Iranian J.Publ. Health.
1982-83, Vol. 11, No. (1-2)

HISTOPATHOLOGY OF LIVER IN CATTLE SPONTANEOUSLY INFECTED WITH FASCIOLA HEPATICA AND FASCIOLA GIGANTICA IN IRAN

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INTRODUCTION

1. Fasciola hepatica (Linnaeus, 1758)

Fasciola hepatica is the most widespread species of liver fluke and is a common parasite of the bile ducts of cattle, sheep and goats. It has also been found in many other animals including marsupials, rodents, the pig, the horse, the rabbit and primates. In view of its wide-spread dissemination throughout the world it is not surprising that this species has been the subject of most of the research on fascioliasis. Extensive reviews have been provided by Dawes & Hughes (1964), Pantelouris (1965) and Sinclair (1967).

The histopathology of the infected liver has been studied in a variety of hosts, but the most comprehensive data has been obtained from the experimental infection of laboratory animals. Descriptions of the liver lesions in the rabbit have been provided by Urquhart (1956), in the mouse by Dawes (1961) and in the rat by Thorpe (1965). Liver lesions in experimentally infected calves have been described by Ross *et al.* (1966), and Sogoyan (1955), and Ibrovic & call-palla (1959) have described the lesions in sheep. Changes in the human liver have been described by Biggart (1937). Fascioliasis is wide spread in Iran and produce a great economical losses in livestock. This study is contribution to the liver pathology caused by these parasitic agents.

2. Fasciola gigantica (Cobbold, 1856)

There seems to be general agreement that *F.* gigantica and *F. hepatica* are different species requiring different intermediate hosts for their life-cycles. *F. gigantica* occurs in the bile ducts of sheep, cattle, goats, horses and many wild mammals and is the common liver-fluke in widespread areas of Africa and Asia. In some parts of the world, the distribution of *F. gigantica* overlaps that of *F. hepatica.* for instance, in Pakistan, Kendall (1954) found *F. hepatica* predominant in the highlands and *F. gigantica* in the lowlands, with mixed infections in an intermediate zone. Sahba *et al.* (1972) reported the predominance of *F. gigantica* in the lowlands of southwest of Iran, while Rafyi and Eslami (1971) reported the predominance of *F. hepatica* in other parts of the country.

There is also considerable variation in the susceptibility of different hosts. Davtyan (1956) found *F. gigantica* to be less infective but more pathogenic than *F. hepatica* in sheep and rabbits. On the other hand, *F. gigantica* was more ineffective and less pathogenic in cattle. Boray (1963) succeeded in experimentally ineffecting guinea-pigs and mice but found albino rats to be resistant.

MATERIAL AND METHODS

Livers were collected from cattle slaughtered in different parts of Iran, i.e. North (Caspian area), Teheran and south (Khuzestan area). A total of 1500 infected liver were examined. The specimens were selected among the most severely affected livers. The gross pathology of each liver was recorded. From each of the 150 selected livers representative portion of tissue were fixed in 10% neutral formal saline. The tissues were then processed, cut of 5μm and stained with haematoxylin and eosin. Selected sections were stained with Masson's trichrome for fibrous tissue, Von Kossa's stain for calcium, PAS for mucopolysaccharides and Parl's stain for iron-positive pigmentations.

RESULTS

Gross Pathology

1. Fasciola Hepatica,

The livers were usually irregular in shape, with prominent bile

ducts visible on the visceral surface as greyish-white, firm-branching streaks. These streaks were more pronounced in the left lobe and gradually became narrow and disappeared at the periphery.

In heavily infested livers the left lobe was completely or partially reduced in size, hard in consistency, and the surface was granular and uneven. This atrophic change was apparently due to a large amount of fibrous tissue in the poral tracts, which produced pressure on the arteries resulting in ischaemia and atrophy of the left lobe. The atrophied left lobe produced compensatory hypertrophy of the other lobes, which gave the liver a round, bulky appearance. In most livers the hepatic lymph nodes were moderately enlarged and the gall bladder distended, containing a few flukes. A number of focal lesions were present, which consisted of young flukes which had apparently failed to reach the bile ducts. These had become encapsulated and had then died, producing a dirty brown, greasy mass under the liver capsule, particularly on the dorsal surface attached to the diaphragm. Some fibrin tags, indicating the penetration of young flukes through the capsule, could be traced on the liver surface.

On the cut surface there was a considerable amount of diffuse connective tissue thickening of the biliary tracts. The main bile duct wall consisted of hard fibrotic tissue which gave the appearance of a pipe-stem, with some calcification on the inner layer of the ducts (Fig. 1).

2. Fasciola gigantica

Livers affected with *F. gigantica* grossly seemed to be of a more normal consistency when compared with those affected by *F. hepatica.* The bile ducts were less prominent, less fibrotic and less thickened. On the cut surface, the secondary bile ducts were affected and more thickened than by *F. hepatica.* Diffuse scarring and haemorrhage were considerable in the deeper parts. Fibrotic scars on the surface of the liver were more noticeable (Fig. 2).

HISTOPATHOLOGY

For convenience, the changes produced in the liver by these flukes have been divided into two groups: (a) parenchymal changes caused by the migration of young flukes, and (b) changes in the bile

Thickening of the main bile ducts was present but not as much as with *F. hepatica* infestation; instead the smaller bile ducts showed much more thickening. Glandular hyperplasia of the main bile duct mucosa was also present. Calcium deposition on the surface mucosa of the ducts was rare. Hypertrophy of blood vessels was more pronounced and some showed thrombosis. Monolobular fibrosis in those parts which were not disrupted by the massive migratory tracks was pronounced and constricted the liver lobules and produced pseudolobulation.

DISCUSSION

The macroscopic liver lisions of fascioliasis described here resemble those described by many other workers. Histopathological changes of the liver were discussed in acute and chronic phases.

1. Acute phase

a) Parenchymal damage due to migratory larvae

In the present observation liver damage due to the migration of young flukes was seen to be serious and comparatively more severe in *F. gigantica* than in *F. hepatica,* showing a markedly destructive and haemorrhagic appearance. (Rahko (1969), Boray (1967) and Sinclair (1967) described the way in which migration of immature flukes produces severe haemorrhagic tracks through the liver substance.

b) Eosinophil infiltration

In the present investigation, eosinophil infiltration was one of the prominent features, particularly in the early stage and migratory phase of infestation. The function of eosinophil granulocytes is virtually unknown. It is generally assumed that eosinophils are attracted by immune complexes and will be stimulated by histamine release and are concerned in detoxification of histamine in antigen-antibody reactions (Jennings, 1970). Hottendorf & Nielson (1966) described an accumulation of eosinophils around collagen necrosis in canine mast-cell tumours, and suggested that components of necrotic collagen tissue are released histamine and, by mast-cells, attract the eosinophils infiltrate. Flagstad & Nielsen (1972), in their experi-

mental *F. hepatica* infection in calves, mentioned the accumulation of eosinophils associated with the cell damage caused by the migration of young flukes, but commented that eosinophils are few in the livers of calves with a hypoplastic thymus. They suggest that normal thymus lymphocytes are stimulated by necrotic material produced by damaged hepatocytes or collagen in liver, the thymus lymphocytes in turn stimulating the bone marrow to produce more eosinophils. Recently Hsu *et al* (1977) suggested that eosinophils seem to be closely associated with T. cells and that in the destruction of schistosomula eosinophils play an important role. In invitro studies they also showed that with immune serum, eosinophils from both normal and immunized hosts can be attracted to schistosomula and enhance their destruction but with normal serum only sensitized eosinophils from immunized hosts can play this role.

2. Chronic phase

a) Bile duct changes

In the present observation, glandular hyperplasia of bile duct walls, which produced a thick and adenomatous picture was marked. It was mostly seen in the main ducts containing many adult flukes. This condition has been reported by other workers and has also been produced experimentally in laboratory animals infested with *F. hepatica* (Urquhart, 1956; Dawes, 1963 and Thorpe, 1965). The nature of this changes has been related to many factors, particularly mechanical irritation caused by the motion of the parasites and chemical substances and toxins produced by flukes.

Chang (1964) also reported a primary neoplasia (adenocarcinoma) of the bile ducts of the liver of the cat infested with *Clonorchis sinesis* and he related the causes of the condition to the chemical stimulants of fluke pro ducts combined with mechanical irritation by flukes. Biliary proliferation and formation of numerous simple bile ductules in the fibrotic tracts was rather a characteristic picture in cattle, and has also been reported by many other workers (Morril & Shaw, 1942; pantelouris, 1965; Dow *et al.*, 1967; Nieberle & Cohrs, 1967).

Calcium deposition in the main bile ducts, which limited to *F. hepatica* infestation, was also interesting. This condition is attributed to prolonged survival and irritation by mature, spiny *F. hepatica* flukes, and has been reported by many workers (Dixon, 1964; pantelouris, 1965; Rahko, 1969; and others). There is a suggestion that, in the bile ducts of cattle, increased fibrosis and calcium deposition

usually reduce the accomodation available to the parasite, so that the life-span of flukes may be reduced to as little as 9-10 months (Ross *et al.*, 1966; Dixon, 1964).

However, in *F. gigantica* in cattle, which produces much less calcification of ducts, the parasite certainly can live much longer.

b. Vascular changes

Thrombotic lesions of hepatic veins and medial hypertrophy of arteries has been described by Dow *et al* (1967), Rahko (1969) and Flagstad & Nielsen (1972). Similar changes were observed in the present study. It has been suggested that the cause might be toxins and metabolic products liberated by the parasite, or an immunological reaction causing such a vasculitis, as cochrane (1968) reported, activation of vascular lesions by antigen-antibody reactions are characteristic of various immunological conditions.

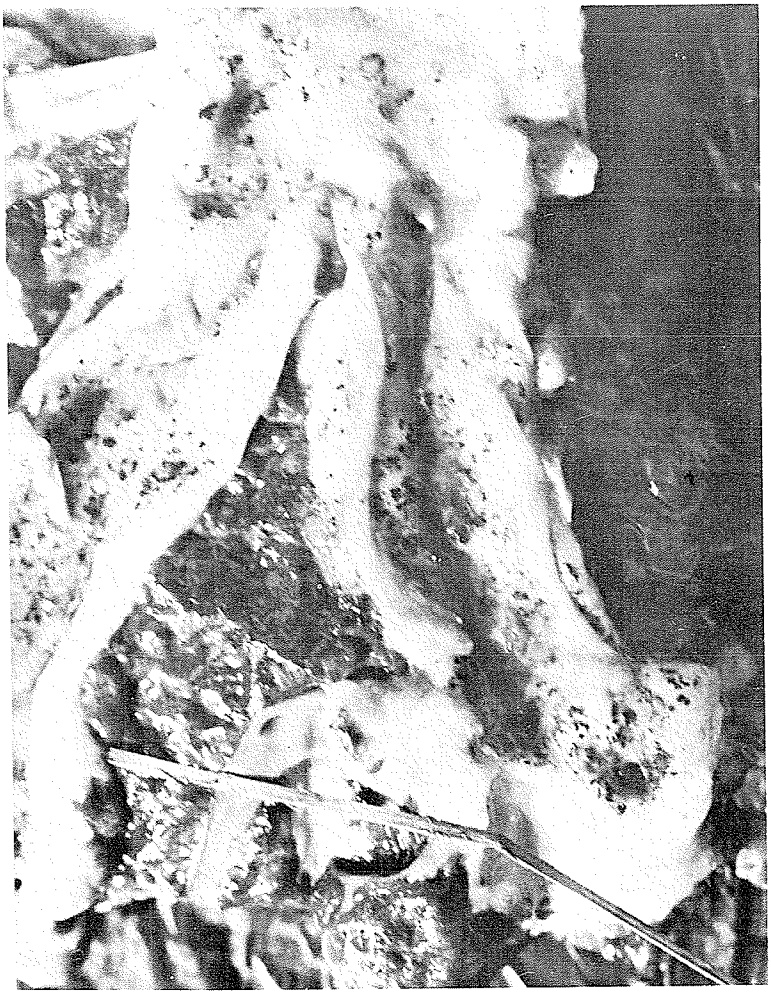
SUMMARY

A total of 1500 infected liver with *F.* hepatica and *F. gigantica* from slaughtered cattle in different parts of Iran were studied. Grossly the livers were usually irregular in shape, with prominant bile ducts on the visceral surface as greyish-white, firm-branching streaks. The atrophy of the left lobe due to fibrosis and isckemia were noticed. On the cut surface the main bile ducts wall consisted of hard fibrotic tissue with pipe-stem appearance and calcium deposition on the inner layer of the ducts in *F. hepatica* infection. In the livers infected with *F. gigantica,* grossly seemed to be of a more normal consistency with much less calcium deposition in the ducts. In histopathological changes there were evidence of different stages of parenchymal damages, varying from fresh burrow with young flukes inside to old and healed nodules due to migration of the young flukes. A large number of eosinophils, lymphocytes plasma cells, disintegration of hepatocytes and coagulation necrosis were seen. The thickening of the bile ducts wall and the intense fibrous tissue proliferation in the portal areas compressed the adjacent liver cells, causing pressure atrophy of the liver. In very chronic cases glandular hyperplasia of ducts wall which lined by columnar epithelial cells with vaculated cytoplasm were marked. In some ducts compact aggregation of fibroblasts which

gave a smooth appearance to the bile ducts wall were noticed. The monolobular fibrosis in both *F. hepatica* and *F. gigantica* infections were a typical feature.

ACKNOWLEDGEMENTS

This study was supported by the School of Public Health and Institute of Public Health Research, Tehran University. We are indebted to Dr. A. Nadim, the Dean of the School of Public Health, and also deeply appreciate the technical assistance of the staff of Khuzestan Medical Research Station and the parasitology department of the School of Public Health, Tehran University.



g. 1.

Liver, showing hard fibrotic bile duct (pipe-stem appearance) with calcium deposition on the inner layer of the duct. *F. hepatica* in cattle.

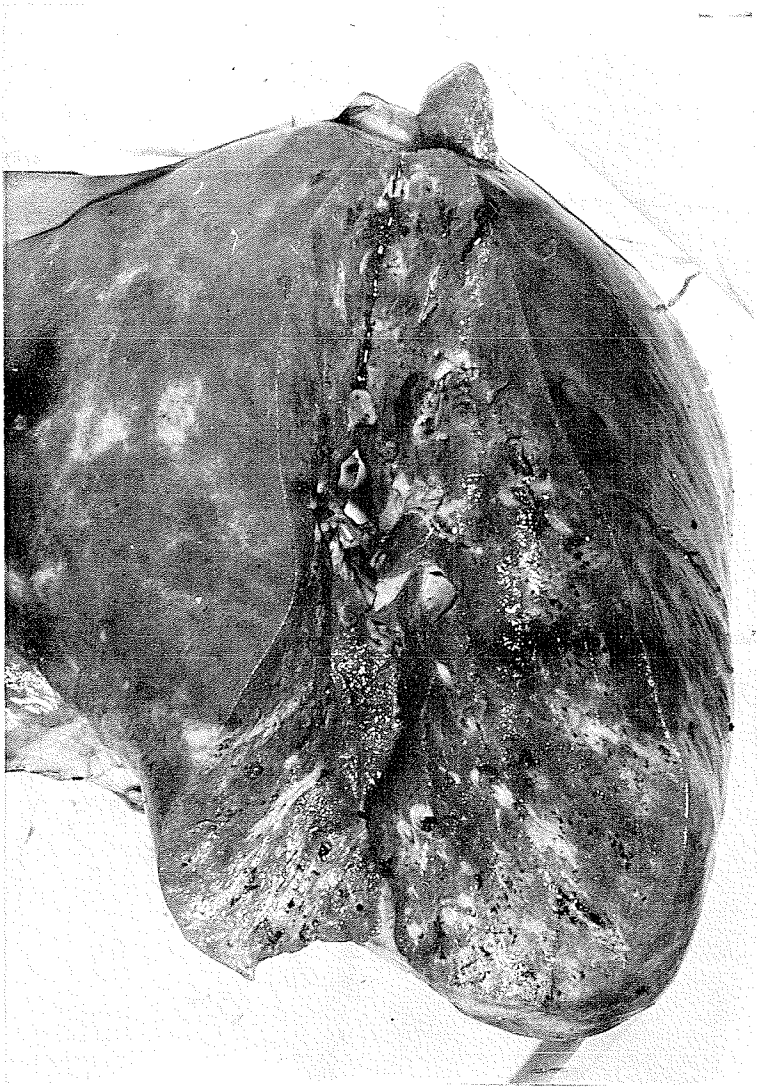


Fig. 2,
Incision of liver, showing diffuse fibrosis and multiple haemorrhagic foci with scarring of the surface. *F. gigantica* in cattle.

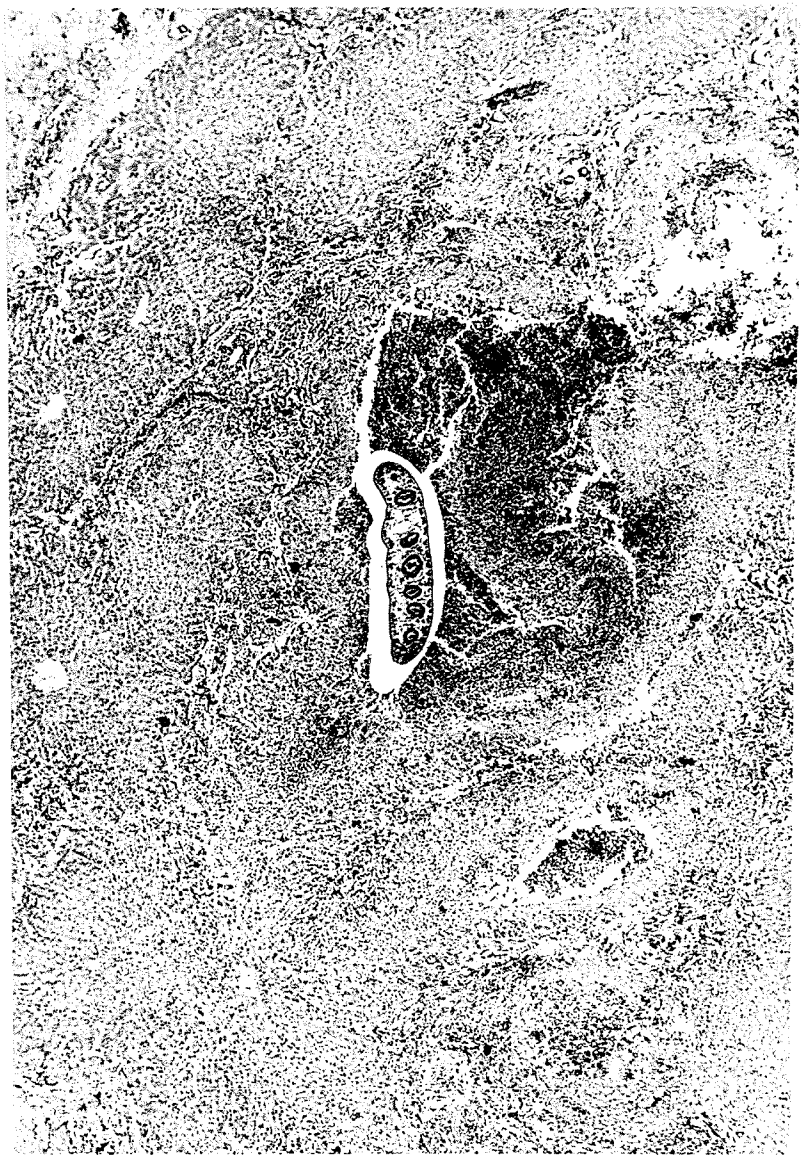


Fig. 3,

Young migrating fluke in liver Parenchyma with intense cellular reaction and necrotic materials in cattle. H. & E x 60.

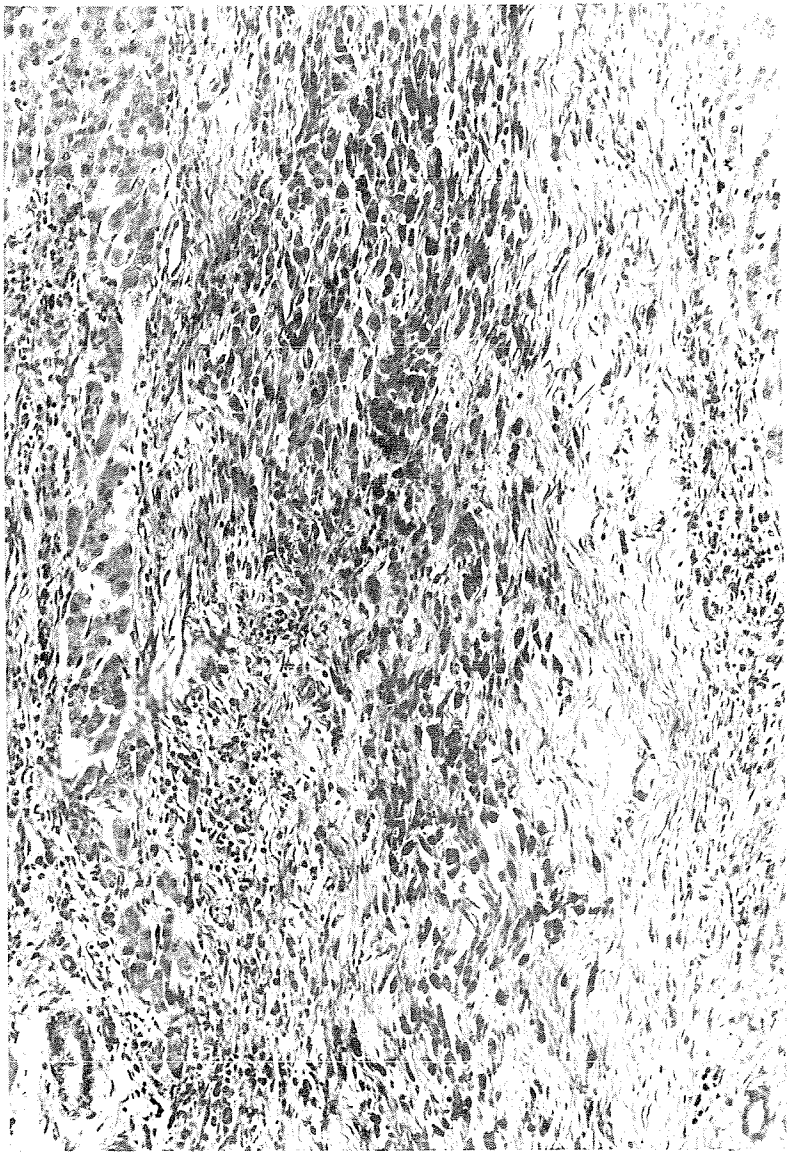


Fig. 4,

Heavy fibrosis of portal tract with pigment depositions F, hepatica in cattle. **H. & E.** x 240.

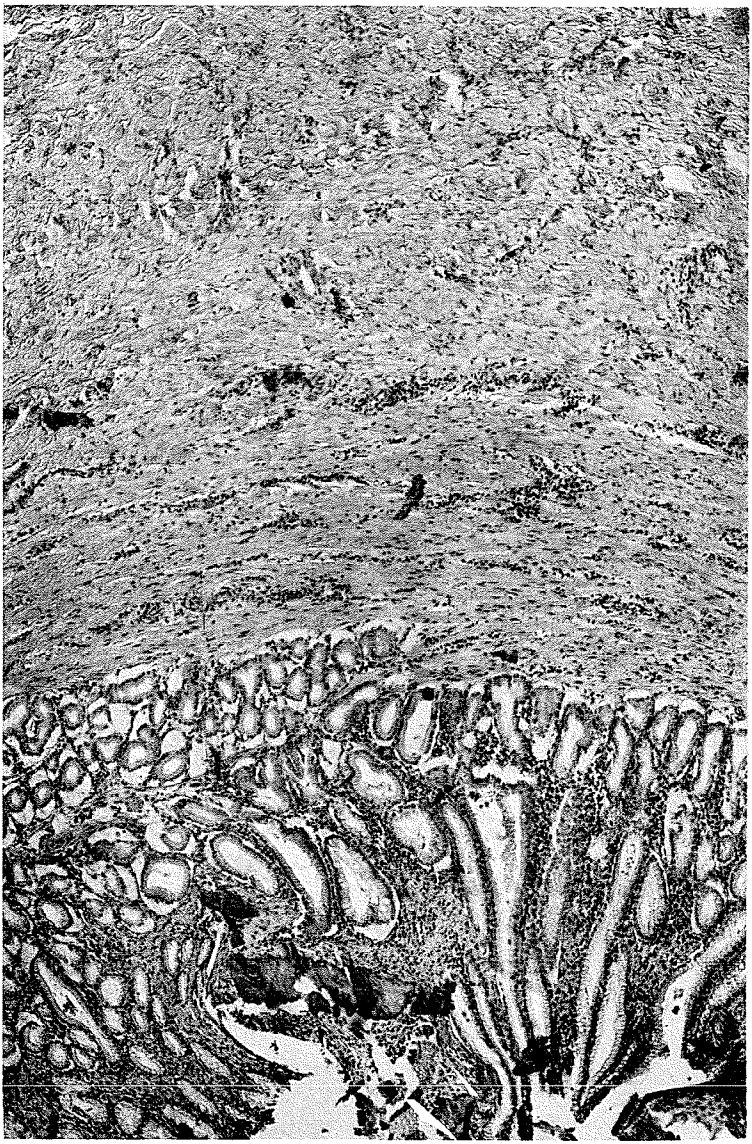


Fig. 5,

Glandular hyperplasia and calcification of mucosal epithelium of main bile duct with severe thickening of the wall, *F. hepatica* in cattle. H. & E. x 120.

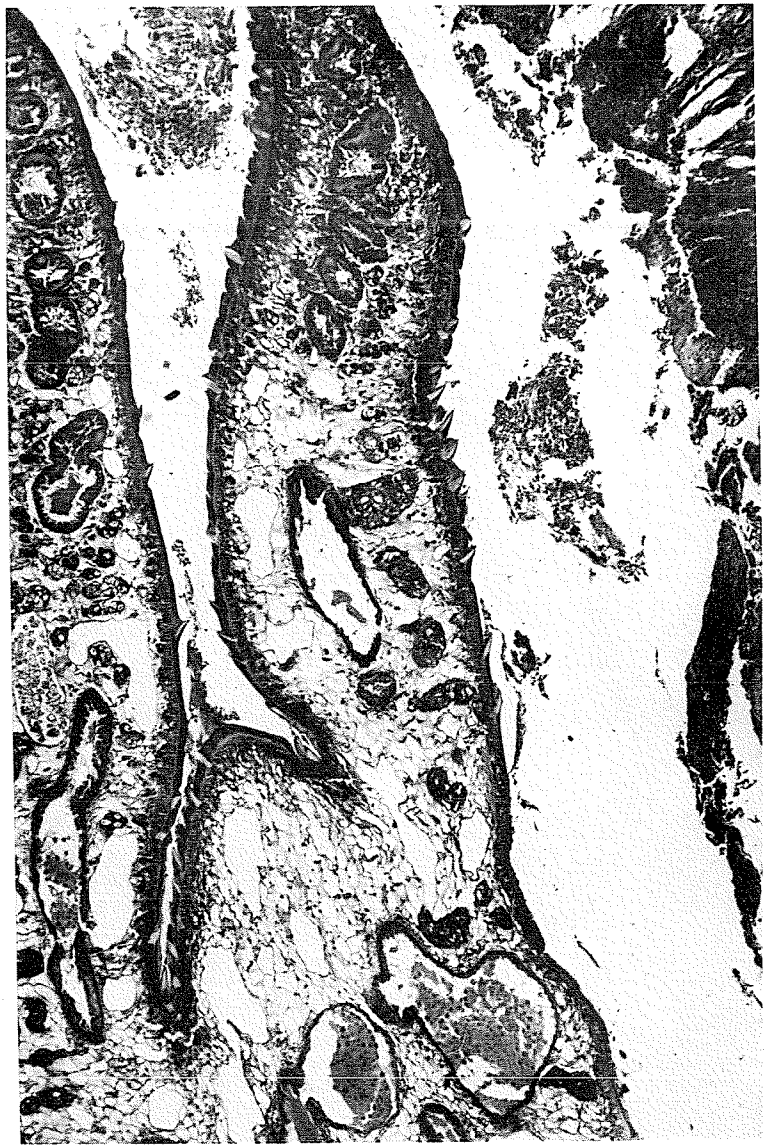


Fig. 6,
Main bile duct containing adult fluke with sharp cuticular spines causing mechanical damages to superficial layer of the duct. *F. hepatica* in cattle. H. & E. x 120.

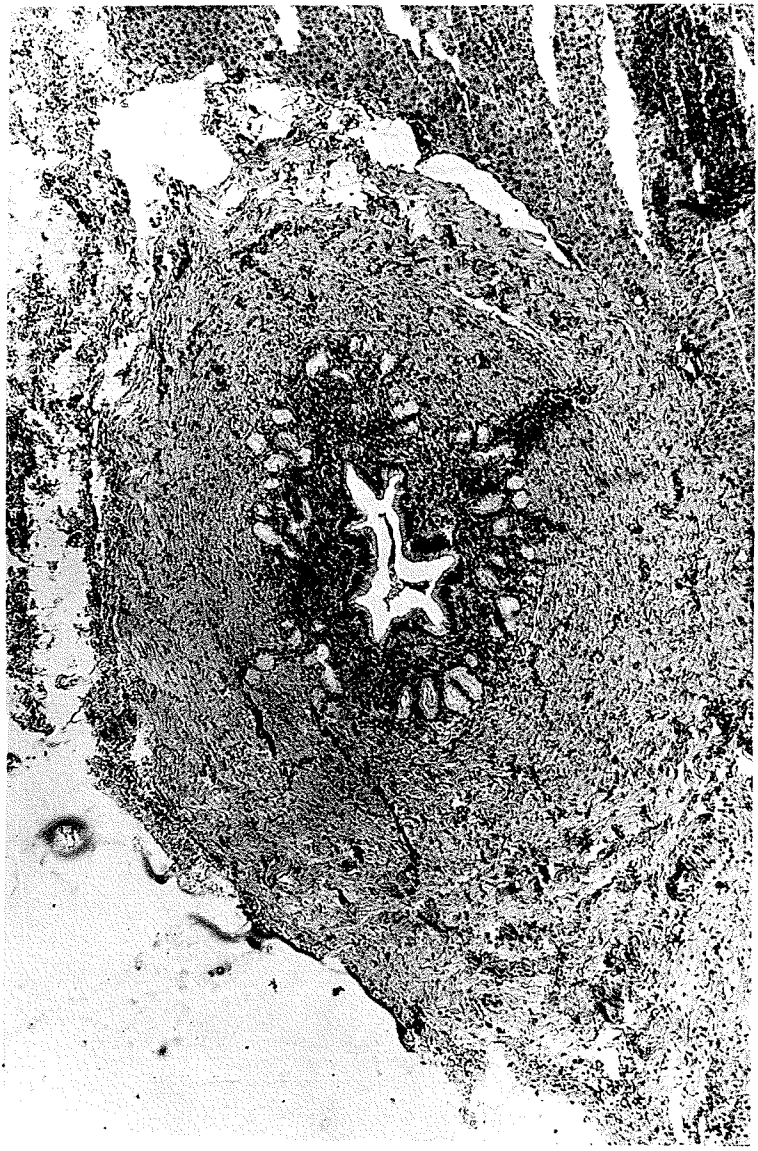


Fig. 7,

Medium size bile duct with severe cholangitis, narrow lumen and thickening of the wall. *F. hepatica* in cattle. H. & E. x 120.

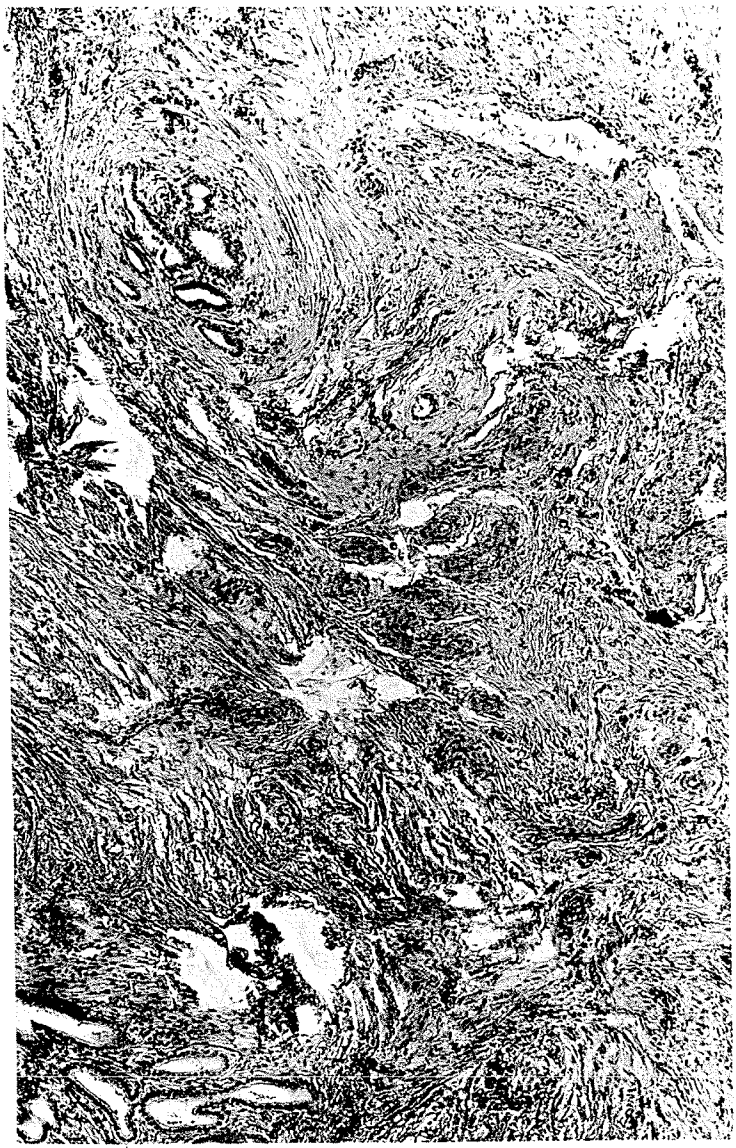


Fig. 8,
Coarse irregular fibrosis of liver. *F. hepatica* in cattle H. & E. x 120.

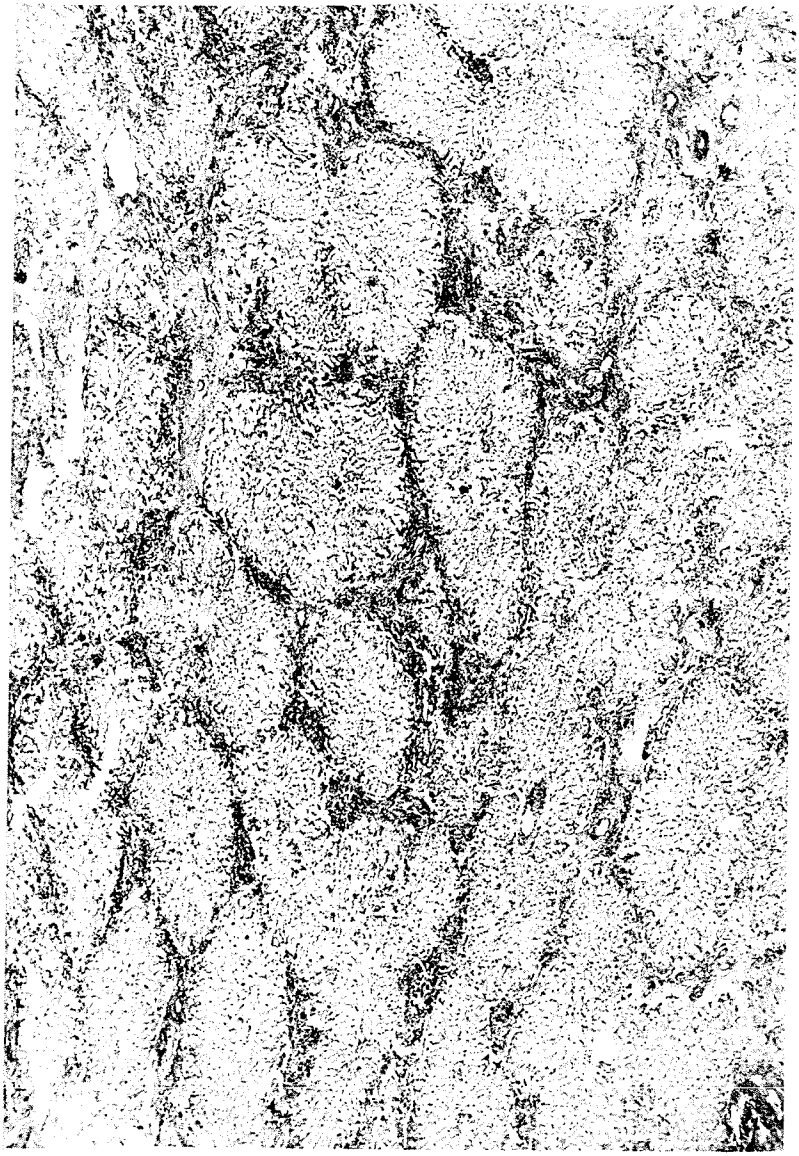


Fig. 9,
Typical monolobular fibrosis of liver. *F. hepatica* in cattle. H. & E. x 60.

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