent of the snails died and 16 snails survived the course of prepatent period but only one of them (3.3 per cent) began to shed the cercariae. Out of 39 snails which were exposed to 120 miracidia in a mass infection, 76.9 per cent of the snails died during the prepatent period and only 7.69 per cent of the exposed snails became positive for the cercariae.
(v) **12-13 weeks old**

During February, 1967 and March, 1967 a total number of 30 snails were individually exposed to 2-3 miracidia each at room temperature ranging between 16.7° and 26.7°C. By the end of March and during the course of 40 days post-infection, mortality was 40 per cent. Of the 18 snails which survived the above period 3 snails (10 per cent) became positive for the cercariae. When 40 snails were exposed to 112 miracidia in a mass infection during the same period, 52.5 per cent of the snails died while 17.5 per cent became positive for the cercariae.

Another group of snails of the same age was exposed to miracidia during June, 1967 and July, 1967 at room temperature, the range being 28.9° to 39.9°C. Out of 35 snails which were individually exposed to 2-3 miracidia each, 82.8 per cent of the snails died during the next 38 days. Of the six snails that survived this period, five (14.28 per cent) began to shed the cercariae. When a mass infection of 22 snails was carried out with 70 miracidia, 50 per cent of the snails died during the prepatent period and 13.63 per cent of the snails became positive for the cercariae.

During September, 1967 and October, 1967 when the room temperature was from 19.4° to 32.3°C, 30 snails were individually exposed to 2-3 miracidia each. Of these,
76.6 per cent died and out of the 7 snails which survived for 37 days, 6 snails became positive for the cercariae. When 38 snails were exposed to 140 miracidia in a mass infection during the same period, 47.3 per cent of them died and 18.42 per cent became positive for the cercariae during the prepotent period.

**Infection of mature G. convexiusculus with miracidia**

As many as 2002 specimens of G. convexiusculus were exposed to miracidia of G. explanatum in ten batches from March, 1967 to December, 1967. Out of the total number of snails exposed, 1440 were field collected and 562 were laboratory bred. The results are summarized in table VI. The number of snails exposed per month varied from 70 to 480 while the total number of miracidia given per batch of snails in the collective infections varied correspondingly from 200-1230. The mortality, on an average, in the snails during the prepotent period was 70.62 per cent. Of the 588 snails that survived the prepotent period, 54 snails became positive and started shedding the cercariae. The percentage of infection varied from 1.21 to 4.58 and the average percentage calculated on the basis of the total number of snails exposed was 2.78.
In laboratory-bred snails the mortality rate during the prepatent period varied from 69.04 to 80.48 per cent, the average being 74.09 per cent. In field collected snails the mortality rate varied from 54.28 to 79.55 per cent, the average being 66.78 per cent. The percentage of infection varied from 1.21 to 3.1 (average 1.91 per cent) in the laboratory-bred snails while the range in field collected specimens was from 1.66 to 4.58 per cent, the average being 3.35 per cent. Not much difference was found in the mortality and infection rates of the laboratory-bred and field collected specimens.
<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Period</th>
<th>Source of snails</th>
<th>Total No. of snails exposed</th>
<th>Method of exposure</th>
<th>Total No. of miracidia given</th>
<th>No. of snails surviving the pre-patent period</th>
<th>No. of positive snails</th>
<th>Percentage of infection</th>
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<td>1</td>
<td>March, 67</td>
<td>F.C.</td>
<td>190</td>
<td>Mass infection</td>
<td>500</td>
<td>67</td>
<td>6</td>
<td>3.15</td>
</tr>
<tr>
<td>2</td>
<td>April, 67</td>
<td>F.C.</td>
<td>70</td>
<td>-do-</td>
<td>200</td>
<td>32</td>
<td>3</td>
<td>4.28</td>
</tr>
<tr>
<td>3</td>
<td>May, 67</td>
<td>F.C.</td>
<td>235</td>
<td>-do-</td>
<td>800</td>
<td>103</td>
<td>9</td>
<td>3.82</td>
</tr>
<tr>
<td>4</td>
<td>June, 67</td>
<td>L.B.</td>
<td>126</td>
<td>-do-</td>
<td>500</td>
<td>39</td>
<td>2</td>
<td>1.58</td>
</tr>
<tr>
<td>5</td>
<td>July, 67</td>
<td>L.B.</td>
<td>129</td>
<td>-do-</td>
<td>430</td>
<td>29</td>
<td>4</td>
<td>3.10</td>
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<tr>
<td>6</td>
<td>August, 67</td>
<td>F.C.</td>
<td>480</td>
<td>-do-</td>
<td>1120</td>
<td>115</td>
<td>8</td>
<td>1.66</td>
</tr>
<tr>
<td>7</td>
<td>Sept., 67</td>
<td>F.C.</td>
<td>240</td>
<td>-do-</td>
<td>1230</td>
<td>72</td>
<td>11</td>
<td>4.58</td>
</tr>
<tr>
<td>8</td>
<td>Oct., 67</td>
<td>F.C.</td>
<td>225</td>
<td>-do-</td>
<td>1125</td>
<td>46</td>
<td>6</td>
<td>2.66</td>
</tr>
<tr>
<td>9</td>
<td>Nov., 67</td>
<td>L.B.</td>
<td>225</td>
<td>-do-</td>
<td>1200</td>
<td>69</td>
<td>4</td>
<td>1.77</td>
</tr>
<tr>
<td>10</td>
<td>Dec., 67</td>
<td>L.B.</td>
<td>82</td>
<td>-do-</td>
<td>482</td>
<td>16</td>
<td>1</td>
<td>1.21</td>
</tr>
</tbody>
</table>

F.C. = Field collected.
L.B. = Laboratory-bred.
Number of cercariae produced daily by infected *G. convexiusculus*

One series of experiments was carried out to determine the number of cercariae produced by *G. convexiusculus* experimentally infected in the laboratory with one or more miracidia each. The observations on uni-miracidial and multi-miracidial infections were confined to only five infected snails of each group which were maintained separately and the number of cercariae shed daily was counted as metacercarial cysts encysted on the leaves and on the sides of the containers. These were counted and removed at the end of the day.

In the uni-miracidial infection, out of five infected snails one shed the cercariae for five days and died on the seventh day and the other shed the cercariae for six days and died on the eighth day. The remaining three snails lived for a period of 11, 19 and 24 days after the initial production of cercariae during which period the cercariae were shed on 10, 18 and 23 days respectively, the missing day being the day on which the snails died. One snail shed only one cercariae, the other four, and the third snail shed six cercariae on the first day. The snail that shed four cercariae on the first day, five were shed on the second day, five on the third day, nine on the fourth day, eight on the fifth day, only two on
the sixth day. However, the maximum number was eleven which was reached on the eighth day. The number of cercariae shed during the following 16 days varied from 1-8. The total number of cercariae shed during the 23 days that the infection was patent by this snail was 107. In the other two snails the similar pattern in the shedding of cercariae was observed. The total number of cercariae shed by one snail was 49 over a period of ten days and by the other 84 over a period of 18 days. In uni-miracidial infection the minimum, maximum and the average number of cercariae produced by an individual snail comes to 26, 107 and 60 respectively.

The five snails which were exposed to 2-3 miracidia per snail, the cercariae were shed for a period of 5 to 16 days. The number of cercariae shed daily by each of these snails varied from 1-10 and the number of cercariae produced per day by these snails was almost similar to the number produced by the snails infected with a single miracidium. In multi-miracidial infection the minimum, maximum and the average number of cercariae produced by a snail was 16, 57 and 41 respectively.

Experimental infection of final host (buffalo-calf)

Two clean buffalo-calves (BC-284 and BC-9) were given orally 3,000 and 2,000 metacercariae of G. explanatum respectively. One buffalo-calf (BC-284), however, died
four days after infection due to pneumonia. The other animal (BC-9) started passing the eggs of the parasite in the faeces on the 89th day postinfection and continued to do so till it was sacrificed on 144th day postinfection. The parasites numbering 237 (i.e., 11.85 per cent of the total given) were all mature and recovered from the ductus choledochus and ductus hepatocysticus.

**Route of migration of *G. explanatum* in goat-kids**

Each of the 11 clean goat-kids were given per os 600-2,400 metacercariae of *G. explanatum* (Table VII). Two of these kids (K-33 and K-34) died in 3 and 4 days after infection respectively, due to pneumonia. The remaining nine kids were sacrificed at 7, 12, 12, 14, 14, 15, 19, 21 and 28 days postinfection respectively. The control kid (No.35) was maintained exactly in the same way as the other animals of the experiment. The results of necropsy of these animals have been summarized in table VII.

(i) **7th day postinfection**

One kid (K-31) had been fed with 1,000 metacercarial cysts, and the kid was sacrificed after seven days. On autopsy no immature parasites were found in any of the compartments of the stomach. Immature parasites were, however, present in the small and large intestine. In the anterior third portion of the small intestine only three immature
parasites were present while 43 and 31 specimens were counted from the middle and the posterior portions respectively. All the three parasites in the anterior portion of the small intestine were recovered from an area anterior to the opening of the ductus choledochus communis. The first 60 cm of the large intestine lodged six parasites. The other organs, such as ductus choledochus communis, pancreatic duct, ductus hepatocysticus, ductus cysticus, gall-bladder, bile-ducts of the liver, the spleen and the kidneys did not have any parasites. The total number of immature parasites recovered was 83 i.e., 8.3 per cent of the total number of cysts administered.

The immature parasites were found over the villi of the mucosal layer of the duodenum (Fig. 1 & 2). A large number of microtome sections of the intestine were examined and the parasites were never found inside the villi, submucosa, muscular and serous layers or in the blood vessels of the duodenum.

(ii) 12th day postinfection

The two goat kids (K-41 and K-43), which received 1,500 cysts each, were sacrificed and autopsied 12 days after infection. In both the animals, the parasites were not encountered in the rumen, reticulum, omasum or abomasum. In kid K-41, there were 36 immature parasites in the anterior third, 19 in the middle and 7 in the posterior third
of the small intestine, whereas in kid K-43, 21, 30 and 18 parasites were recovered from the three parts respectively. In both the animals the large intestine did not show any parasites. However, 12 parasites were found for the first time in the lumen of the ductus choledochus communis of one kid (K-41) and only one parasite was present in the pancreatic duct. In kid K-43, the ductus choledochus communis had seven parasites in its lumen whereas two were recovered from the pancreatic duct. In kid K-41, six parasites from ductus hepatocysticus, seven from gall-bladder and two from the smaller bile-ducts of the liver were recovered while in the case of kid K-43, five parasites had reached the ductus hepatocysticus. Only one parasite was found in the ductus cysticus, and the gall-bladder contained six of them. One parasite was also present in the bile-duct of the liver. In both the animals the spleen and kidney did not show any parasites. The total recovery of parasites comes to 6.0 per cent in K-41 and 6.06 per cent in K-43 of the total number of metacercarial cysts administered.
## Table VII

Showing data on the recovery of immature parasites of *G. explanatum* from goat-kids

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Animal number</th>
<th>No. of cysts given</th>
<th>Duration of infection (in days)</th>
<th>Number of worms recovered from different organs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rumen</td>
<td>Reticulum</td>
</tr>
<tr>
<td>1.</td>
<td>K-33*</td>
<td>600</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>K-34*</td>
<td>650</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>K-31</td>
<td>1000</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>4.</td>
<td>K-41</td>
<td>1500</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>5.</td>
<td>K-43</td>
<td>1500</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>6.</td>
<td>K-5</td>
<td>670</td>
<td>14</td>
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</tr>
<tr>
<td>7.</td>
<td>K-32</td>
<td>1000</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>8.</td>
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<td>15</td>
<td>0</td>
</tr>
<tr>
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<td>19</td>
<td>0</td>
</tr>
<tr>
<td>10.</td>
<td>K-51</td>
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<td>21</td>
<td>0</td>
</tr>
<tr>
<td>11.</td>
<td>K-52</td>
<td>800</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>12.</td>
<td>K-35</td>
<td>Control</td>
<td></td>
<td></td>
</tr>
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</table>

* Died due to pneumonia.
- Indicates that these organs were not examined because of the putrefaction already set in.
# Indicates that these organs were preserved for histological examination.

Sacrificed on 5-7-1967
(iii) 14th day postinfection

Two goat-kids (K-5 and K-32) which received 670 and 1,000 metacercariae respectively, were sacrificed 14 days postinfection. In both the animals the immature parasites were not found in any part of the stomach. In K-5, 18 immature specimens were recovered from the anterior and two from the middle third of the small intestine respectively, while the posterior third of the small intestine and the caecum did not contain any parasites. In K-32, there were 29 parasites in the anterior third of the small intestine, whereas the middle and posterior third regions of the small intestine and the caecum did not show any parasites. In Kid K-5, a total of 15 parasites were found in the ductus choledochus communis and four in the pancreatic duct. The gall-bladder and the ductus cysticus did not contain any parasites, though five parasites were recovered from the ductus hepatocysticus. In addition, seven parasites were found in the bile-ducts of the liver. The total percentage recovery of the immature parasites comes to 8.3 per cent. The ductus choledochus communis along with a portion of the duodenum, pancreas, gall-bladder, ductus cysticus, ductus hepatocysticus and portions of the liver of kid K-32 were fixed and processed for histological studies. The material is described below.
An examination of the serial sections of the duodenum portion into which the ductus choledochus communis opens by means of a papilla, revealed the presence of immature parasites in the lumen of the papillar projection of the ductus choledochus communis (Fig. 4). In the subsequent series of sections (Figs. 5, 6, 7, 8, 9 & 10) the immature parasite was found in the ductus choledochus communis. At this stage the musculature of the oral and ventral sucker of the parasites was well developed. The parasite, was seen attached to the superficial mucosal tissue by means of its acetabulum while its anterior end was embedded deep in the lamina propria of the duct.

(iv) 15th day postinfection

The kid K-38, was infected with 2,400 metacercariae and was sacrificed and autopsied 15 days postinfection. There were 36 and 10 immature parasites present in the anterior and middle third regions of the small intestine respectively. The rumen, reticulum, omasum, abomasum, the posterior part of the small intestine and the caecum were devoid of any parasites. The ductus choledochus communis had ten parasites whereas three were found in the pancreatic duct. As many as 19 specimens were recovered from the ductus hepatocysticus. The gall-bladder and the ductus cysticus contained six and two parasites respectively. In addition, 11 parasites were collected from the bile-ducts
of the liver. The spleen and kidneys were negative for the parasites. The total recovery of parasites was 4.04 per cent of all the metacercariae given.

(v) 19th day postinfection

One kid (K-42) was infected with 1,500 metacercariae, and it was sacrificed and autopsied 19 days postinfection. The rumen, reticulum, omasum, abomasum, the posterior part of the small intestine and the caecum did not contain any parasites. In all ten specimens were recovered from the anterior and two from the middle third regions of the small intestine respectively, while 28 parasites were found in the ductus choledochus communis. The pancreatic duct and the ductus cysticus did not contain any parasite. Seven parasites were recovered from the ductus hepatocysticus and two from the gall-bladder. In addition, nine specimens were found in the bile-ducts of the liver. The spleen and kidneys contained no parasites. The total percentage recovery of the parasites was 3.86 per cent of the total number of metacercariae given.

The histological examination revealed the presence of immature parasites deep in the serous glands of lamina propria of ductus choledochus communis (Fig. 11). In another series of sections the parasite was found still deeper in the muscular portion of the duct (Figs. 12 & 13).
(vi) 21st day postinfection

One kid (K-51) which was infected with 800 metacercariae was sacrificed and autopsied 21 days postinfection and 14 immature specimens were recovered from the anterior third of the small intestine. The other two parts of the small intestine, caecum and the various compartments of stomach did not show any parasites. The ductus choledochus communis contained 22 parasites. The pancreatic duct and the ductus cysticus were negative for the parasites but the gall-bladder contained two specimens. In addition, 16 parasites were recovered from the ductus hepatocysticus and eight from the bile-ducts of the liver. There were no parasites in the spleen and kidneys. The total recovery of parasites was 7.75 per cent of the total number of metacercariae given.

While examining the serial sections of the ductus choledochus communis and the pancreas, an immature parasite was found embedded in the glands of pancreas (Figs. 14, 15 & 16). In addition, migratory tracts in the pancreas were also observed in a few sections.

(vii) 28th day postinfection

One kid (K-52) was infected with 800 metacercariae and it was sacrificed and autopsied 28 days postinfection. No parasites were found in the rumen, reticulum, omasum,
abomasum, small intestine and the caecum. Twenty-nine parasites were recovered from the ductus choledochus communis whereas the pancreatic duct contained only four specimens. The parasites were not found in the gall-bladder and the ductus cysticus. On the other hand, 11 parasites were recovered from the ductus hepatocysticus and three from the bile-ducts of the liver. The spleen and kidneys did not contain any parasites. The total recovery of parasites was 5.37 per cent of the total number of metacercariae given.

The immature parasites were also seen in the sections of ductus hepatocysticus (Fig. 17).

**Route of migration of *G. explanatum* in buffalo calves**

The results of the experiments using eight buffalo calves (BC-10, BC-3, BC-40, BC-4, BC-6, BC-7, BC-8 and BC-9), each infected with 2,000 - 4,000 metacercariae of *G. explanatum* have been summarised in table VIII. The calf BC-5 was maintained in the same way as the experimental animals and served as a control.
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Buffalo No.</th>
<th>calf No.</th>
<th>No. of cysts given</th>
<th>Duration of infection (in days)</th>
<th>Number of parasites recovered from different organs</th>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>Rumen</td>
<td>Reticulum</td>
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<tr>
<td>2.</td>
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<tr>
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<tr>
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</tr>
<tr>
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</tr>
<tr>
<td>8.</td>
<td>BC-9</td>
<td></td>
<td>2,000</td>
<td>144</td>
<td>0</td>
</tr>
</tbody>
</table>
(i) 6th day postinfection

The buffalo-calf (BC-10) which received 2,000 metacercariae of *G. explanatum* was sacrificed and autopsied six days postinfection. No immature parasites were found in any of the compartments of the stomach. The parasites were, however, recovered from the small and large intestines, 19 from the anterior third, 16 from the middle third and 36 from the posterior third of the small intestine. The first 60 cm of the large intestine contained only ten parasites. The total number of parasites recovered from this animal comes to 4.05 per cent of all the metacercariae fed.

(ii) 19th day postinfection

From buffalo-calf (BC-3) which received 3,000 cysts and was autopsied after 19 days, two parasites were recovered from the abomasum while the other compartments of the stomach did not contain any parasites. The anterior third of the small intestine contained 108 specimens, while 266 and 51 parasites were recovered from the middle and posterior third regions of the small intestine respectively. Only two parasites were found in the anterior region of the large intestine. As shown in fig. 22, 11 parasites had reached the ductus choledochus by this time. The total recovery of parasites in this animal comes to 18 per cent of all the metacercariae fed.
In microtome sections, the immature parasites were found either free in the lumen of the small intestine attached to the villi of the mucosa or even embedded in the mucosa. In addition, a few of them were found embedded deep in the glandular region of the submucosa (Figs. 18, 19 & 20). The blood vessels of the submucosa did not contain any parasite.

(iii) 21st day postinfection

The other buffalo-calf (BC-40) which received 4,000 cysts and which was sacrificed after 21 days, showed only five parasites in the abomasum while the remaining three compartments of the stomach did not contain any parasites. The anterior third of the small intestine had 241 parasites and the middle and posterior third regions contained 339 and 144 parasites respectively. The caecum was found to harbour only four parasites. In the anterior part of the small intestine a large number of parasites in various stages of development were found present around the opening of ductus choledochus in the intestine (Fig. 21). In addition, some of the parasites had already gained entrance into the ductus choledochus (Fig. 22) and even reached the ductus hepatocysticus (table VIII). The gall-bladder, ductus cysticus, pancreatic duct and the bile-ducts of the liver, however, did not contain any parasites. The
total recovery of parasites comes to 19.6 per cent of all the metacercariae fed.

In the serial sections of the ductus choledochus the parasites were found either free in the lumen (Figs. 23 & 24) or attached superficially to the epithelial lining but dragging parts of the tissue into their acetabulum (Fig. 25). Though a large number of microtome sections were examined, the parasites were not found inside the submucosa or in the blood vessels.

(iv) 28th day postinfection

In one buffalo-calf (BC-4), which was given 2,000 cysts and autopsied after 28 days, only one parasite was found in the abomasum whereas the other three compartments of the stomach did not contain any parasites. The anterior third of the small intestine contained 336 parasites while 142 and 95 parasites were recovered from the middle and the posterior third regions of the small intestine. The caecum did not have any parasites. The ductus choledochus had 102 parasites and most of them were attached to the wall. Eight parasites had migrated into the gall-bladder and one was present in the ductus cysticus. The ductus hepatocysticus contained five specimens. The total recovery of parasites comes to 34.5 per cent of all the metacercariae given.
The histological examination of the ductus choledochus and ductus hepatocysticus showed that the parasites were either attached to, or were lying adjacent to the mucosal lining of the ducts (Figs. 26 & 27). The parasites were also observed in sections of the gall-bladder (Fig. 28).

(v) 35th day postinfection

One buffalo-calf (BC-6) was infected with 2,000 metacercarial cysts and it was sacrificed 35 days after infection. On autopsy, 277 parasites were recovered from the anterior third of the small intestine while the middle and posterior third regions contained 92 and two parasites respectively. The large intestine and the stomach compartments did not contain any parasite. There were 112 specimens occupying the ductus choledochus. The gall-bladder and the ductus cysticus did not reveal any of the parasites but 12 parasites were recovered from the ductus hepatocysticus and three from the bile-ducts of the liver. The total number of parasites recovered works out to be 24.9 per cent of all the cysts administered.

(vi) 43rd day postinfection

One calf, BC-7, was infected with 2,000 metacercarial cysts and it was sacrificed and examined 43 days
postinfection. The abomasum of the calf contained only four parasites whereas the anterior and middle third regions of the small intestine contained 216 and 35 immature specimens respectively. The posterior third region of the small intestine and the caecum did not contain any parasites. There were 189 parasites present in the ductus choledochus and two were recovered from the ductus cysticus. In addition, 16 and 5 immature parasites had migrated into the ductus hepatocysticus and the bile-ducts of the liver respectively. The percentage recovery of immature parasites comes to 23.35 per cent of all the metacercariae given.

(vii) 49th day postinfection

One buffalo-calf (BC-8) was given 2,000 cysts and was sacrificed and autopsied 49 days postinfection. Five parasites were present in the abomasum. The anterior third of the small intestine had 61 immature parasites while the middle third contained eight parasites only. A total of 218 parasites were recovered from the ductus choledochus. The gall-bladder, ductus cysticus and the bile-ducts of the liver did not contain any parasite. The total recovery of the parasites comes to 15 per cent of all the metacercariae fed.

The histological examination revealed the presence of parasites in the dilated ductus hepatocysticus (Fig.29).
The parasite had reached the proximal part of the duct where the duct enters the liver substance.

In calf BC-9, which was infected with 2,000 metacercarial cysts, and sacrificed and examined 144 days postinfection, the ductus choledochus was packed with mature parasites, which were either attached to the wall or were free in the lumen (Figs. 30 & 31).

Morphology of immature stages of *G. explanatum*

The following description is based on a study of a large number of permanent, stained, slightly flattened specimens made from material collected from laboratory infected experimental animals sacrificed at various predetermined intervals. The measurements of the various organs in different individuals were taken and their range and average (in parentheses) are given from a study of several specimens.

Studies on immature stages from goat-kids

Parasites recovered on 7th day postinfection

(Fig. 32)

The immature parasites were recovered from the posterior part of the duodenum of a goat-kid (K-31). The parasites measured 0.71-0.96 (0.86) mm in length and 0.18-0.37 (0.3) mm in maximum breadth. The oral sucker was 0.08-0.13 (0.11) mm in diameter while the oesophagus measured 0.08-0.1 (0.09) mm in
in length, the latter dividing into two intestinal caeca which extended up to the anterior margin of the acetabulum. Among the different genital organs, only a mass of cells representing the ovary was present just anterior to the acetabulum. Other structures could not be seen or identified as such since the pigment was distributed densely throughout the body. The mass of cells that would eventually form the genital papilla was situated at the level of the caecal bifurcation, and consisted of an elongated packed area of cells. The genital pore was not developed. The acetabulum was oval with an elliptical opening and measured 0.18-0.28 (0.24) mm in diameter.

Parasites recovered on 12th day postinfection

(Fig. 33)

The parasites of this age were obtained from the middle part of the duodenum of a goat-kid (K-41). The parasites showed the same general features as the previous one. They measured 0.73-1.06 (0.9) mm in length and 0.28-0.44 (0.37) mm in maximum breadth. The oral sucker measured 0.13-0.16 (0.14) mm in diameter and the oesophagus was 0.08-0.11 (0.098) mm long. The nerve mass was present at the oesophageal level and it extended on either side of the oesophagus. The ovary was represented by a rather dense mass of cells present anterior to the acetabulum. The cells representing the uterus extended anteriad as a median
longitudinal streak of nuclei staining dark red and which joined the rudiments of the genital papilla located at the level of intestinal bifurcation. The testes were present as two small masses of nuclei staining dark red, situated medially one behind the other along the uterine course and anterior to the ovary. The pigment was less than in the previous stage. The acetabulum measured 0.24-0.33 (0.28) mm in diameter.

**Parasites recovered on 14th day postinfection**

(Fig. 34)

The parasites were recovered from the ductus choledochus communis of a goat-kid (K-5). They measured 0.74-1.03 (0.91) mm in length and 0.23-0.42 (0.36) mm in maximum breadth. The oral sucker was 0.09-0.17 (0.13) mm in diameter and the oesophagus measured 0.08-0.11 (0.1) mm in length. The ovarian mass was situated just anterior to the acetabulum from which the uterus ran as a faint line. The rudiment of the genital opening was located at the level of the caecal bifurcation where the uterus came to an end. The testes exhibited the same general morphological features as in the previous stage. The pigment cells distributed on the general body surface of these parasites were faint, and less dense compared to the earlier stages. The acetabulum measured 0.24-0.31 (0.29) mm in diameter.
Parasites recovered on 15th day postinfection
(Fig. 35)

The immature parasites were obtained from the ductus choledochus communis of a goat-kid (K-38) and measured 0.89-1.33 (1.12) mm in length and 0.3-0.42 (0.36) mm in maximum breadth. The oral sucker was 0.13-0.17 (0.15) mm in diameter while the oesophagus measured 0.08-0.13 (0.11) mm long. The state of development of the genital organs was more or less the same as in the previous stage except that the testes had grown in size. The acetabulum measured 0.27-0.37 (0.31) mm in diameter.

Parasites recovered on 19th day postinfection
(Fig. 36)

The parasites were recovered from the ductus hepato-cysticus of a goat-kid (K-42). They measured 1.04-2 (1.5) mm in length and 0.31-0.59 (0.47) mm in maximum breadth. The oral sucker measured 0.13-0.22 (0.18) mm in diameter and the length of the oesophagus was 0.14-0.26 (0.18) mm. This stage resembled the earlier stages in the extent of development of the genital organs but in addition, the two vasa efferentia from the respective testes were clearly seen while the rudiment of the genital pore was slightly anterior to the intestinal bifurcation. The uterus was more prominent than in the previous stage and was situated medially. The density of the pigment was still more reduced as compared to the earlier
stages. The acetabulum measured 0.31-0.44 (0.38) mm in diameter.

On the 19th day a few parasites were also recovered from gall-bladder (Fig.37) and these measured 0.85-1.45 (1.06) mm in length and 0.35-0.44 (0.39) mm in maximum breadth. The oral sucker was 0.11-0.18 (0.14) mm in diameter and the oesophagus measured 0.08-0.2 (0.13) mm in length. The genital organs were in the same state of development as in the parasites of the same age recovered from the ductus hepatocysticus. The acetabulum measured 0.27-0.41 (0.32) mm in diameter.

On the 19th day postinfection a few parasites were recovered from the ductus choledachus communis (Fig.38). They measured 1.44-1.9 (1.6) mm in length and 0.5-0.6 (0.53) mm in maximum breadth. The oral sucker was 0.18-0.27 (0.22) mm in diameter whereas the oesophagus measured 0.17-0.31 (0.23) mm in length. The genital organs were better developed than in the parasites recovered from the ductus hepatocysticus and the gall-bladder. The acetabulum was 0.4-0.49 (0.43) mm in diameter.

**Parasites recovered on 21st day postinfection**

(Fig.39)

The developing parasites were obtained from the ductus choledochus communis of a goat-kid (K-51). They measured
1.78-2.07 (1.94) mm in length and 0.82-1.02 (0.89) mm in maximum breadth. The diameter of the oral sucker ranged from 0.27 to 0.34 (0.3) mm and the oesophagus was 0.11-0.21 (0.16) mm long. The male and female genital organs were better developed than in the previous stages and the rudiment of the genital pore was anterior to the intestinal bifurcation. The pigment was lightly distributed over the general body surface. The acetabulum measured 0.55-0.7 (0.67) mm in diameter.

Parasites recovered on 28th day postinfection (Fig. 40)

The specimens were obtained from the ductus choledochus communis of a goat-kid (K-52) and these measured 2.18-2.72 (2.45) mm in length and 0.75-0.84 (0.8) mm in maximum breadth. The diameter of the oral sucker ranged from 0.32 to 0.33 (0.326) mm and the oesophagus was 0.26-0.31 (0.28) mm long. In addition to the genital organs described in the earlier stages there appeared a transverse vitelline duct on each side, present between the terminal portion of the caeca and the anterior border of the acetabulum. The pigment was poorly distributed. The diameter of the acetabulum ranged from 0.6 to 0.71 (0.65) mm.
Immature stages from buffalo calves

Parasites recovered on 6th day postinfection

(Fig. 41)

The parasites were obtained from the middle part of the duodenum of a buffalo-calf (BC-10). The parasites measured 0.91-0.96 (0.94) mm in length and 0.31-0.45 (0.4) mm in maximum breadth. The oral sucker was 0.17-0.2 (0.19) mm in diameter while the oesophagus measured 0.054-0.089 (0.07) mm in length. The two intestinal caeca extended up to the anterior margin of the acetabulum. Among the genital organs, only a mass of cells representing the ovary was seen situated anterior to the acetabulum. Other structures could not be seen as the pigment was densely distributed throughout the body of the parasite. The genital pore rudiment in the form of an elongated condensed mass of cells without an opening was present at the level of caecal bifurcation. The acetabulum measured 0.34-0.41 (0.38) mm in diameter.

Parasites recovered on 21st day postinfection

(Fig. 42)

The parasites were collected from the ductus choledochus of an experimentally infected buffalo-calf (BC-40). The parasites measured 1.72-2.1 (1.94) mm in length and 0.74-0.96 (0.81) mm in maximum breadth. The diameter of the oral sucker ranged from 0.3 to 0.34 (0.31) mm and the oesophagus
was 0.1-0.2 (0.15) mm long. The anterior and the posterior testes were quite prominent. The ovary was represented by a mass of cells present just anterior to the anterior margin of the acetabulum while the uterus was represented by a chain of very faintly staining cells running from the ovary towards the anterior side. The rudiment of the genital pore was slightly anterior to the intestinal bifurcation. There were a few pigment cells distributed over the general body surface. The acetabulum measured 0.62-0.72 (0.66) mm in diameter.

Parasites recovered on 43rd day postinfection (Fig. 43)

The parasites were obtained from the ductus choledochus of a buffalo-calf (BC-7) and measured 3.85-5.25 (4.57) mm in length and 1.88-2.17 (2.01) mm in maximum breadth. The oral sucker measured 0.48-0.57 (0.52) mm in diameter and the oesophagus was 0.27-0.46 (0.34) mm long. The testes in these specimens were more conspicuous than in the previous stage, and gave rise to two vasa efferentia which united to form the vas deferens. The latter was much convoluted anteriorly and opened very close to the female genital opening. The ovary was larger than in the previous stage. The Mehlis' gland was prominent and situated close to the ovary. The vitelline follicles could not be identified as such but the transverse vitelline ducts were present
in between the ovary and the acetabulum. The uterus was comparatively prominent and convoluted at the terminal end. The acetabulum measured 1.36-1.75 (1.53) mm in diameter.

Parasites recovered on 49th day postinfection (Fig. 44)

The parasites were collected from the ductus choledochus of a buffalo-calf (BC-8). The parasites measured 4.35-5.79 (5.24) mm in length and 1.54-2.16 (1.82) mm in maximum breadth. The diameter of the oral sucker varied from 0.58 to 0.66 (0.63) mm and the oesophagus was 0.13-0.51 (0.37) mm long. The genital organs were better developed than in the previous stages. The testes had attained an elliptical shape and the two vasa efferentia had joined to form a much convoluted vas deferens. The ovary was slightly bigger than in the previous stage, and the Mehlis' gland was situated medial to it. The vitelline duct and the uterus were more prominent and the genital opening was anterior to the caecal bifurcation. The body was devoid of all pigment cells. The acetabulum measured 1.57-1.69 (1.63) mm in diameter.
Parasites recovered on 144th day postinfection
(Fig. 45)

The adult parasites were obtained from the ductus
choledochus of a buffalo-calf (BC-9). The parasites mea-
sured 10.27-13.33 (12.2) mm in length and 4.71-5.29 (4.94)
mm in maximum breadth. The oral sucker was 0.8-0.97 (0.9)
mm in diameter while the oesophagus measured 0.28-0.75
(0.52) mm in length and the intestinal caeca bifurcated
at the level of the genital pore and ended blindly at the
level of the ovary anterior to the acetabulum. The aceta-
bulum was fairly well developed and measured 3.55-3.76
(3.64) mm in diameter.

The male genital organs consisted of two well deve-
loped testes situated one behind the other. The anterior
testis measured 1.07-2.76 x 2.93-3.85 mm while the posterior
testis measured 2.03-2.86 x 3.04-3.76 mm. The two vasa
efferentia joined to form the vas deferens anterior to the
testes. The vesicula seminalis was quite prominent and the
genital pore was situated at level, or just posterior to
the intestinal bifurcation. The ovary was rounded in shape
and present in between the posterior testis and the aceta-
bulum. The Mehlis' gland was present very close to the
ovary. The vitelline follicles were well developed and
situated mainly on the lateral sides of the body extending
from the level of the oesophagus to the anterior border
of the acetabulum. The uterus was well developed and contained eggs which measured 0.117-0.131 (0.12) x 0.055-0.069 (0.062) mm in size.

On the 144th day postinfection, a few adult parasites were also recovered from the gall-bladder (Fig. 46) and they measured 10.53-12.64 (11.49) mm in length and 4.14-4.51 (4.38) mm in maximum breadth. The oral sucker was 0.32-0.89 (0.86) mm in diameter while the length of oesophagus ranged from 0.24 to 0.69 (0.43) mm. The acetabulum was well developed and measured 2.62-3.69 (3.31) mm in diameter. The genital organs were similar in state of development to the stage recovered from the ductus choledochus. The anterior testis measured 1.24-2.17 x 2.88-3.27 mm while the posterior testis measured 1.95-2.48 x 3.10-3.15 mm in size. The eggs measured 0.124-0.131 (0.126) x 0.062-0.069 (0.066) mm in size.
Susceptibility of some laboratory animals to experimental infection with G. explanatum

To study the relative susceptibility of some of the laboratory animals and to ascertain the possible route of migration in them, a few rabbits, white rats and guinea-pigs were used.

Rabbit: Following the feeding of 100 metacercariae to each of the two rabbits, both the animals started passing intact cysts with the faeces as early as six hours postinfection. Rabbit No.1 was then sacrificed immediately and the stomach, intestine, ductus choledochus, abdominal cavity and the liver were thoroughly examined. No immature stages were encountered in any of these locations, though 59 per cent of the cysts were present in the intestine and 5 per cent in the stomach. All the cysts were intact. In addition, 25 per cent of the cysts, all still intact, were recovered from the faeces. The faeces of rabbit No.2 were examined up to 10 hours post-infection and 68 per cent of all the metacercariae given were voided in the faeces. It was then sacrificed and examined. The stomach did not contain any cysts. However, 18 per cent of the cysts administered were present in the contents of the intestine. No immature stages were found in the stomach, intestine, ductus choledochus, abdominal cavity or the liver.
In another experiment six rabbits (R-3, R-4, R-5, R-6, R-7 and R-8) were each given 300 metacercariae per os. After 24 hours of infection, rabbit R-3 was killed and the stomach, intestine, abdominal cavity, ductus choledochus and liver were thoroughly searched for the developing stages, but no immature stages of the parasite could be recovered from these locations. However, 6 per cent of the cysts administered were obtained from the intestinal washings. Rabbit No.4 was sacrificed two days postinfection and its examination did not reveal the presence of any cysts or immature stages of the parasite in the teased material of stomach, intestine, abdominal cavity, liver and the ductus choledochus.

The other infected rabbits (R-5, R-6, R-7 and R-8) were sacrificed 7, 14, 21 and 28 days postinfection respectively. In all the cases, the stomach, intestine, liver, abdominal cavity and ductus choledochus were free of immature stages.

**White rats:** Each of the ten white rats were individually infected per os with 200 metacercariae of *G. explanatum* and killed at 8 and 24 hours, and 2, 4, 7, 14, 21, 28, 35 and 42 days postinfection. The first two rats which were sacrificed at 8 and 24 hours postinfection revealed the presence of intact cysts in the contents of the intestine and the faeces but the stomach was devoid
of any cysts. The immature parasites were not found in any part of the intestine, ductus choledochus, abdominal cavity or the liver. The other experimental rats were sacrificed as stated above and their viscera searched both grossly as well as histologically by examining microtome sections but in no case the immature stages of the parasites were found.

**Guinea-pigs:** Each of the ten guinea-pigs were infected individually *per os* with 150-500 metacercariae of *G. explanatum*. They were later sacrificed at 6 hours, and 1, 3, 7, 12, 16, 18, 21, 28 and 35 days postinfection respectively. In only one case (6 hours postinfection) a few cysts were found in the intestinal contents but immature stages of the parasites could not be recovered from the intestine, abdominal cavity or the liver. The other experimental guinea-pigs were autopsied and in no case the excystment of the metacercariae was witnessed.

**Excystment of metacercariae of G. explanatum**

To determine the site of excystment of metacercariae of *G. explanatum* in the gastro-intestinal tract of final host, each of the five 1-month old goat-kids were fed 1,500-2,000 metacercariae which were encysted on leaves. Subsequently, these animals were killed at different intervals of time, ranging from 6 to 72 hours
postinfection. A detailed examination of rumen, reticulum, omasum, abomasum and intestine was carried out.

The excystment of metacercariae occurred in the small intestine between 6 and 12 hours after the cysts had been fed to kids. Several portions of the small intestine were examined and only on one occasion the emergence of immature parasite from the inner cyst was observed under the microscope. At 6 hours postinfection a large number of metacercariae enclosed within their cysts were found distributed in the lumen of the small intestine up to about six meters from the pylorus. These metacercariae showed varying stages which were associated with the emergence of the trematode from within the cyst. In addition, a few metacercariae with intact cysts were also present in the contents of the abomasum. In one case two young parasites were found together about two metres away from the pylorus. These immature parasites were embedded in the mucosa and did not show any movement.

The metacercariae having only the inner cyst wall were examined under the microscope, and the individual inside the cyst could be seen moving by extending and contracting the body. However, at 12 hours postinfection, a number of immature parasites and a few encysted metacercariae were found in the small intestine up to about
six metres away from pylorus. At 24, 48 and 72 hours postinfection, the excysted parasites were recovered as far away as about 10 metres from the pylorus. In the animal examined 72 hours postinfection, one encysted metacercaria was found deeply embedded in between the villi about 20 cm from the pylorus.

Excystment of metacercariae in Mouse

The "sausage" experiment was performed in a large number of mice infected with the metacercariae of *G. explanatum*. In two mice the duodenum was ligatured near its junction with the pylorus and the other ligature was put about 5 cm away from the first. In the "sausage" of the duodenum thus formed an inoculum of 200 metacercariae was injected. Later, the mice were sacrificed after 30 minutes and 60 minutes respectively. The "sausages" were examined grossly and histologically. In no case the excystment was witnessed.

Similarly in two other mice a part of the small intestine was used to form a "sausage" beginning at 15 and 25 cm from the pylorus and each injected with 200 metacercariae. The mice were sacrificed 90 and 120 minutes postinfection respectively. The gross examination and microtome sections revealed the presence of intact
cysts in the lumen and no free immature stages were found either in the lumen or penetrating the tissues of the small intestine.

In another experiment using six mice, the "sausages" formed in the small intestines of each were injected with 100 metacercariae. The first mouse was killed 150 minutes postinfection. The metacercariae of *G. explanatum* did not excyst during this period and on cutting microtome sections of a portion of the "sausage", the cysts were found in the lumen as well as adjacent to the mucosa. Similarly when the second mouse was sacrificed after 180 minutes of infection, the excystment was not witnessed. The remaining four mice were examined at 210, 240, 270 and 300 minutes postinfection. A careful examination of the serial sections of the intestine "sausages" of these mice, did not show the excystment of the metacercariae. However, the intact cysts were found in the lumen of the intestine even after 300 minutes postinfection (Fig. 48).
(4) Pathological study

Goat-kids

During the gross examination of the infected small intestine of the several experimentally infected goat-kids, no significant lesions were found in any one of them, though a few detached parasites were present mixed with the intestinal contents occasionally accompanied by thin dirty mucus. In one case (15 days post-infection) the anterior part of the small intestine showed congestion and a number of parasites were aggregated near the opening of the ductus choledochus communis. The ductus hepatocysticus, ductus choledochus communis, pancreatic duct, gall-bladder, ductus cysticus and the liver did not reveal any gross pathological lesions, though sometimes parasites were present in some of these organs.

The sections of the duodenum (seven days post-infection) showed extensive superficial desquamatory changes (Figs. 1 & 2) and moderate linear hypertrophy of the mucosa, to which a few parasites were attached. The epithelial cells showed proliferative changes resulting in moderate linear hypertrophy of the glands. The connective tissue of the tunica propria was much proliferated and infiltrated with mononuclear and fibroblasts.
The epithelial degenerative and necrotic changes were insignificant. The submucosa and the Brunner's glands were not much affected excepting for the cellular reaction at places adjoining the muscularis mucosae.

The other series of sections (14 days postinfection) through the junction of the ductus choledochus communis with the duodenum showed superficial degeneration and desquamation of the villi, epithelial and connective tissue proliferative changes in the mucosa which was heavily infiltrated with mononuclear cells and fibroblasts. The majority of the epithelial cells showed degenerative changes and many cells were desquamated. In the submucosa the Brunner's glands were not hypertrophied and presented moderate infiltration with the mononuclear cells. The musculature of the duodenum was thinned out and on the side of the ductus choledochus communis it was continuous with the duct's musculature. The ductus choledochus communis contained developing stages in its lumen, together with the cellular debris resulting from superficial desquamation of the duct epithelium. The deeper epithelium of the duct was much hypertrophied and convoluted, and the inter-glandular connective tissue was much proliferated and infiltrated with mononuclears and fibroblasts. There was considerable hyperplasia of the
serous glands also. A large number of the proliferated mucous epithelial cells showed degeneration and desquamation. The musculature and the serous layer of the duct which were continuous with their counterparts of the duodenum, however, showed nothing of interest.

In an infection of a longer standing (19 days old infection), the parasites were observed in the lumen of the ductus choledochus communis showing much proliferated mucous glands which were surrounded with lymphocytes, neutrophils and fibroblasts, accompanied with extensive epithelial desquamation filling the lumen of the duct with cellular debris (Fig. 48). The serous layer and the muscular layer of the duct showed hyperplastic changes.

In the other sections, the parasite was within the acini of the mucous gland of ductus choledochus communis (Fig. 49). The glands were moderately hypertrophied and were accompanied with increased connective tissue activity and mild mononuclear infiltration. The serous glands were also somewhat hypertrophied. The blood vessels were congested and the muscular coat was thinned out and replaced by thick bands of connective tissue.

Where the parasites had entered deep into the ductus choledochus communis wall, both the serous and the mucous glands were hypertrophied and there was marked hyperplasia of the interglandular connective tissue. The
blood vessels were congested and the duct's musculature was mostly replaced by the loose connective tissue fibres.

In an older infection (21 days postinfection), the parasites had penetrated beyond the wall of the ductus choledochus communis and were present embedded deep in the pancreatic tissue (Figs. 14 & 15). There was much proliferation of the interglandular connective tissue and the mucous and serous glands were hypertrophied, more so the serous glands. The pancreatic glands showed hyperplastic changes and there was a marked increase in the interglandular and interlobular connective tissue, with the result that the pancreas appeared to be separated into several lobules.

Upon arriving in the ductus hepatochlysticus the parasite was found in the lumen of the duct producing moderate proliferative and desquamative changes. The subepithelial glandular tissue was mildly infiltrated with the mononuclear cells. At places the serous glands showed hyperactivity and the musculature was hypertrophied.

**Buffalo calves**

The following account is based on the study of a large number of sections obtained from the tissues of the experimentally infected buffalo calves. In the early stages of infection, the gross lesions were mostly observed
in the anterior and middle parts of the small intestine. These consisted of thickening and oedema of the mucosa giving it a corrugated appearance, and a number of developing stages were present either free or attached to the mucosa, mixed with dirty mucus. Occasionally pin-point hemorrhages were found in the anterior part of the small intestine. In the later stages of infection, the lesions were encountered in the ductus choledochus. Many parasites were attached superficially to the mucosa and were found to have dragged parts of the mucosa of the ductus choledochus into their acetabula and at places the mucosa of the ductus showed pin-point haemorrhages. In advanced stages of infection a large number of papillomatous projections were found throughout the wall of the ductus choledochus conforming to the contours of the acetabulum of the parasite. The lumen of the duct was packed with parasites. The gall-bladder did not show any abnormal feature though occasionally it contained parasites. The ductus hepatocysticus and the larger bile-ducts of the liver showed nothing abnormal in spite of the fact that a few developing parasites were sometimes found in them. The liver proper did not show any abnormality.

The sections of the posterior part of the duodenum (19 days old infection) showed the presence of a large number of parasites in the lumen at various stages of
development along with a large amount of detritus consisting of the desquamated superficial mucosal tissue (Fig. 50). The mucosa showed the epithelial hyperplastic changes and the mononuclear infiltration in the tunica propria together with significant superficial desquamation. At places the damaged superficial mucosa showed necrotic changes. Some of the immature parasites were seen attached to the superficial mucosa holding mucosal tissue by means of their acetabula. A few parasites were usually found embedded in the mucosa with leucocytic infiltration in some cases. The blood vessels were congested but haemorrhages were not so evident. The lumen of some of the glands showed excessive mucin secretion and the epithelial cells at such places were degenerating and desquamating. There was proliferation of connective tissue of the tunica propria, which was also infiltrated with leucocytes. Though the immature stages of the parasites were not seen in the submucosa of the sections, the deeper glandular tissue had become hyperplastic (Fig. 51) and was sometimes seen to have entered the submucosa. The blood vessels in the submucosa were mildly congested associated with slight proliferation of the connective tissue. The musculature did not show any significant abnormality excepting for a few patches of slight cellular infiltration. The serosa also did not show any abnormality.
In the middle part of the duodenum there was extensive superficial degeneration and desquamation of the mucosa and a number of parasites were either attached to the mucosa superficially or were free in the lumen. The parasites had dragged the mucosal tissue into the acetabulum and such portions showed necrotic changes. At a few other places, there were concavities lined by the necrotic tissue and these were probably the sites where the parasites had been attached earlier. The oral suckers of some of the parasites lying free in the lumen of the duodenum showed some degenerated epithelial cells. The mucosa, in general, showed necrotic and proliferative changes and there were marked areas of degeneration and desquamation. The lumen of some of the glands showed accumulation of mucus as evidenced by eosinophilic homogeneous masses. At places, there was complete degeneration of the glands which were replaced by proliferated connective tissue of the tunica propria and infiltrated with a large number of lymphocytes, neutrophils and fibroblasts. The tunica propria in general showed proliferation of the connective tissue infiltrated with leukocytes and fibroblasts. There were some areas marked by heavy cellular infiltration in the deeper mucosa and also on the submucosal side of the muscularis mucosae. At places, the muscularis mucosae was breached and the
glandular tissue of the mucosa had invaginated into the submucosa. The blood vessels in the mucosa were congested and the Brunner's gland showed mild proliferation. In some areas, there was much increase in the connective tissue of the submucosa which had hypertrophied. The musculature and serosa did not show any significant pathological changes.

The section of the duodenal wall around the opening of the ductus choledochus (19 days old infection) showed comparatively large number of parasites in the lumen, amidst extensive tissue debris consisting of extensively desquamated superficial mucosa, degenerated epithelial cells and large number of erythrocytes. The parasites were attached to the mucosa of the duodenum with the help of the acetabulum and a few parasites in the lumen showed similar degenerated cellular debris inside their oral cavities or in their caeca, or in both. In addition, the section of the duodenal wall in this region also showed many parasites embedded both in the superficial as well as the deeper portions of the mucosa. The mucosa, in general, showed extensive epithelial proliferative changes of the epithelial lining, desquamation of a large number of epithelial cells, and the tunica propria also showed extensive proliferation of the connective tissue infiltrated with a large number of
lymphocytes and fibroblasts. Many necrotic patches were evident in the superficial mucosa.

The blood vessels were congested and at places many small foci of haemorrhages were evident. The cellular reaction in the tunica propria was more intense towards the superficial mucosa. The parasites in the mucosa were present in the clear spaces which were lined by a thin layer of fibrous connective tissue. Some mitotic patches were observed in the middle of the mucous layer also. The submucosa showed congested blood vessels and the presence of some remnants of the parasites. However, these parasites, in general, were not accompanied by the proliferation of the connective tissue or that of the Brunner's gland cells or, by any significant cellular reaction. At places, however, the hyperplastic glandular tissue had breached the muscularis mucosae and had entered the submucosa. The muscular coat showed nothing abnormal excepting for some focal patches of cellular infiltration. The serosa was almost normal.

At 21 days postinfection, the ductus choledochus showed extensive proliferative and infiltrative changes in the duct epithelium, together with a few parasitôs attached superficially to it and dragging parts of the tissue into their acetabulum (Fig. 26). The superficial lining epithelium, in general, showed degenerative changes
and desquamation. The deeper epithelial lining showed intense proliferative changes resulting in convolution and thickening of the mucosa. The lumen of the glands showed the presence of coagulated mucus. In the tunica propria there was much proliferation of the connective tissue and this was infiltrated with leucocytes, predominantly lymphocytes, and with the fibroblasts (Fig. 52). Besides, there were many focal patches of cellular reaction, more towards the superficial mucosa and particularly around the area where the parasite was attached.

In many places there was evidence of epithelial degeneration and desquamation. The muscularis mucosae at places had also proliferated and its fibres had penetrated between the islands of glandular tissue. In the submucosa the blood vessels were congested and the connective tissue fibres were moderately proliferated together with mild cellular infiltration which was more towards the muscularis mucosae.

At 28 days postinfection, the ductus choledochus revealed widespread superficial necrosis of the mucosa due to presence of many parasites in the lumen. There was acute proliferation and convolution of the epithelium resulting in the formation of many wide clear spaces which were sometimes filled with mucin, desquamated epithelial
cells and cellular debris. The blood vessels were congested and there was much proliferation of the connective tissue of the tunica propria which was infiltrated with a large number of lymphocytes and fibroblasts. The tunica propria now consisted of proliferated tissue and the thick bands of connective tissue fibres ran amidst various mucosal glands which appeared as islands surrounded by connective tissue. At places the epithelial cells lining the wide spaces were degenerated and desquamated with the result that some of these were only lined by the basement membrane. There were also some focal areas of degenerated tissue in the mucosa. The superficial necrosis of the mucosa was quite extensive and showed many concave areas suggestive of parasite attachment sites. There were some focal areas of heavy cellular reaction in the middle as well as in the deeper mucosa (Figs. 53, 54 & 55). The fibrous tissues separating the mucous gland layer from the serous gland layer were also proliferated, enclosing various groups of glands in between.

The serous glands were also showing proliferative changes with some wide spaces filled with cellular debris. At places there were mild cellular infiltrations. The outer muscular layer showed nothing abnormal excepting for vasular congestion. In addition to the above changes in the ductus choledochochus, another series of section of
this organ showed degeneration and desquamation of the glandular epithelial cells and much proliferation of the inter-glandular connective tissue which was infiltrated with leucocytes and fibroblasts. The acini of many of the glands contained coagulated mucus or cellular debris. There was much linear hypertrophy of the glands (Fig. 56) together with the degeneration of the lining epithelial cells. Some conspicuous areas of cellular reaction were present at various depths of the glandular region. The serous glands, as well as the connective tissue between the serous and the mucus layers, showed proliferative changes. The blood vessels were congested and the musculature was almost normal.

The sections of the ductus choledochus in a case of advanced infection (43 days old infection), showed acute proliferative changes and linear hypertrophy resulting in the total hypertrophy of the duct epithelium. The superficial lining epithelium was usually regenerated but many areas were denuded of the epithelium and showed necrotic changes. The parasites were attached superficially showing papillomatous elevations (Fig. 60), and at places the plugs of mucosa which were dragged and detached by the acetabulum of the parasite were present (Fig. 57). The deeper epithelial cells were much proliferated but the superficial
layers showed degeneration and desquamation.

Due to the acute proliferation, the acini of many of the mucous glands were lined with multi-layered epithelium (Fig. 58), and some of the acini contained mucus and desquamated cellular debris. At places, the epithelial proliferative changes were so severe so as to give an impression of neoplastic changes evinced by multi-layered, actively dividing epithelial cells. The inter-glandular connective tissue was also proliferated and this was much more marked towards the lumen of the duct (Fig. 61), and it was heavily infiltrated with mononuclear cells and the fibroblasts. The connective tissue between the mucous glands and the serous glands was mildly proliferated and infiltrated. The serous glands were also hypertrophied and their acini were filled with secretion. A few patches of intense cellular infiltration, consisting of lymphocytes, neutrophils and fibroblasts, were present between the mucous and the serous areas (Fig. 59). The blood vessels were much congested and a few small focal haemorrhages were evident. The muscular layer did not show much abnormality excepting for the congested blood vessels and a few areas of proliferated connective tissue and infiltration with fibroblasts.

The other series of sections from a more advanced stage of infection (49 days old infection) in the ductus
choledochus (Fig. 62), showed increased papillomatous thickening, desquamation of the superficial lining epithelium at many places, and pronounced hypertrophy of the mucous layer. Large areas of the inter-glandular tissue were occupied by extensively proliferated connective tissue and this was more marked towards the lumen where considerable parts of the tissue presented necrotic changes. The connective tissue throughout was mildly infiltrated with the various cells. A large number of epithelial cells showed degenerative changes, but desquamation was not widespread. The serous glands were not much hypertrophied. The muscular layer did not show any abnormality.

The sections of the ductus choledochus at a later stage of infection (144 days old infection), showed acute epithelial proliferative changes and many papillomatous elevations due to the attachment of the parasites (Fig. 63). Due to the proliferative changes of the epithelium and the subepithelial stroma, the duct wall was hypertrophied. At places, there were islands of epithelium amidst highly proliferated connective tissue, which was infiltrated with leucocytes predominated by mononuclear cells. The division of the nucleus of epithelial cells at many places showed neoplastic tendency evinced by mitotic figures and many layers of nuclei. In general, there was superficial degeneration and desquamation of the epithelium and the papillomatous
elevations were constituted mostly by the highly proliferated connective tissue. At many places, the epithelial cells were degenerated and desquamated and replaced by the connective tissue. There was congestion of the blood vessels and significant proliferation of the fibrous tissue in the duct wall. Sometimes, particularly at the site of papillomatous elevations, there were a few foci of mononuclear infiltration in the fibrous wall adjacent to the epithelium.

The sections of the ductus hepatocysticus (35 days old infection) with some parasites in the lumen, showed epithelial proliferative changes and hypertrophied changes of the duct wall. The epithelium of the duct was much proliferated and convoluted due to the presence of the parasites, and the epithelial region was highly infiltrated with mononuclear cells and the fibroblasts. There was widespread superficial desquamation, resulting in the loss of superficial lining epithelium associated with many patches of superficial necrosis. Some raised or depressed areas which were present superficially and confirming to the contours of the acetabulum, were indicative of the parasite attachment. The interepithelial connective tissue was also much proliferated, and there were many focal areas of heavy cellular reactions and this was more prominent in the area adjoining the fibrous duct wall.
The duct wall presented hypertrophy with proliferated fibrous tissue and the fibres were more mature towards the hepatic lobules and immature towards the duct epithelium. The blood vessels were mildly congested, and at places, proliferated epithelium had also entered these. The hepatic cells showed various grades of retrogressive changes and the Kupffer cells were prominently displayed (Figs. 64 & 65). The cirrhotic changes were not evident.

The sections of ductus hepatocysticus (43 days old infection), showed proliferation of epithelial cells and of the connective tissue, moderately infiltrated with leucocytes and fibroblasts. The superficial epithelial lining was desquamated and the superficial tissue showed moderate necrotic changes. At places, pronounced papillomatous elevations were observed towards the lumen of the duct due to acetabular attachment, accompanied with more cellular infiltrations and more proliferation of the connective tissue. At other places, cavities were superficially notched due to the desquamation of the tissue following attachment of the parasite. The acini of some of the mucous glands showed desquamated cellular debris and coagulated mucus. At many places, the epithelial cells showed degeneration and desquamation. Sometimes, focal areas of dense cellular infiltration were also observed in the epithelium adjacent to the

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fibrous wall. The fibrous wall showed congestion of the blood vessels and an increase in the fibrous tissue. The hepatic cells showed early degenerative changes, and mild periportal cellular infiltration. The Kupffer cells were not prominent and the cirrhotic changes were absent.

The sections of ductus hepatocysticus (144 days old infection), showed parasite in its lumen and epithelial lining of the duct showed mild proliferative changes with leucocytic infiltration. The blood vessels were mildly congested and there was a moderate thickening of the duct wall. In the adjoining hepatic tissue, the hepatic cells showed various retrogressive changes and a proliferation of the Kupffer cells. The portal areas were infiltrated with mononuclear cells and the fibroblasts and the blood vessels were congested.

The section of the affected liver parenchyma showed early degenerative changes of the hepatic cells evinced by degenerating nuclei and a slightly granular nature of the cytoplasm. The Kupffer cells were proliferating and prominent. The bile-duct did not show any significant changes. At places, however, fine streaks of connective tissue were perceptible in the interlobular areas. The central veins of some of the lobules were comparatively widened.

In the gall-bladder (28 days old infection), there was moderate proliferation of the lining epithelial
cells and infiltration with the mononuclear cells. The blood vessels were highly congested and the connective tissue of the mucosa was proliferated. At places the lining epithelial cells were degenerated and desquamated (Fig. 66) and a few necrotic foci were present in the mucosa. Sometimes the connective tissue fibres were seen running in between the muscle fibres interrupting their continuity. The serous layer showed nothing abnormal.

In addition, mucous nature of the sucker and the internal sacs of the parasite showed an intense PAS-positive reaction (Fig. 68) in comparison to the outer structures of the parasite. The treatment with silver did not alter the subsequent PAS reaction in the vessels and the infected tissues. After acetylation all the structures became PAS-negative but deacetylation restored the PAS-positive reaction as before.

When sections stained with the regular PAS routine were counterstained with Celestine Blue and safranin yellow, most of the PAS-positive areas were masked by the safranin yellow except for the parasites and the glandular structures.
(5) Histochemical study

I. Carbohydrate and carbohydrate-containing tissue elements

(a) Periodic acid-Schiff reaction

The goblet cells of the epithelial lining, Brunner's glands and masses of secreted mucus in the lumen of the duodenum gave PAS-positive reaction in both the normal and the infected duodenum. A stronger reaction was, however, observed in the region of the Brunner's glands of the infected duodenum (Fig. 67). In addition, musculature of the suckers and the intestinal caeca of the parasite showed an intense PAS-positive reaction (Fig. 68) in comparison to the other structures of the parasite. The treatment with saliva did not alter the subsequent PAS reaction in the normal and the infected tissues. After acetylation all the structures became PAS-negative but deacetylation restored the PAS-positive reaction as before.

When sections stained with the regular PAS routine were counterstained with Celestine Blue and Naphthol yellow S, most of the PAS-positive areas were masked by the Naphthol yellow except for the parasites and the goblet cells which retained the red colour.

A much stronger PAS-positive staining reaction was given by the goblet cells of the epithelial lining of the
mucosa, mucous and serous glands of the infected ductus choledochus than by these cells and tissues of the normal organ (Figs. 68 & 69). In addition, the lumen of these glands showed the presence of PAS-positive material. Other structures in the tissue gave a weaker PAS-positive reaction in both the normal and the infected tissue. The suckers of the parasite were, however, intensely PAS-positive. The prior treatment with the saliva did not alter the subsequent PAS reaction in normal as well as the infected tissues. After the acetylation all the structures became negative when subjected to the regular PAS routine. However, after the deacetylation the test gave similar results as seen with the regular PAS routine. With the Celestine Blue and Naphthol yellow S staining, the red colouration of the serous and mucous glands and of the tissues of the parasite remained as such while the other structures stained in shades of yellow.

(b) Best's carmine test for glycogen

The mucosa and the submucosa of the infected and the normal duodenum were negative for glycogen, though a very faint reaction was observed in the muscular layer. In the infected section the body of the parasite along with the suckers showed a strong positive reaction for glycogen (Fig. 71). When treated with saliva and then stained with Best's carmine, the stained areas became negative.
In sections of the ductus choledochus only the muscular layer showed a light pinkish colour. However, the suckers and the parenchyma of the parasite gave positive reaction for glycogen. When the tissue was first treated with saliva, and then stained with Best's carmine, there was no staining reaction seen.

(c) Hale's dialysed iron method for acid mucopolysaccharides

The goblet cells of the villi, Lieberkühn's glands and Brunner's glands of the infected duodenum showed a much stronger positive reaction for acid mucopolysaccharides than the normal tissue. In addition, deep blue-stained masses were also seen in the lumen of the duodenum. The intestinal caeca of the parasite gave a faint positive reaction.

In the sections of the infected and the normal tissues of the ductus choledochus, faint positive reactions were observed in the epithelial lining of the mucosa, mucous and the serous glands of the lamina propria. In the infected tissue, in addition, the intestinal caeca of the parasite gave a faint staining reaction.

(d) Alcian blue method for acid mucopolysaccharides

Though the sections of the infected and the normal tissues of the duodenum gave a similar staining reaction,
the intensity of colour was more in the region of the
goblet cells of the lamina epithelialis, glands of
Lieberkuhn and the Brunner's glands of the infected
duodenum (Fig. 72). In addition, blue-green masses
were also seen around the parasites present in the lumen,
but the parasites did not show any positive staining
reaction with this routine.

In the case of the infected ductus choledochus
the positive staining reaction was more in the glandular
epithelium of the infected tissue than in the normal
tissue. The parasite did not give any positive reaction.

(e) **Mucicarmine test for mucopolysaccharides**

Both the normal and the infected tissues of the
duodenum showed various shades of pink and red stain. A
positive reaction was shown by the parasite at the site
of its attachment to the mucosa. The sections of the
ductus choledochus did not give any reproducible results.

(f) **Schmorl's thionin method for metachromasia**

A violet colour (β-metachromasia) was seen in the
epithelial lining cells of the mucosa in the sections of
the infected and the normal duodenum. The parasite did
not reveal any metachromasia with this routine. In the
case of sections of infected ductus choledochus, β-meta-
chromasia was observed only in the glandular region of
the lamina propria.

(g) **Toluidine blue method for metachromasia**

The results were more or less similar to those
obtained with thionin as described above though the meta-
chromatic staining reaction was less intense and fugitive.

II. Proteins

(a) **Millon reaction (Baker modification)**

The muscularis mucosae and the epithelial lining
of the villi of the normal duodenum gave a faint positive
reaction while in the infected duodenum the reaction was
more or less same except that the lumen of the duodenum
contained the parasites which had been stained light-pink.
A differential staining reaction was found in the sections
of the ductus choledochus.

(b) **Mercury-Bromphenol blue method**

The muscular layer of the normal duodenum was
stained deep blue whereas the staining of the villi varied
from light blue to deep blue. Similar results were obtained
with the infected tissue except that deep blue masses were
seen scattered in the lumen of the duodenum along with the
deep blue stained parasites.

In the normal and infected sections of the ductus
choledochus, a positive reaction was observed in the region
of the muscular layer, epithelial lining cells and glands of the lamina propria and which varied from light blue to deep blue. In the infected duct the posterior sucker of the parasite took deep blue colour. The results with this histochemical reaction were on the whole unsatisfactory in as much as no specific difference could be observed.

(c) Ninhydrin-Schiff method

The muscular coat, muscularis mucosae and the intermuscular connective tissue were stained pinkish red in the sections from the normal as well as the infected duodenum. However, in the infected duodenum there was a stronger staining reaction in the region of the epithelial lining containing the goblet cells and the crypts of Lieberkuhn. Besides, bright pink stained masses were also observed in the lumen along with the pink stained parasites. The intestinal caeca of the parasite gave a strong reaction. The deamination completely suppressed the Ninhydrin-Schiff reaction in all the tissues.

(d) The DMAB-nitrite method for tryptophan

Light blue staining reaction was observed in the different layers of the normal as well as the infected duodenum. However, the duodenal glands and the intestinal caeca of the parasite were strongly positive for this test.
(e) The coupled tetrazonium reaction

The muscular layer, intermuscular connective tissue, muscularis mucosae, and the glandular epithelial cells of villi and the submucosal glands took various shades of reddish brown in the normal and infected sections of the duodenum but the muscular coat of the infected duodenum stained bright red and the parasites appeared as brownish red bodies.

III. Lipids

For the demonstration of lipids in the tissues of duodenum and ductus choledochus, the Sudan black B staining and Acetone-Sudan black methods were employed but they did not give satisfactory results.

IV. Nucleic acids

(a) Feulgen reaction

The Feulgen reaction revealed only the nuclei as red bodies in a clear matrix.

(b) Galloccyanin-chromalum method

No conclusions could be derived with this staining reaction as there was diffuse staining of all the tissue components.

(c) Methyl-green-Pyronin Y method for DNA and RNA

The cytoplasm of the epithelial cells lining the
villi and the glands in the duodenum and the ductus choledochus were pyroninophilic but this reaction appeared to be comparatively weak in the sections of the same region obtained from the infected animals.

V. Enzymes

(a) The calcium-cobalt method for alkaline phosphatase

A positive reaction for the alkaline phosphatase activity was observed in the epithelial lining of the villi and the glands of Lieberkühn of the normal and the infected duodenum. In addition, a very strong alkaline phosphatase activity was seen in the intestinal caeca of the parasite. The epithelial lining of the mucosa of the normal and the infected ductus choledochus gave a faint reaction for the alkaline phosphatase activity. However, a stronger reaction was observed in the intestinal caeca of the parasites in the infected ductus choledochus.

(b) Modified lead nitrate method for acid phosphatase (after Takeuchi and Tanoue)

No acid phosphatase activity was observed in the normal and the infected portions of the duodenum and the ductus choledochus. The parasites also gave negative results.
VI. Connective tissue

(a) Mallory's P.T.A.H. method

The muscularis mucosae and the intermuscular connective tissue of the duodenum gave a strong positive reaction for collagen while the muscular layer took light blue stain in both the normal and the infected tissues. The connective tissue fibres of the hypertrophied glandular epithelium in the section of the infected duodenum were stained light reddish brown and the parasites took light to deep blue colour. The sections of the ductus choledochus did not show any difference in the normal and the infected tissues.

(b) Eosin-Gram-Weigert method

This staining routine gave unsatisfactory results which could not be properly interpreted.

(c) Van Gieson method

A positive reaction for the presence of collagen was observed in the muscularis mucosae and the intermuscular connective tissue of the infected duodenum. The sections of the infected ductus choledochus showed a stronger reaction for collagen in the muscular layer and the papillomatous projections of the mucosa.

(d) Masson's trichrome stain

The sections of the infected tissue of the ductus
choledochus gave strong staining reaction for collagen as most of the papillomatous projections contained deeply stained collagen fibres.

VII. Amyloid

(a) **Dahlia method**

The normal and the infected tissues of the duodenum and the ductus choledochus did not show the presence of amyloid as all the structures were stained blue.

(b) **Modified Congo red method**

The test did not give a positive reaction for the presence of amyloid in the sections of the duodenum and the ductus choledochus.

VIII. Calcium

(a) **Von Kossa method**

The test did not give positive reaction for the presence of calcium deposits in the lesions of the duodenum and the ductus choledochus.

IX. Pigments

(a) **Gmelin reaction for bilirubin and haematoidin**

Both the normal and the infected tissues of the duodenum and the ductus choledochus gave negative reaction.

(b) **Iodine method for bile pigments**

The test did not give positive result for the
presence of bile pigments in the sections of the duodenum and the ductus choledochus.

(c) Ferric Iron method for bilirubin

A staining reaction for the presence of bilirubin was not seen in the sections of the duodenum and the ductus choledochus.

In the present study, a systematic survey on the frequency of occurrence of O. procyonis in buffalo, sheep and goat was carried out. Since cattle were not slaughtered in the area under survey, observations on them could not be carried out. A total of 1748 buffaloes were examined for about 49 months, the incidence of infection being 78.76 to 92.06 per cent and the infection was observed throughout the year. Of the 337 sheep examined during this period the infection with this parasite ranged from 2.08 to 9.52 per cent, while in the case of goats, out of 77 animals, the range of infection was 0 to 4.75 per cent, the infection being observed only in the month of
DISCUSSION

(1) Incidence of *G. explanatum* in vertebrate hosts

*G. explanatum* (Creplin, 1847) Nasmak, 1937 has been reported from *Bos taurus indicus* and *Bubalis bubalis* as a common amphistome parasite from India, Burma, Ceylon, Indo-China, Philippines and Celebes Islands (Nasmak, 1937; Dawes, 1946; Willmott, 1950; Crusz, 1952; Yamaguti, 1954; Kulasiri and Seneviratne, 1956; Singh, 1958; Mukherjee, 1960 and Sen Gupta, 1966).

In the present study, a systematic survey on the frequency of occurrence of *G. explanatum* in buffalo, sheep and goat was carried out. Since cattle were not slaughtered in the area under survey, observations on these could not be carried out. A total of 1748 buffaloes were examined for about 19 months, the incidence of infection being 73.78 to 92.06 per cent and the infection was observed throughout the year. Of the 337 sheep examined during this period the infection with this parasite ranged from 2.08 to 9.52 per cent, while in the case of goats, out of 77 animals, the range of infection was 0 to 4.76 per cent, the infection being observed only in the month of March, 1967 (Table I).

A very high incidence of infection was encountered in buffalo which could be due to several reasons. Apparently
there is no immunity developed following previous infections and therefore a massive infection can be built up over a period of time. Also because the adult parasites are well tolerated by the host, a cumulative infection can be built up without distressing the host or the individual animal reacting strongly to the infection. It follows that the rate of incidence would naturally be higher in the older animals than in the younger animals. In India, all the buffaloes that are slaughtered are old animals and therefore the present survey has shown such a high incidence of infection.

It has been said for good reasons that the nature of parasitism in a host is reflected in its habits, specially where food is concerned. The buffalo being a water-loving animal, as a rule feeds on the aquatic weeds or those that are growing in swampy areas or on the banks of the rivers and the ponds. As a study of the life-history of *G. explana-tum* has shown, like the other amphistomes and *F. gigantica*, the metacercariae are found in large numbers attached to the aquatic weeds at water level. And this is exactly what forms a major part of the food of buffalo. In consequence, a buffalo is more likely to pick up this infection than other animals like cattle, goat or sheep. In fact, of all these animals it is only buffalo that will actually enter...
a sheet of water to feed on the aquatic vegetation.

In the present survey besides *G. explanatum*, concomitant infections with *F. gigantica* and hydatid cysts were encountered frequently in a number of livers examined. Thapar (1956) while surveying U.P., Bihar, Bengal (including East Bengal), Assam and Orissa for helminth parasites reported that of these, amphistomes were abundantly found in buffalo, cattle, sheep and goat and *G. explanatum* was encountered in all the four ruminants. But he never found the parasites in the bile-duct of the liver and recovered the specimens most commonly either from rumen or duodenum. Varma (1957) reported that 57.1 per cent of the buffaloes examined were infected with *G. explanatum* and stated that this parasite was not found in any other host. But in the present studies, though the buffaloes have been observed to be more frequently infected, the sheep and goats have also been observed to act as definitive hosts, though the incidence of infection in these animals was much less.

In the present survey the adult parasites were recovered from the ductus choledochus, ductus hepatocysticus and occasionally from the gall-bladder and rumen of buffaloes and ductus choledochus communis and ductus hepatocysticus of sheep and goats but never from the liver parenchyma of buffaloes even when the intensity of infection
was high. Willmott (1950) reported that her specimens of *G. bathycotyle* came from the rumen of Zebu in Ceylon. Crusz (1952) in his extensive survey found no specimens of *Gigantocotyle* in any site other than the liver and he did not mention whether they were present in the gall-bladder. Nasmak (1937) pointed out that *G. explanatum* had not been found in organs other than the liver, bile-ducts and the gall-bladder except in one collection which came from the duodenum. He did not comment on the site of infection of *G. bathycotyle* although in some of the collections which he examined the stomach had been cited. He mentioned, however, that if any location outside the liver was possible it would be the duodenum and not the stomach. However, Kulasiri and Seneviratne (1956) reported the presence of these parasites in the liver substance, portal veins and the bile-ducts though the parasites were not mature. Further, they also reported the absence of these parasites from the gall-bladder of buffalo, although in the present study the parasites have been occasionally found in this organ. It is apparent that many identifications of the parasite, specially in the older papers, are incorrect as they refer to species other than *G. explanatum*.
(2) Incidence of natural infection of 
_G. explanatum_ in snail

Sewell (1922) described _Cercaria indicae XXVI_ from 
_Indoplanorbis exustus_, which resembles the present cercaria 
which however develops only in _G. convexiusculus_ (Hutton). 
Chatterjee (1931), however, reported that _C. indicae XXVI_ 
Sewell, 1922 would develop into some species of _Param-
phistomum_. Later, Rao and Ayyar (1932) proved through 
their transmission experiments that _C. indicae XXVI_ was 
the cercaria of _P. cervi_. The present cercaria from _G. 
convexiusculus_ has proved to be the cercaria of _G. ex-
planatum_. It shows no difference in morphology from 
the cercaria of _G. explanatum_ described by Singh (1958) 
and _Cercaria gyraulusi_ Peter and Srivastava, 1960 from 
_G. convexiusculus_.

In the present studies a systematic survey on 
the intensity of _G. explanatum_ infection in _G. con-
 vexiusculus_ was carried out. A total of 7081 snails 
was examined over a period of about 19 months, the 
incidence of infection being 0.81 per cent. The infection 
was observed during rainy season and early winter months 
(table II). A very high incidence of infection was en-
countered during August, September and October which could 
be due to seasonal variations. The drying up of faeces 
deposited on the land due to adverse climatic conditions
and reduction in the available aquatic areas during summer and winter months, are probably the factors responsible for the increased mortality of eggs and low incidence of infection in the intermediate host. On the other hand, once the rains set in, not only the area under water increases providing suitable breeding grounds for the snail but the temperature of water helps in better development of the eggs of the parasites. In short, the ideal conditions are created for the propagation of the parasite.

The *G. explanatum* infection in *G. convexiusculus* which is the larval stage of *Gastrothylax crumenifer*, was encountered mainly in the rainy season. During this period *Cercaria chungathi* Peter and Srivastava, 1960, was also found infecting *G. convexiusculus*. There was no case of any snail being infected by both the amphistomes simultaneously. Some other cercariae belonging to pleurolophocerca, diplocotylea, echinostome, xiphidiocercous and furcocercous cercariae were encountered frequently in summer months in a number of snails examined, at which time infection with cercaria of *G. explanatum* and *Cercaria chungathi* was not at all present. Mukherjee (1960) also reported the above types of cercariae from *G. convexiusculus*. 
(3) Life-history study

As early as 1892, Sonsino described an amphistome cercaria which is now known as Cercaria pigmentata. Looss (1900) described the same cercaria and showed that it was the larval stage of Paramphistomum cervi. Later, Sewell (1922) reported Cercaria indicae XXVI from Indoplanorbis exustus, Cercaria indicae XXIX from Lymnaea acuminata, Lymnaea succinea and Gyraulus euphraticus and Cercaria indicae XXXII from Amnicola travancorica. Singh (1958) stated that Cercariae indicae XXVI Sewell, 1922 resembles the cercaria of G. explanatum. The present studies have confirmed that G. convexiusculus (Hutton) is the only known intermediate host of G. explanatum. Also, the cercariae which were obtained from G. convexiusculus experimentally infected with the miracidia of G. explanatum were morphologically identical with Cercaria gyraulusi Peter and Srivastava, 1960. Srivastava (1944) described the life history of G. explanatum from India and he incriminated Indoplanorbis exustus as its intermediate host. Later, Singh (1958) failed to infect I. exustus with the miracidia of G. explanatum but succeeded in recovering cercariae from G. convexiusculus. Mukherjee (1960) also agreed with Singh (1958) that G. convexiusculus is the intermediate host of G. explanatum. It, therefore, appears almost certain that the snails that were
identified as young specimens of *I. exustus* by Srivastava, were actually the adult specimens of *G. convexiusculus*.

As regards the biology of *G. convexiusculus* under laboratory conditions, the data on the egg-laying capacity of snails and the subsequent development may not be strictly comparable with what may happen under field conditions but they give valuable information.

During the present experimental infections, a large number of laboratory-bred *G. convexiusculus* of different age groups were infected individually as well as *en masse* with the miracidia of *G. explanatum*. It was observed that the snails of the various age groups readily became infected and started shedding the cercariae, there being no age resistance for the snails of the various ages were equally susceptible. However, the extent of establishment of infection and the mortality afterwards among the snails varied depending on whether the snails were infected individually or collectively. It was observed that in many cases there was a heavy mortality among the snails which were exposed to mass infection compared to those that were exposed to infection individually. In the latter case, 2-3 miracidia per snail were used and this ratio was generally maintained in the mass infection also.

The difference in the rate of mortality can be explained only in one way. It is almost certain that in any
population of snails some of the snails would be refractory due to one reason or the other, some of these being rather ill-defined and not well understood. In those cases, where the snails are infected singly, a refractory snail would not be attacked by the miracidia, or even if these do gain an entrance they are quickly contained. At the same time, the susceptible snail would be infected by a maximum of 2 to 3 miracidia. In case of mass infection, this condition works to the disadvantage of the susceptible snails. In a group, many of the snails which were refractory would not attract the miracidia and therefore a larger number of miracidia, more than its share of 2 to 3 miracidia would fall to the share of a susceptible snail and eventually penetrate it. This naturally leads to a heavy mortality because of the extensive destruction of the glands of the snail by the developing larval stages of the trematode. This explanation naturally presupposes that the miracidia are actually attracted to a susceptible snail only and further than, even if the miracidia make a contact with a refractory snail, they would not attack it or penetrate it and thus would be available to the susceptible snails. It is conceded that the evidence for this is not wholly satisfactory in spite of the numerous reports to the effect that miracidia are actually attracted towards a
susceptible snail.

This, of course, does not always happen for in one batch the mortality among the individually infected snails was noticeably higher than in the group which was exposed en masse. In the below 4-weeks old group of snails the mortality among individually infected ones was 75.5 per cent as against 78.01 per cent in the mass infection and the establishment of infection was more in the case of individually infected ones being 8.88 per cent, while in the group of mass infection it was 2.83 per cent.

In the snails whose age varied between 4 and 12 weeks the incidence of infection was variable but in general, lower than in the snails which were less than 4 weeks old. This would have ordinarily been interpreted as an indication of the age-resistance in the snails but for the figures obtained for the snails which were 12-13 weeks old. In this group, the incidence of infection in the individually infected snails was as high as 14.72 per cent and in the group of snails exposed to mass infection it was still higher, being 17 per cent. In fact, the last figure happens to be the highest obtained experimentally of all the groups.

When the field collected and mature laboratory-bred snails were infected, the percentage of establishment of infection varied from 1.21 to 4.58 and the average mortality
was 70.62 per cent. There was no significant difference found in the mortality and the infection rates of the laboratory-bred and the field collected specimens. Evidently, there is no indication of any age resistance being there and the variations observed in the various age-groups must have been caused by other undetermined factors.

Singh (1958) observed that snails (G. convexiusculus) of all ages could readily be infected with the miracidia of G. explanatum. Tandon (1957) observed that it was possible to infect even the youngest G. convexiusculus which was 0.5-1 mm in diameter with the miracidia of G. crumenifer, even though some died a day or two after being infected and only 15-20 days old and older snails were able to bear infection. In addition, he noticed that very heavy infection killed even the largest snail after a few days of its being infected. Bennett (1936) tried very young, medium-sized and old snails of Fossaria parva and found that the miracidia of Cotylophoron ctylophorum (=P. microbothrioides) could infect snails of all ages equally well. He also commented that since large number of miracidia were used, most of the snails died before cercariae were shed.

Under laboratory conditions during winter months the prepatent period of G. explanatum infection in the
intermediate host varied from 36 to 40 days, in summer months from 34 to 38 days while in the rainy season it ranged from 35 to 37 days. Singh (1958) reported that the cercariae were shed 25 to 64 days after the snails were exposed to miracidia. However, Mukherjee (1960) stated that under laboratory conditions during August to October (rainy season) the mature cercariae of G. explanatum emerge from the infected snail from the 29th to 34th day of infection. Further, he noted that the cercariae during the winter months take longer to emerge. This is, of course, to be expected for other factors remaining the same, an increase in temperature within certain limits, will reduce the time taken for the intramolluscan development of the parasite. Dinnik and Dinnik (1954) observed that in case of P. microbothrium, the mature cercariae began to emerge from the snails on the 43rd to 46th day after exposure to miracidia. Bennett (1936) reported that the time required for the development of cercaria of C. cotylophorum (=P. microbothrioides) in molluscan host varied directly with the temperature and the infested snails kept under natural temperature conditions shed cercariae in 30 to 91 days.

In the present studies buffalo-calves have been experimentally infected for the first time as a definitive host for G. explanatum. One buffalo calf was infected with
2,000 metacercariae and the eggs of the parasite appeared in the faeces, for the first time on the 89th day following infection. In this animal as many as 237 metacercariae (11.85 per cent) had succeeded in establishing themselves as mature parasites, mostly present in the ductus choledochus, though a few were also present in the ductus hepatocysticus and the gall bladder. Singh (1958) had infected a goat-kid with the metacercariae of *G. explanatum* and had reported that the parasites reached maturity in less than 16 weeks after the metacercariae enter the host. According to Mukherjee (1960) *G. explanatum* takes 151-161 days (about 21-23 weeks) to reach maturity in the goat kid, which was comparatively much higher. These figures are higher than in the present study and this may be due to individual variation. Also, it is possible that a small number of eggs appearing in the faeces during early patent period were missed. This is quite likely unless a procedure was employed which concentrated the eggs.

Jain (1968) concluded that the specimens of *G. bathycotyle* (which is accepted here as a synonym of *G. explanatum*) became mature in 90 to 103 days in kids following infection with the metacercariae. However, among other amphistomes, Le Roux (1930) reported that *C. cotylophorum* reached maturity in 16 weeks. Rao and
Ayyar (1932) and Vaidyanathan (1941) recovered adults of *Fischederius elongatus* in 8-9 weeks time. Brumpt (1936) indicated that *P. cervi* reached maturity in 76 days. Peter and Mudaliar (1948) reported that *Gastrodiscus secoundus* requires 98 days to reach maturity, while a maximum of 90 days was required by the specimens of *Pseudodiscus collinsi* to attain maturity (Peter and Srivastava, 1960). Peter and Srivastava (1961) reported the prepatent period of *Gastrothylax crumenifer* to be 118 days in kids and 114 days in buffalocalf. Dinnik and Dinnik, 1962 found the eggs of *P. microbothrium* in the faeces of calves for the first time 87 to 107 days postinfection. However, Horak (1967) reported for the same parasite 56 days in cattle, 69 days in goat and 93 days in sheep. It is obvious that the time required for attaining maturity varies considerably depending upon the species of amphistome as also the host.

The present studies on *G. explanatum* have shown that the excystment of the metacercariae occurs in the small intestine from about 30 cm to about 6 metres from the pylorus and takes between 6 and 12 hours after the cysts had been fed to goat kids. In this connection, it has to be borne in mind that the rate of passage of food stuff in ruminants is broadly governed by three factors,
namely the species of animal, age of animal (i.e. the development of rumen) and the nature of food stuff including the size of the food particle. It, therefore, follows that if any one of these three factors is changed, the time and place of the excystment will vary with it. Bennett (1936) reported that the metacercariae of *G. cotylophorum* excyst in the upper part of the duodenum of the calf but some may be carried as far posterior as about 2 metres. Dawes (1961b) observed the excystment of the metacercariae of *F. hepatica* in the intestine of mouse, within 2–3 hours after cysts had been fed.

In the present studies goat-kids as well as buffalo-calves were used to determine the behaviour of metacercariae of *G. explanatum* when they are in the process of migrating from the intestine to the bile-ducts of liver which is the seat of predilection. It is obvious from the data presented in table VII, and from the excystment experiment on goat-kids, that infection of the final host takes place as a result of the metacercariae freeing themselves from the cysts in the small intestine, after which they migrate till they reach the opening of the ductus choledochus communis and gain entrance into it. By using as many as 14 goat kids, it was possible to follow the path of migration.
The excystment of metacercariae occurs in the intestine between 6 and 12 hours after the cysts have been fed to kids. The excysted parasites were, however, recovered as far away as about 10 metres from the pylorus between 24 and 72 hours postinfection. At seven days postinfection the maximum number (51.8 per cent) of the parasites were found in the middle third while the posterior third of the intestine had about 37.3 per cent of the parasites. Some parasites were found in the anterior part of the large intestine and also in the anterior third of the small intestine.

Subsequently, the migrating stages of the parasites reach the ductus hepatocysticus and the bile-ducts of the liver without the parasites penetrating the tissues of the liver. This migratory route of G. explanatum has been followed step by step, experimentally for the first time in goat kids. That the parasites do not adopt any alternative route has been confirmed by the fact that neither any developing stages of the parasite were ever collected from the peritoneal cavity of the experimental animals nor any parasite was ever found attached to the surface of the intact abdominal visceral organs. Some other trematodes are known to migrate through the blood vessels but in the present case no parasites were ever encountered inside the blood vessels of the small intestine.
Similarly the data on the route of migration of *G. explanatum* in the buffalo-calves (table VIII) (which is considered to be the most suitable definitive host), have shown that the parasites migrate from the intestine to the seat of predilection i.e., the bile-duct through the opening of ductus choledochus. The other two possible routes i.e. by way of portal vein and through the abdominal cavity were not used because in all the animals examined, there was no evidence of the presence of parasites either in the peritoneum, or in any of the blood vessels of the various organs as shown by an examination of the microtome sections, though a few parasites were recovered from the abomasum.

Besides the ductus choledochus and ductus hepatocysticus which are the usual sites for the final localization of parasites in buffalo-calves, developing stages were also encountered, though in less numbers, from the ductus cysticus, gall-bladder and bile-ducts of the liver. In goat-kids, in addition to the above locations, a few parasites were also encountered in the deeper layers of ductus choledochus communis, pancreatic duct and the pancreas. The only reasonable explanation for this somewhat erratic behaviour on the part of a few parasites in goat, appears to be because of the goat being not a very suitable definitive host. It is a well known phenomenon
in parasitism that the parasites behave abnormally in an abnormal host, the more dissimilar the host to the normal host, the more abnormal the behaviour. This conclusion drawn is supported by the fact that in goat-kids the recovery of immature stages of *G. explanatum* was lower and ranged from 3.86 to 8.3 per cent as against the buffalo-calves in which it was 4.05 to 34.5 per cent. Further, in sheep and goat the bile-duct joins the pancreatic ducts to form a common bile duct (ductus choledochus communis), which opens into the duodenum while in the case of cattle and buffalo both the pancreatic duct and the bile duct (ductus choledochus) open independently into the intestine.

Earlier Kulasiri and Seneviratne (1956), while studying the histopathology of livers of buffalo infected with *G. explanatum*, said that the histopathological picture of the material suggested a transperitoneal infection of the liver and subsequently of the bile-ducts as occurs in *Fasciola hepatica*. They recorded sexually mature adult parasites in the cystic spaces or cylindrical dilatations of numerous bile-ducts of the liver but the gall-bladder did not contain any parasite, even though the dilated cystic duct was packed with parasites right up to the neck of the gall-bladder. Out of the many sections examined by them only on two occasions parasites were encountered
within the veins. In one case the parasite was enclosed in granulation tissue while in the other, the parasite was calcified, thus indicating that they did not commonly travel in veins.

Singh and Kuppuswamy (1969) while studying the histopathology of the caprine liver infested with *G. explanatum*, supported the transperitoneal route of migration but they did not record the presence of pre-adult stage of parasite in the liver parenchyma or the portal vein as reported by Kulasiri and Seneviratne (loc.cit.). However, Singh and Kuppuswamy (loc. cit.) recorded one parasite from the gall-bladder which they considered had possibly migrated into it after the death of the host when the sphincter muscles at the mouth of the gall-bladder had relaxed.

The route of migration of other amphistomes in ruminants differs significantly from that of *G. explanatum*, as in the former, the young stages migrate forward in the intestine till they reach the rumen—the most common habitat of the majority of the amphistomes (LeRoux, 1930; Srivastava, 1938; Dinnik and Dinnik, 1962; and Horak, 1967). However, in *Fasciola* on the whole, three possible routes of migration have been adopted. These are: (i) through the choledochus duct (Leuckart, 1881), (ii) through the blood stream (Lutz, 1892, 1893; Railliet, Moussu and
Henry, 1913; Compes, 1923; Noller, 1925; Marek, 1927; Noller and Schmid, 1928; Curtze, 1932; Dizier, 1933; Bugge, 1935; Sogoyan, 1955; and Enigk and Duwel, 1959), and (iii) through the peritoneal cavity (Sinitsin, 1914; Shirai, 1927; Susuki, 1931; Shaw, 1932; Vogel, 1934; Schumacher, 1938; 1956; Krull and Jackson, 1943; Ono and Isoda, 1952; Dawes, 1961a, 1961b, 1963a; and Srivastava, 1962). But it is now generally accepted that the young flukes migrate across the abdominal cavity. The present studies have shown that in this respect the young *G. explanatum* behave differently from the immature specimens of *Fasciola hepatica* and *F. gigantica*.

Among the other small flukes of liver, the most commonly accepted route of migration is the same as in *G. explanatum* in the present study, i.e. through the choledochus duct as in *Clonorchis sinensis* (Mukoyama, 1921; Faust and Khaw, 1927; and Hsu and Wang, 1938), *Opisthorchis felineus* (Vogel, 1934) and *Dicrocoelium dendriticum* (Krull, 1958; and Svadzhyan, 1959).

In the present studies where goats were used as a definitive host for *G. explanatum* the parasites were, however, encountered in the pancreatic duct and the pancreas. It is quite obvious that parasites had migrated to these locations because of the positive chemotaxis to pancreatic juice which is, however, less marked than in
the case of bile as described by Yoshida (1931) and Iwata (1937) for *Clonorchis sinensis*.

The immature parasites recovered from experimentally infected animals at varying intervals postinfection revealed lesser rate of growth of parasites between six and 21 days and accelerated growth between 21 and 49 days. Thus the 21 days old parasites measured on an average 1.94 mm only whereas the 49 days old parasites measured 5.24 mm. At 19 days postinfection the parasites recovered from the gall-bladder were smaller in size than those recovered from ductus choledochus communis and ductus hepatocysticus. Perhaps the gall-bladder does not provide ideal conditions for the parasites.

The genital organs in the six days old parasite were not clearly seen due to intense distribution of the pigments, even though the rudiment of ovary could be identified. In 12 days old parasite the pigmentation was scanty and the genitalia comprising rudiments of ovary, testes and uterus were seen. However, by 19th day the vasa efferentia were developed. By 28th day the parasites showed the developing vitelline ducts and the vas deferens. The 43 days old parasites showed the vas deferens and the developing genital pore, which were still better developed by the 49th day postinfection.
Deiana, Lei and Arru (1962) described the morphology of the immature stages of *Paramphistomum cervi* recovered from goats in an outbreak. They noticed that in the parasites which were still present in the duodenum of the host, the genital organs were in a very early stage of development. They observed the two testes, ovary and the genital pore present in the form of small areas of aggregates of cells, and the uterus running as a streak of granules from the ovary to the genital pore. Whenever a natural case of amphistomiasis is investigated, usually a large number of parasites evidently belonging to many distinct species of amphistomes are recovered. It is rather difficult to identify these specimens with any degree of certainty and to assign them to the various species. Accordingly a number of amphistome species have been incriminated as the cause of amphistomiasis. The present study would help in the identification of the young specimens of *G. explanatum*, and thus help in telling us as to how far this parasite is responsible for causing amphistomiasis.

In all the cases investigated in the present study, the parasites recovered from the intestine measured 0.71 to 1.06 mm in length. The specimens which were larger had already migrated into the bile-duct. Since the parasites which have been found responsible for producing the
acute stage of amphistomiasis are considerably larger than these parasites, *G. explanatum* is obviously not responsible for producing the disease. At least it can be said that even though a few immature specimens of *G. explanatum* may be present along with others in a case of amphistomiasis, they could not be contributing much to the pathology and pathogenicity.

The number of individuals gaining entry into the host in the present study may have some bearing on their migration behaviour. It has been shown at least in one species, *Paramphistomum microbothrium* (Horak and Clark, 1963) that as many as 172,000 ± 3,000 metacercariae of *P. microbothrium* have to be given to a host to produce acute amphistomiasis. In the present study a much smaller number of metacercariae was used and therefore it can at best be called a logical presumption that an increase in the number of parasites will not materially alter their behaviour in the host.

Moreover, the lesser ratio of the acetabulum to the body length due to the exceptionally large acetabulum helps in distinguishing specimens of *G. explanatum* from those of other immature amphistomes. This ratio in the present material obtained from the intestine of the host during the first two weeks postinfection ranged between 1:3.21-3.6 and in the specimens recovered from the bile-
duct it ranged between 1:2.93-3.35. These observations do not agree with those of Patnaik (1964) who obtained the immature forms of *Paramphistomum explanatum* from the jejunal and duodenal mucosa of naturally infected buffalo-calves. His specimens measured 8 mm in length and 3.8 mm in maximum width and the acetabulum was 3.4 mm in diameter and its ratio to the body length was 1:2.3. This was probably due to the fact that in a massive infestation the migration of the parasites in the intestine would be tardy for some individuals and so fairly grown up parasites were recovered from the duodenum and jejunum by Patnaik (1964).

Kulasiri and Seneviratne (1956) found a specimen of pre-adult *G. explanatum* inside a thrombosed vessel of liver. In section, the fluke measured 0.49 mm in length, and the breadth "above" the acetabulum was 0.16 mm. The acetabulum was 0.175 mm in diameter and thus the ratio between its diameter and body length was 1:2.8. Almost similar ratios have been obtained in the specimens recovered from the bile-duct in the present studies.

To determine the susceptibility of rabbits, white rats and guinea-pigs as definitive hosts, they were experimentally infected *per os* with the metacercariae of *G. explanatum*. The rabbits started passing out the intact
metacercarial cysts with the faeces as early as six hours postinfection. They were killed after varying intervals of time, starting 6 hours and up to 28 days postinfection. In none of the rabbits any immature stage was found either in the intestine or in the liver and there was no evidence that the metacercariae had even excysted.

Like rabbits, white rats and guinea-pigs were also completely refractory to the metacercariae of *G. explanatum*, as their faeces revealed the presence of intact cysts. The gross and histological examinations of the intestine and liver did not produce any evidence regarding the excystment of the metacercariae. However, Abdel Ghani (1960) successfully infected one guinea-pig with the metacercariae of *Paramphistomum cervi* and subsequently recovered the immature and mature parasites. The author could not find a detailed published account of this article in original for comparison.

In addition to *per os* method, the excystment of the metacercariae was also studied using the "Sausage" method. The present investigations indicated that when the viable metacercariae of *G. explanatum* were inoculated into the first two inches of the small intestine of mouse, the cysts did not excyst within 30-60 minutes. Similarly, the excystment did not occur in the "Sausages" of the intestine even up to 300 minutes postinfection and the intact cysts
were found during the gross and histological examinations of the "sausages". It is, therefore, concluded that the conditions required for the excystment of the metacercariae of *G. explanatum* are wanting in the intestine of rabbit, mouse and guinea-pig.

(4) **Pathological study**

The present studies on gross pathology of the intestine infested with the migrating stages of *G. explanatum* revealed no significant lesions in the experimentally infected goats. However, a few detached parasites were present in the intestinal contents mostly in the anterior and middle parts of the small intestine, occasionally mixed with thin, dirty mucus. Near the opening of the ductus choledochus communis in the duodenum a large number of immature stages were found associated with slight congestion of that part.

The present studies on buffalo-calves showed lesions which were more or less similar to those found in goat, except that the lesions were more severe in the former. Thus the intestinal mucosa showed characteristic oedematous thickening with corrugated appearance and the migrating stages were either free in the lumen or were attached to the mucosa, mixed with dirty mucus. The pin-point haemorrhages were rare.
Patnaik (1964) held that P. *explanatum* when present in large numbers in buffalo-calves could be highly pathogenic as its young stages might invade the deeper layers of the intestine. Since the acetabulum of these parasites are more powerful and larger than those of other amphi- stomes, a greater area of the intestinal mucosa of the host might evidently be involved due to their attachment during the course of migration. However, in the majority of amphistomiasis cases reported by the earlier workers, the gross lesions consisted of moderate to marked thickening of the infected parts of the intestine resulting in corruga- tions, especially of the duodenum. The mucosa of the small intestine was congested and haemorrhagic and it was covered by a thick catarrhal exudate giving an over all picture of acute catarrhal enteritis (Simson, 1926; LeRoux, 1930; Pande, 1935; Srivastava, 1938; Maqsood, 1944; Guilhon and Priou- zeau, 1945; Mudaliar, 1945; Iyer, 1949; Nobel, 1956; Boray, 1959; Ahluwalia, 1960; Varma, 1961; Butler and Yeoman, 1962; Horak and Clark, 1963; Horak, 1967; and Sharma Deorani and Katiyar, 1967).

The lesions observed in the present studies in goats and buffalo-calves appeared mild compared to the reported natural infections. This is explained by: (i) the much smaller number of metacercariae administered in the present cases, (ii) the infection by only one species, viz.,
G. explanatum, and (iii) the absence of any subsequent infection, or a concomitant infection, since only one dose of infection was given.

The present studies on histopathology of the duodenum showed that in an early infection among goats, immature parasites caused superficial desquamation and moderate linear hypertrophy of the mucosa accompanied with moderate proliferation of fibroblasts and infiltration with mononuclears. The histopathological findings of Sharma Deorani and Katiyar (1967) on the intestine of sheep and goats infected with immature amphistomes revealed the changes in the mucosa and submucosa according to stages of infection which were characterised in the early infection by infiltration of only large mononuclears in the mucosa and submucosa but with the advancement of the infection the eosinophils and fibroblasts appeared.

The present studies on the histopathological changes in the duodenum of buffalo-calves showed a large number of developing stages in the lumen at various stages of development along with a large amount of detritus consisting of desquamated, superficial mucosal tissue. The parasites had dragged the mucosal tissue into the acetabulum and the necrotic changes were also encountered in such regions. The deeper glandular tissue had become hyperplastic and was accompanied by excessive mucus secretion brought about
by the irritation caused by the presence of the parasites. The oral cavities and the caeca of some of the parasites lying free in the lumen showed degenerated epithelial cells. The upper and middle layers were infiltrated with large number of lymphocytes, neutrophils and fibroblasts. The Brunner's glands showed hypertrophy.

Patnaik (1964), however, observed the strangulation and necrosis of mucosa in buffalo-calves that was drawn as a plug inside the acetabulum of *P. explanatum*, followed by a severance of such portions, especially in the duodenum. The immature stages were also embedded in the submucosa and some had even reached the muscularis mucosae accompanied with lymphocytic infiltration around them. The present findings on the histopathology of the duodenum of buffalo-calves resembled closely the histopathological changes in the intestine associated with other amphistome parasites (Pande, 1935; Srivastava, 1938; Mudaliar, 1945; Nobel, 1956; Boray, 1959; Ahluwalia, 1960; Varma, 1961; Butler and Yeoman, 1962; and Horak, 1967).

On entering the ductus choledochus communis in goats, the migrating stages caused superficial desquamation of the mucosa with the proliferation of the glandular connective tissue and infiltration with mononuclears and fibroblasts. However, in the older infections the parasites had reached the acini of the mucous glands, the latter became hypertrophied,
there was increased connective tissue activity and mononuclear infiltration. In addition, these stages were encountered in the deeper layers of the ductus choledochus communis, the wall of which they penetrated and were at times also observed embedded deep in the pancreatic tissue. However, studies on the ductus choledochus of the buffalo-calves revealed no such penetration and migration into the pancreatic tissue. This difference in the behaviour of the parasites may be due to the fact that the parasites are not so well adapted to goat as they are to buffalo, and also to the anatomical differences in the bile-ducts of the two animals.

The pancreatic glands showed hyperplastic changes amidst marked increase in the interglandular and interlobular connective tissues around the area of localisation of parasite. In Clonorchis sinensis infestation in man, Hou (1955) observed some degree of connective tissue proliferation around the affected pancreatic ducts and infiltration by a few mononuclear cells. He also observed adenomatous tissue formation in the affected ducts which was not observed in the present cases. The necrosis of pancreatic tissue was absent in both these infections.

The changes encountered in early stage of infection in ductus choledochus of buffalo-calves, in the present study, consisted, in general, of superficial desquamation,
degeneration of mucosa and proliferation of the deeper layer. The lumen of the glands showed presence of coagulated mucus. Besides, many focal patches of cellular reactions were observed particularly in the area where the parasite was attached. Subsequently the duct showed extensive superficial necrosis of mucosa and many concave or protruded areas suggestive of parasite attachment. The focal areas of heavy cellular reaction in the middle as well as in the deeper mucosa were also evident. In addition, there was much linear hypertrophy of the mucous and serous glands. This eventually resulted in the total hypertrophy of the duct epithelium and the papillomatous elevations were quite prominent which were formed as a result of increased proliferation of connective tissue and hyperplasia of the glandular region. The mucous glands showed multilayered epithelium while at places the epithelial proliferative changes were so severe so as to give an impression of neoplastic changes characterised by multilayered, actively dividing cells.

The advanced stages of infections showed increased papillomatous thickening and pronounced hypertrophy of the mucous layer. However, in the older infections (144 days postinfection) the papillomatous elevations due to attachment of the parasites were very prominent and were formed mostly by the highly proliferated connective tissue.
The division of the nucleus of epithelial cells at many places showed neoplastic tendency evinced by mitotic figures and many layers of nuclei. Sharma Deorani (1965) noticed, in addition to raised papillae in the duct wall, the linear hypertrophy of the bile-duct epithelium with much cellular infiltration. Sen Gupta (1966) observed almost similar changes in the common bile-duct.

In the ductus hepatocysticus in goats, moderate proliferative and desquamatory changes were observed accompanied with mild infiltration with mononuclears. From the naturally infected goats, Singh and Kunnuswamy (1969) reported heavy infection of hepatic bile-ducts with *G. explanatum* which showed desquamation of biliary epithelium, hypertrophy of biliary wall and hyperplasia of mucous glands. The liver showed increased capsular thickening and haemorrhagic tracts containing tissue debris, erythrocytes, neutrophils and free macrophages were found. The lymphocytic and mononuclear infiltrations were also noticed in the portal areas as well as in the hepatic lobules.

In the present studies the sections of ductus hepatocysticus of infected buffalo-calves revealed more or less similar desquamatory, proliferative and inflammatory changes as seen in the early stages of infection of the ductus choledochus. The hepatic cells showed various grades of retrogressive changes with mild periportal cellular
infiltration consisting of mononuclear cells and fibroblasts and Kupffer cells were prominently displayed. However, the cirrhotic changes were not evident. The bile-ducts in the parenchyma did not show any significant change. Even though the central veins of some lobules were widened the interlobular areas generally showed fine streaks of connective tissue only. However, Kulasiri and Seneviratne (1956) reported monolobular cirrhosis of liver infested with *G. explanatum* which was due to the widespread blocking of bile-ducts by the parasites. In addition, extraordinary muscular hypertrophy and subendothelial proliferation of vessels were also encountered. They further suggested that this type of reaction might follow as a result of hepatic hypertension, either from a diffusible chemical substance or substances elaborated by the parasite, or from widespread granulomatous lesions in the portal tracts involving blood vessels. Sharma Deorani (1965) reported periportal and perilobular cirrhosis. However, Sen Gupta (1966) observed haemorrhagic tracts and monolobular cirrhosis in the parenchyma of the liver infested with *G. explanatum*. In the present study the gall-bladder mucosa showed desquamation and moderate proliferation of epithelial cells and infiltration with mononuclear cells around the area of parasitic attachment. However, Kulasiri and Seneviratne (1956) reported an over all picture of muscular hypertrophy followed by pressure atrophy.
The earlier reports on fascioliasis, clonorchiasis, opisthorchiasis and dicrocoeliasis have shown similarity in the general pathological changes to those of G. explanatum infection even though they differed in some aspects which were mostly correlated with their peculiar migrating habits and seat of predilection. Thus, in cases of fascioliasis, calcification of the bile-ducts in the liver parenchyma has been observed to be a common feature (Svitil, 1934; Finzi, 1941; Sinclair, 1949; Stemmermann, 1953; Isoda, 1954; Sugiura and Fugio, 1954; Gordon, 1955; and Lapage, 1956). The existence of migratory tracts in the liver parenchyma in cases of fascioliasis associated with immature parasites have been reported earlier (Sogoyan, 1955; Lapage, 1956; Urquhart, 1956; Greshan and Jennings, 1962; and Dawes, 1961, 1963). The neoplastic changes in the liver associated with clonorchiasis have been reported by Chin, Lei and Wang (1956), Hou (1956), Lingard, Huestis and McLean (1958) and several others. Cholangitis and pericholangitis leading to obstructions associated with fascioliasis have been reported by Bonciu et al. (1954), Lapage (1956) and Greshan and Jennings (1962). In cases of clonorchiasis and opisthorchiasis also the above lesions have been observed by several workers.

The present study has shown that none of these changes are associated with G. explanatum infection. The earlier
reports of the haemorrhagic migratory tracts and cholangitis and obstruction of the bile-ducts are attributed to the presence of concomitant infection with fascioliasis. This is quite possible since the material upon which these investigations were made, was obtained from the slaughter houses and were all cases of natural infection. During the present study also, a large number of animals was found naturally infected with both G. explanatum and F. gigantica. In fact, the latter infection is more common in certain areas.

(5) Histochemical study

The term mucopolysaccharide, introduced by Meyer (1938) includes mucin and its homologues. The highly specialised 'goblet cells' of the epithelial lining in the gastro-intestinal tract secrete this component and in a number of parasitic infections it has been reported that there is increased secretion and excessive accumulation of mucin (Sawada et al., 1956; Kuwamura, 1958; Dhar and Singh, 1963). The Brunner's glands, the epithelial lining of the ductus choledochus, and the glands of Lieberkuhn in animals infected with G. explanatum showed excessive mucin secretion and some of it even accumulated in masses in the lumen of the duodenum and the ductus choledochus.

An increased secretion of mucin has invariably been associated with irritation, mechanical or chemical, or both, due to the presence of a parasite (Dhar and Singh,
1963). According to Florey (1954) the mucus dilutes and transports the irritants during the process of removing it from the body.

In certain parasitic infections, an increased mucin production has been associated with hyperplasia of the mucin producing cells. In *G. explanatum* infection, the irritation to the mucosal lining of the duodenum and bile-ducts was apparently quite severe and hence a permanent damage and desquamation of the epithelium and hyperplasia of glandular elements was common.

The result of the histochemical analysis of the infection with *G. explanatum* indicated that there was no marked alteration in the polysaccharide, protein and lipid contents of the duodenum and bile-duct and there was very little, if any, altered activity for alkaline or acid phosphatases. In a number of other parasitic infections, however, the alkaline phosphatase is produced in larger quantities particularly at the sites where there is an intimate contact of the parasite with the host tissue (Kuwamura, 1958; Dusanic, 1959; Kumar, 1967). This is probably explained by the fact that wherever the parasite is attached, there is certain amount of necrosis followed by some regeneration or reparative action resulting in the division of cells of the host. In such cases, it is only natural that more alkaline phosphatase should be produced.
In *G. explanatum* infection, there was no indication of amyloid deposits or calcification of parasites. There was also no evidence for the deposition of bile pigments and hence obstructive jaundice was not present.

The important findings reported in this thesis are enumerated here.

1. The incidence of *G. explanatum* infection in buffalo, sheep and goat was studied and of these buffalo is the only important host in which the incidence was 84.34 per cent out of 1753 animals examined; in sheep and goat it was 1.34 and 0.70 per cent respectively.

2. The incidence of *G. explanatum* infection in the intermediate molluscan host *i.e.*, *Boreus complicatus* (button) showed seasonal variations. During a 6 months period of 19 months, 7627 snails were examined, of which 32 (i.e., 0.31 per cent) were found infected with the cercariae of *G. explanatum*.

3. The time required for the hatching of the cercariae of *G. explanatum* varied from seven to 22 days.
SUMMARY

Some aspects on the early stages of *G. explanatum* (Creplin, 1847) Nasmak, 1937 infection in the buffalo-calf and goat have been investigated. In addition, the geographical distribution of amphistomiasis, in general, has been reviewed.

The important findings reported in this thesis are enumerated here.

1. The incidence of *G. explanatum* infection in buffalo, sheep and goat was studied and of these buffalo is the only important host in which the incidence was 84.21 per cent out of 1758 animals examined; in sheep and goat it was 1.24 and 0.79 per cent respectively.

2. The incidence of *G. explanatum* infection in the intermediate molluscan host i.e., *Gyraulus convexiusculus* (Hutton) showed seasonal variations. During a continuous period of 19 months, 7081 snails were examined, of which 58 (i.e. 0.81 per cent) were found infected with the cercariae of *G. explanatum*.

3. The time required for the hatching of the miracidium of *G. explanatum* varied from seven to 22 days during the different parts of the year.

4. The biology of *G. convexiusculus* (Hutton) comprising its egg laying capacity, nature of egg masses,
development of eggs and hatching and growth of snails under laboratory conditions was studied. The eggs of snail hatched as early as 6th day at 32.2° to 37.7°C, and the newly hatched out snails began to lay eggs on 26th day, when their shell measured 4.5 to 4.8 mm in diameter.

(5) It has been confirmed that *G. convexiusculus* (Hutton) is the only known intermediate host of *G. explanatum*. In both the buffalo-calf and the goat-kid the infection was established and the trematode did not escape in the faeces. The metacercariae were fed to a buffalo-calf and the metacercariae were fed to a buffalo-calf.

(6) Infection of laboratory bred snails, both individually and en masse with the miracidia of *G. explanatum* showed that snails of all ages readily became infected. The extent of establishment of infection and the mortality afterwards among the snails varied depending on whether the snails were infected individually or collectively.

(7) Each infected snail shed one to ten cercariae per day. The minimum, maximum and the average number of cercariae shed by a snail infected by a single miracidium was 26, 107 and 60 respectively while a snail infected with many miracidia the figures respectively were 16, 57 and 41.

(8) The cercariae obtained from natural infections and morphologically identical with *Cercariae gyraulusi* Peter and Srivastava, 1960, gave rise to adult *G. explanatum* when the metacercariae were fed to a buffalo-calf.
(9) In the buffalo-calf the eggs of the parasite appeared in the faeces, for the first time on 89th day following infection and 11.85 per cent had succeeded in establishing themselves as mature parasite in the ductus choledochus and ductus hepatocysticus.

(10) The excystment of metacercariae occurs in 6 to 12 hours in the small intestine about 25 cm to 6 metres from the pylorus in goat kid.

(11) In both the buffalo-calf and the goat-kid the excysted parasites migrate till they reach the opening of the ductus choledochus communis and gain entrance into it and eventually reach the bile-ducts of the liver.

(12) The morphology of the migrating immature stages of G. explanatum recovered from goat kid and buffalo-calf, has been described for the first time. Between 21 and 43 days there was accelerated growth of parasites in buffalo calves, and between 43 and 49 days the genital organs were fairly well developed.

(13) A number of feeding experiments with rabbit, rat and guinea-pig showed that these animals were refractory to infection. The metacercariae did not excyst in the intestine "sausage".

(14) The gross lesions in the small intestine of experimentally infected goat-kid and buffalo-calf were mild and showed, in general, the characteristic oedema
and thickening of the mucosa with corrugated appearance, mixed with dirty mucus. Microscopically, the chief pathological changes consisted of widespread superficial desquamation, degeneration and necrosis of the mucosa. The deeper glandular tissue had become hyperplastic and was accompanied by excessive mucus secretion and at places was infiltrated by leucocytes and fibroblasts.

The mucosa and deeper glandular tissue of ductus choledochus was damaged by the parasites and areas of heavy cellular reactions were evident. The mucous glands showed multi-layered epithelium while at places the characteristic multilayered, actively dividing cells gave an impression of neoplastic changes. In some, the papillomatous elevations due to attachment of parasite were very prominent. The hepatic cells showed various grades of regressive changes with mild periportal cellular infiltration consisting of mononuclear cells and fibroblasts. The Kupffer cells were prominently displayed.

(15) The presence of *G. explanatum* has been reported for the first time from the pancreas of a goat-kid.

(16) The Brunner's glands, epithelial lining of the ductus choledochus and the glands of Lieberkühn in infected animals showed excessive mucin secretion and some of it even accumulated in masses in the lumen of the duodenum and the ductus choledochus.
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REFERENCES


N.B. *(1) References marked thus have not been seen in original. Either they were cited by other workers or their abstracts were seen.

(2) As far as possible the titles of journals have been abbreviated in accordance with world list of abbreviations published in *Helm. Absts.*, 35(1) : iv-Lii.


Dawes, B. 1946. The Trematoda. Cambridge Univ. Press.


________ 1963c. Some observations of *Fasciola hepatica* L. during feeding operations in hepatic parenchyma of the mouse, with notes on the nature of liver damage in this host. *Parasitology*, 53: 135-143.


Mukoyama, T. 1921. Experimental studies on the route of migration by Clonorchis sinensis in the final host, Nippon Byori Gakkai Kaishi, 11: 443-446.


Peter and Srivastava, 1955 and its relationship to Gastrothylax crumenifer (Creplin).
Parasitology, 51 : 111-115.


* Podlesni, G.V. 1959. Massive infection of young cattle with

Price, E.W. & McIntosh, A. 1944. Paramphistomes of North
American domestic ruminants. J. Parasit.,

* Priouzeau, M. 1947. La paramphistomose bovine en Vendee,

* Railliet, A., Moussu, G. & Henry, A. 1913. Recherches expérimen-
tales sur la development de la Douve

Rama Krishnan, M. 1951. An outbreak of acute amphistomiasis
among cattle in Nellore district. Indian vet.

amphistome cercariae and their adults. Indian

Roach, R.W. & Lopes, V. 1966. Mortality in adult ewes resulting
from intestinal infestation with immature
paramphistomes, complicated by severe fascioliasis.

* Roneus, O. 1963. Parasitic lesions in swine, experimentally
produced by visceral larva migrans of T. cati.

(#) Robeßts, F.H.S. 1939. The gastrointestinal helminths of cattle
in Queensland: their distribution and pathogenic


Susuki, S. 1931. Researches into the life-history of *Fasciola hepatica* and its distribution in Formosa, especially on determination of the first intermediate host and some experiments with larvae freed from their cysts artificially. Taiwan Igakki Zasshi, 30: 97-102.


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<tr>
<td>acet.</td>
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<td>m. gl.</td>
<td>Mehlis' gland</td>
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EXPLANATION TO FIGURES
Fig. 1  Section of duodenum of goat-kid showing the immature parasite. (H & E) Ca. 55x.

Fig. 2  Showing the immature parasite with the prominent acetabulum. (H & E) Ca. 11x.

Fig. 3  Section of duodenum of goat-kid at region where ductus choledochus communis opens through a papilla. (H & E) Ca. 16x.

Fig. 4  Section showing entry of immature parasite (marked by arrow) in lumen of papillar projection of ductus choledochus communis in duodenum. (H & E) Ca. 16x.
Fig. 5  Section showing parasite in papillar projection of ductus choledochus communis. (H & E) Ca. 16x.

Fig. 6  Showing parasite in ductus choledochus communis. (H & E) Ca. 55x.

Fig. 7  Section of ductus choledochus communis showing parasite with prominent musculature of oral sucker and acetabulum. (H & E) Ca. 55x.

Fig. 8  Showing parasite with a part of epithelial mass in its acetabulum and its anterior end embedded in the lamina propria of ductus choledochus communis. (H & E) Ca. 55x.
Fig. 9  Section of ductus choledochus communis showing attachment of parasite to superficial mucosa. (H & E) Ca. 55x.

Fig. 10  Showing parasite in lumen of ductus choledochus communis. (H & E) Ca. 240x.

Fig. 11  Showing parasite in serous glands of ductus choledochus communis. (H & E) Ca. 240x.

Fig. 12  Section of ductus choledochus communis showing parasite in muscular layer, (H & E) Ca. 55x.
**Fig. 13** Same section as in Fig. 12 showing musculature of acetabulum. (H & E) _Ca._ 240x.

**Fig. 14** Section of pancreas of goat-kid showing presence of immature parasite. (H & E) _Ca._ 55x.

**Fig. 15** Showing parasite and its migratory tract in pancreas. (H & E) _Ca._ 55x.

**Fig. 16** Magnified view of same parasite (in pancreas) showing well developed acetabulum containing a portion of pancreatic tissue. (H & E) _Ca._ 240x.
**Fig. 17**  Section of ductus hepatocysticus of goat-kid showing parasite in its lumen. (H & E) Ca. 240x.

**Fig. 18**  Section of duodenum of buffalo-calf showing presence of immature parasites in its lumen. (H & E) Ca. 55x.

**Fig. 19**  Showing attachment of parasite to villi of duodenum. (H & E) Ca. 55x.

**Fig. 20**  Section of duodenum of buffalo-calf showing parasite embedded in mucosa. (H & E) Ca. 55x.
Fig. 21  Showing portion of duodenum of buffalo-calf where ductus choledochus opens into it. A large number of immature parasites are present in and around opening.

Fig. 22  Showing lumen of ductus choledochus of buffalo-calf containing migrating stages of parasite.

Fig. 23  Showing parasites in lumen of ductus choledochus of buffalo-calf. (H & E) Ca. 16x.

Fig. 24  Section of ductus choledochus of buffalo-calf containing number of migrating parasites in its lumen cut at various planes. (H & E) Ca. 11x.
Fig. 25  Showing attachment of parasite to epithelial lining with a part of tissue withdrawn into its acetabulum. (H & E) Ca. 55x.

Fig. 26  Section of ductus hepatocysticus showing presence of immature stage in lumen. (H & E) Ca. 11x.

Fig. 27  Magnified view of Fig. 26. (H & E) Ca. 55x.
Fig. 28  Section of gall-bladder of buffalo-calf containing parasite. (H & E) Ca. 20x.

Fig. 29  Section of ductus hepatocysticus of buffalo-calf showing well-developed parasite filling its lumen. (H & E) Ca. 20x.

Fig. 30  Showing a part of ductus choledochus of buffalo-calf packed with mature parasites.

Fig. 31  Showing parasite in ductus choledochus of buffalo-calf.
Fig. 32 7 day old parasite from duodenum of K-31.

Fig. 33 12 day old parasite from duodenum of K-41.

Fig. 34 14 day old parasite from ductus choledochus communis of K-5.

Fig. 35 15 day old parasite from ductus choledochus communis of K-38.

Fig. 36 19 day old parasite from ductus hepatocysticus of K-42.

Fig. 37 19 day old parasite from gall-bladder of K-42.

Fig. 38 19 day old parasite from ductus choledochus communis of K-42.

Fig. 39 21 day old parasite from ductus choledochus communis of K-51.

Fig. 40 28 day old parasite from ductus choledochus communis of K-52.
Fig. 41  6 day old parasite from duodenum of BC-10.

Fig. 42  21 day old parasite from ductus choledochus of BC-40.

Fig. 43  43 day old parasite from ductus choledochus of BC-7.

Fig. 44  49 day old parasite from ductus choledochus of BC-8.

Fig. 45  144 day old parasite from ductus choledochus of BC-9.

Fig. 46  144 day old parasite from gall-bladder of BC-9.
Fig. 47  Section of duodenum of moude showing intact metacercarial cyst in lumen. (H & E) Ca. 55x.

Fig. 48  Section of ductus choledochus communis of goat-kid showing extensive epithelial desquamation. (H & E) Ca. 85x.

Fig. 49  Section of ductus choledochus communis showing parasite in acini of mucous glands, the latter showing hypertrophy and increased connective tissue activity. (H & E) Ca. 55x.

Fig. 50  Section of duodenum of buffalo-calf showing extensive desquamation of superficial mucosal tissue. (H & E) Ca. 85x.
Fig. 51  
Section of duodenum showing hyperplastic changes in glandular tissue. (H & E)  
Ca. 85x.

Fig. 52  
Section of ductus choledochus showing necrosis, degeneration, desquamation and proliferation of connective tissue in tunica propria together with infiltration of lymphocytes and fibroblasts. (H & E) Ca. 85x.

Fig. 53  
Section of ductus choledochus showing heavy cellular reaction in middle part of mucosa. (H & E) Ca. 85x.

Fig. 54  
Section of ductus choledochus showing generalised heavy cellular reaction in deeper part of mucosa and submucosa. (H & E) Ca. 85x.
Fig. 55  Magnified view of cellular reaction zone of Fig. 54. (H & E) Ca. 380x.

Fig. 56  Section of ductus choledochus showing hypertrophy of glands. (H & E) Ca. 85x.

Fig. 57  Section of ductus choledochus showing plug of mucosa detached by acetabulum. (H & E) Ca. 85x.

Fig. 58  Section of ductus choledochus showing acini of many mucous glands with multilayered epithelium. (H & E) Ca. 380x.
Fig. 59  Section of ductus choledochus showing intense cellular reaction consisting of neutrophils, lymphocytes and fibroblasts between mucous and serous areas. (H & E) Ca. 85x.

Fig. 60  Showing papillomatous elevation made by attachment of parasite in wall of ductus choledochus. (H & E) Ca. 85x.

Fig. 61  Section showing increased proliferation of interglandular connective tissue into papillomatous elevation of ductus choledochus. (H & E) Ca. 85x.

Fig. 62  Section of ductus choledochus showing increased papillomatous thickening. (H & E) Ca. 85x.
**Fig. 63** Section of ductus choledochus at advanced stage showing well developed papillomatous elevation. (H & E) Ca. 20x.

**Fig. 64** Section of liver of buffalo-calf showing various grades of retrogressive changes and prominent Kupffer cells. (H & E) Ca. 85x.

**Fig. 65** Magnified view of Fig. 64. (H & E) Ca. 240x.

**Fig. 66** Section of gall-bladder showing degeneration and desquamation of lining epithelial cells. (H & E) Ca. 85x.
Fig. 67  Section of duodenum of infected buffalo-calf showing PAS-positive reaction in glandular region. (PAS) Ca. 240x.

Fig. 68  Section of duodenum showing PAS-positive reaction in musculature of sucker and intestinal caeca of parasite. (PAS) Ca. 240x.

Fig. 69  Section of infected ductus choledochus showing strong PAS-positive reaction in mucous and serous glands. (PAS) Ca. 55x.
Fig. 70 Magnified view of Fig. 69. (PAS) Ca. 240x.

Fig. 71 Section of infected duodenum of buffalo-calf showing positive reaction for glycogen in body and sucker of parasite. (Best's carmine) Ca. 55x.

Fig. 72 Section of infected duodenum showing presence of acid mucopolysaccharides in goblet cells. (Alcian Blue). Ca. 380x.