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Animal Health Research Institute
Assiut Regional Laboratory
Lab. Director Prof. Dr. S. M. Nashed

INVESTIGATIONS ON AN OUTBREAK OF OVINE INFECTIOUS NECROTIC HEPATITIS AT SOHAG GOVERNORATE

(With 12 Figures)

By

SHAHERA, M. R. ABDEL HASEEB; A. H. BAYOUMI* and SANAA, A. H. EL-SHAMY

*: Dept. of Pathology and Clinical Pathology,
Fac. of Vet. Med., Assiut Uinv.

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فحوص على وباء التنكرز الكبدى فى الأغنام فى محافظة سوهاج

شهيرة محمد رشاد عبد الحسيب ، عبد اللطيف حسن بيومى

سناء عبده الشامى

قطيع أغنام بمحافظة سوهاج يتكون من ٨٧ رأساً لوحظت به نسبة نفـوق ٩ % وتلخصـت الأعراض الاكلينيكية فى سرعة وسطحية التنفس والدوار. واشتملت الصفة التشريحية علـى خروج سائل رغوى من الأنف والفم، أحتقان الأوعية الدموية تحت الجلد وأوديما فى تجاويف الجسم وكان تنكرز الكبد آفة ثابتة ومميزة فى كل الحالات. وبالفحص الميكروسكوبى وجـد تفكك وعدم انتظام الخلايا الكبدية وتنكرزها ويحتمل أن يكون سبب التنكرز هو سموم مفـوزة من عصويات موجبه لصبغة جرام. وكذلك وجود أتساع بين الأعمدة الكبدية. وقـد نوقشـت النتائج وأعزيت التغيرات الباثولوجية إلى سموم ميكروب عصوى موجب لصبغة جرام.

SUMMARY

A herd of 78 raising sheep at Sohag Governorate appeared suffering from drowsiness, rapid shallow respiration and 9 % mortality rate. The necropsy findings were frothy fluid from the mouth and nose,

subcutaneous congestion and haemorrhages, hydroperitonium, hydrothorax and hydropericardium. Hepatic focal areas were the constantly observed. The histopathological lesions included cell dissociation, coagulative necrosis and cellular infiltration. Gram positive bacilli were also noticed in necrosed areas. Telangiectasis was also a prominent feature. The lesions could be attributed to the toxins of clostridial infection.

Key Words: *Ovine, Infectious Necrotic Hepatitis.*

INTRODUCTION

The liver is central to the metabolic pathway and has a responsibility for the metabolic function and health of other organs and tissues. Many infectious agents result in focal hepatic necrosis, (Jubb et al., 1985). Black disease or infectious necrotic hepatitis is an enzootic disease, commonly occurs in ovine, (Hiepe, 1970; Bagadi 1974 and Hungerfored 1989), bovine (Niilo et al., 1969 and Blood et at., 1983) It was also recorded in swine (Batty et al., 1964), equine (Gay et al., 1980) and human (Mollaret et al., 1948). The disease has been considered to cause serious economic losses in sheep (Sinclair, 1956). It is primarly an intoxication caused by *Clostridium novyi* which is commonly distributed in the soil. Development of the disease requires combination of initiating factors, (Jubb et al., 1985). Osborne (1958) reported the seasonal incidence of fasioliasis and black disease in sheep. The disease was reported in many areas in the world (Jayaraman and Harbola, 1971 and Bagadi, 1974). The main histologic lesions were described in the liver. It included coagulative necrosis of parenchymal cells, some infammatory cell infiltrations. The presence of the Gram positive bacilli is considered to be diagnostic.

The present investigation describes in details the clinical symptoms, post-mortem lesions and histomorphologic alterations of an outbreak of infectious necrotic hepatitis occurred in sheep at Sohag Governorate.

MATERIALS and METHODS

A herd of 78 raising sheep at Sohag Governorate appeared suffering from drowsiness, rapid respiration and 9 % mortality. Deaths were observed in lambs which ranged from 9 to 16 months of age.

Specimens from liver, spleen, heart, kidneys, intestines, skeletal muscles and lungs were fixed in 10 % neutral buffered formaline, dehydrated, embedded, sectioned and stained with haematoxylin and eosin. Gram stain for tissue sections (Brown and Brenn, 1931) and Prussian blue stain (Bancroft and Stevens 1997) were also used. The macromorphological and histological findings were discribed, illustrated, discussed and the probable aetiological agent was diagnosed.

RESULTS

Clinical symptoms:

As it was noticed by the owner, there were no characteristic signs for the illness and when present it occurred in good conditioned animals and took very short time ranging from 3-9 hours. The animal appeared to be fatigue, move behind the herd then refuse to move, fall down with rapid and shallow respiration, subnormal temperature and soon dies. The owner added that the dead lambs appeared as if falling asleep. Although some animals in the herd showed diarrhoea in addition to some respiratory distress.

Gross pathological findings:

The post-mortem in seven carcasses findings included frothy fluid from the mouth and nose, diffuse subcutaneous congestion and haemorrhages. Variable degress of hydroperitonium, hydrothorax and hydropericardium were noticed. The fluid was straw-coloured. In all dissected animals focal necrotic areas were evident in the liver. They were multiple, yellowish white and of 1- 4 cm in diameter. The rest of the organ appeared dark brown to blackish red. The cut surface revealed dark red areas of haemorrhages in addition to the deep situated necrotic foci. In two cases visible fascioliasis with thickened bile ducts were noticed. In all dissected cases the gall bladder was distended with greenish black secretion. In other organs no pathognomic gross changes could be observed except subendocardial peticheal haemorrhages one case and mild catarrhal enteritis in two cases.

Histopathological findings:

In all the examined cases the hepatopathic alterations were constant and diagnostic. Most of the examined liver sections showed several fields of hepatocellular dissociation. The, hepatocytes were completely seperated from their neighbours, condensed and shrinked, and their nuclei appeared smaller and hyperchromatic. Some hepatocytes

showed features of apoptosis (Fig. 1). The sinusoids were congested. Disse spaces were dilated and filled with edematous fluid and some reticuloendothelial cells were laden with brown haemosederin pigments.

In many areas, irregularly located focal areas of coagulative necrosis could be easily diagnosed. Such areas were usually located near or even in close association with areas of cellular dissociation. The necrosed hepatocytes revealed more cytoplasmic acidophilia and nuclear pyknosis. Other focal areas of incomplete lytic necrosis were also detectable (Fig. 2).

The mostly centrilobular areas of coagulation necrosis were not infrequent and showed Gram positive bacilli either within the central vein or even between the hepatic cords (Fig. 3). The examined cases showed also haemosiderosis and bile pigmentation. The brown haemosederin pigments were found in the Kupffer cells, while the canary yellowish bile pigments were also seen within the reticuloendothelial cells. In some cases the bile canaliculi showed bile casts.

Angiopathic alterations were seen in most of the examined livers. The vascular ramifications revealed endothelial degenerative and proliferative changes, hyaline thrombi, perivascular edema and organization. Diffuse sinusoidal dilatation was noticeable in affected livers (Fig. 4). In the severly affected cases intralobular microscopic areas of sinusoidal dilatations contained haemolysed blood could seen. The most advanced lesions appeared as focal vascular distentions represented in cavernous ectasia of sinusoids which were either communicated together or separated from each other by thin and atrophied hepatic cell cords (Fig. 5).

In most of the examined cases the portal triads showed variable amounts of mononuclear cellular infiltration, mainly lymphocytes and polymorphnuclear leucocytes specially eosinophiles (Fig. 6). The bile ducts within the portal triads revealed either subacute cellular or chronic productive inflammatory reaction (Fig. 7).

Within the hepatic parenchyma irregular tracts of destructed hepatic cords and haemorrhages were noticed and the adjacent areas showed reticuloendothelial cell proliferation (Fig. 8). Only in two cases relatively acellular fibrous scars replacing necrotic parenchyma were observed. The neighbouring paranchyma suffered from pressure atrophy and revealed necrobiotic and lytic changes (Fig. 9).

In most the spleen sections showed excessive accumalations of haemosiderin pigments within the reticular phagocytes in the red pulp.

These haemosiderin pigments stained positively with Prussian blue reaction. In the white pulp the lymphocytes were relatively decreased in number. In the vascular branches specially the central follicular arteries, endothelial proleferative and degenerative changes as well as thrombi could be seen (Fig. 10).

In the heart focal myocardiosis was noticed in three cases. The sarcoplasm of the myofibrils showed coagulation necrosis and lysis. In addition, sarcolemmal proliferation was observed (Fig. 11). In the other cases the myocardial fibers showed loss of striation and granulation of sarcoplasm.

Examination of kidney sections showed well marked tubulonephrosis in addition to erythrocytic extravasation in the collecting tubules of all examined cases. Vacuolar degeneration and necrosis of the renal epithelium could be easily diagnosed (Fig. 12).

Sections taken from the intestine showed mild catarrhal enteritis in two cases and lung congestion and edema was also observed in three cases.

DISCUSSION

In the present work, an outbreak of ovine necrotic hepatitis was described and illustrated. Diagnosis of the disease is based partly on the clinical manifestations and necropsy findings, however the histopathological lesions in the liver and detection of the aetiological agent confirmed the disease.

Infectious necrotic hepatitis is also known as black disease (Bruner and Gillespine, 1966), hense the appearance of flayed skin and dark colouration results from the subcutaneous venous congestion (Jubb et al., 1985). In our findings, irregular focal blackish areas were remarkable in gross examination of the liver in addition to the dark blackish colour of the skin. The blackish areas were either subcapsularly located or even deeply situated in the parenchyma.

Aetiologically, *Clostridium novyi* type B which is a common inhabitant of the intestinal tract, when reachs the hepatic tissue, it multiplies and produces haemolytic, necrotizing and lethal toxins. Arru and Deiana (1973), Orynbaev (1981), Radionov and Islamov (1988), Kulkarni and Pimpale (1989), Pathakand parihar (1991), Pathak and Parihar (1994) and Barker et al (1996) reported the predisposing factors underwhich the clostridium organisms multiply and produce their toxins.

These factors include overdosing of carbohydrates, abrubt changes from milk to solid food and ovine coccidiosis as well as cestode, nematode and trematode infections. Such factors probably iniate an anaerobic environment for the organism. The anaerobic bacilli could be easily recognized in our examined liver sections. The bacilli were Gram-positive (Brown and Brenn, 1931). In our results the organisms could be only seen in the centrilobular areas undergoing coagulation necrosis. It could be never seen in the surrounding healthy hepatic parenchyma. Similar findings were recorded by Jubb et al. (1985). The specific situation of the bacilli could be related to their anaerobic character, this also confirm their production of necrotizing toxins and consequently the primary cause of hepatic coagulation necrosis. Because the liver can not tolerate tissue damage, no or even minimal inflammatory reaction could be seen around the zone of necrosis. In our results many hepatocytes revealed the characterstic features of apoptosis. This could be considered a further explanation for the absence of inflammatory cells surrounding the area of necrosis because apoptosis occurs within intact plasma membrane and the cell contents do not pass to the interstitial tissue. This also explain the hepatic cell dissociation and loss of histological architecture noticed in our results. Degenerative and necrobiotic changes could be markedly seen in the liver, kidneys and cardiac muscle. The microorganism could not be detected in these organs neither in the degenerative foci or adjacent to it but such lesions could be also attributed to the necrotizing toxin which circulate in the blood.

As it is reported by Jubb et al. (1985) and Kulkarni and Pimpale (1989), the bacilli produce also haemolytic toxins. This probably explains the erythrocytic haemolysis seen in all the examined organs. The presence of haemoglobinogenic pigments within phagocytic cells is an indicative factor the erythrocytic haemolysis. In addition vascular degenerative and proliferative changes of endothelium, capillary thrombosis and perivascular edema could be seen in many organs. Such lesions could be assumed as they were reported by Quinn et al. (1994) and Zaitoun et al. (1998) to explain toxin secreted by the microorganism. Cavernous ectasia of sinusoids was a prominent feature and seen in most cases. Although this lesion was not a constant finding and not reported by other investigators, it could not be considered as an accidental Lesion. Ectasia could be attributed to atrophy and coagulative necrosis in hepatic cell cords, lytic effect of the microbial toxins on the reticular framework and the direct vascular effect of the toxin. Jensen et al. (1982) reported and

discussed the correlation between ischamia, hypoxia and portal venopathies in both ovine and bovine livers to expansion and ectasia of hepatic sinsusoids. The author also attributed the haemoglobinogenic pigments to local stasis in such dilated intralobular telangiectatic areas. Regarding the pathogenesis of hepatic telangiectatsis in bovines, Anderson (1966) added the interference with production and maintenance of the intercellular cement substances of the vascular endothelium and pericapillary ground substance and this could also related to the clostridial exotoxins. Moreover reminisceut cell mediated immune reaction was added by Jubb et al. (1985) as an aetiological factor.

In our investigation adult fasciola could be seen grossly in two cases. Microscopic examination revealed sites of acute trumatic haemorrhagic lesions as well as areas of scaring within the hepatic parenchyma. Subacute and chronic cholangitis were also remarkable. The presence of such lesions probably aided for creation of anaerobic enviroment for the organism.

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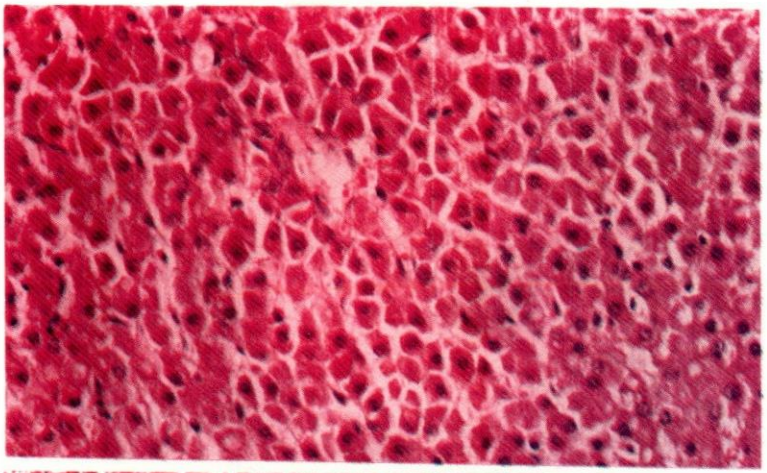
Fig. 12: Kidney showing tubulonephrosis and erythrocytic extravasation within the collecting tubules. H. E., × 250.



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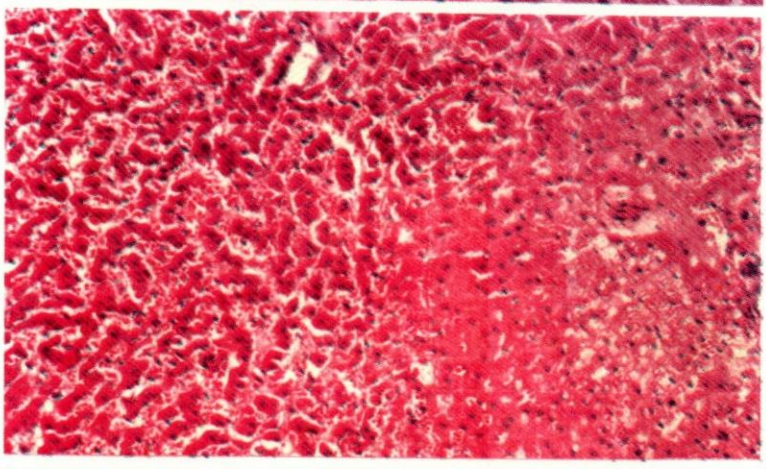

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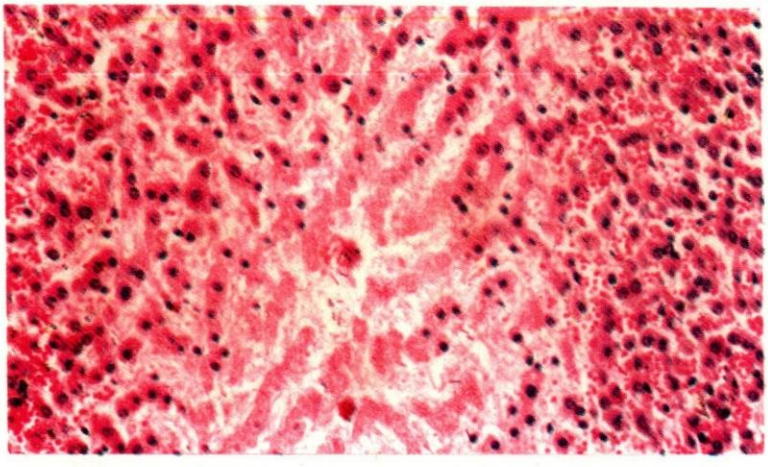

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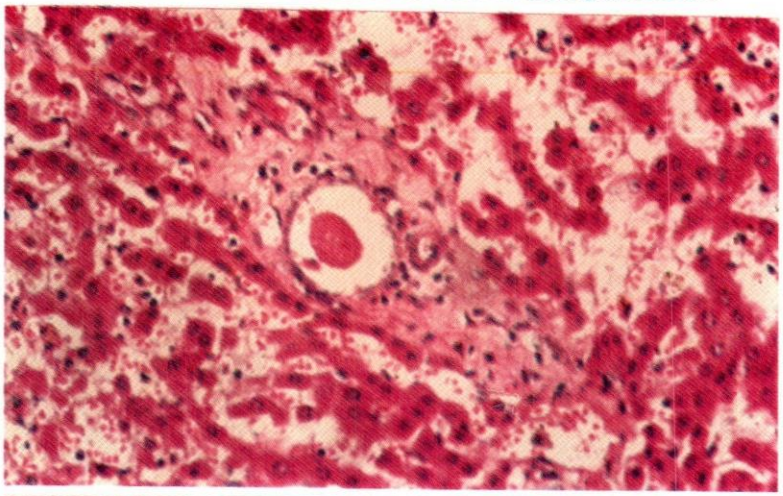

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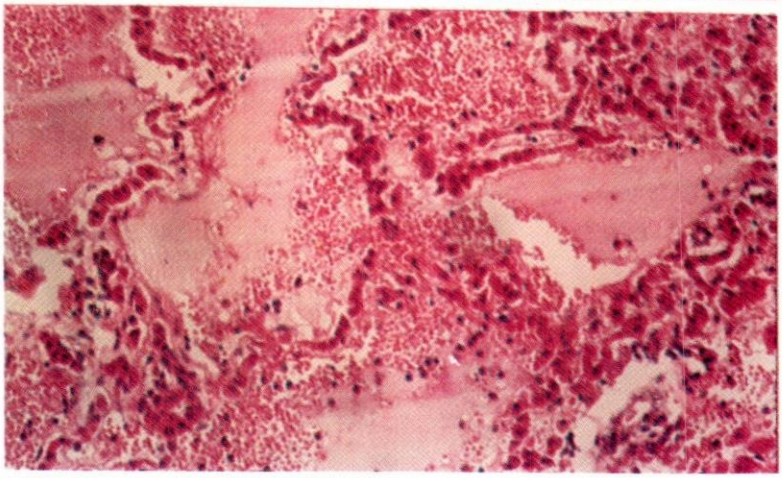

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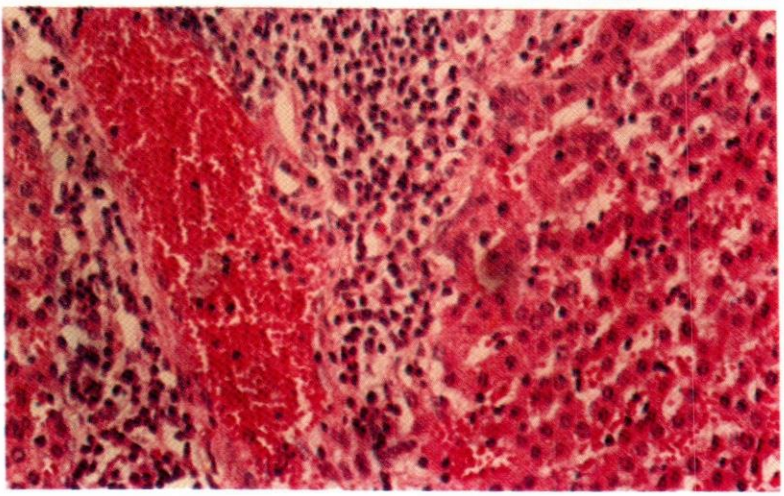

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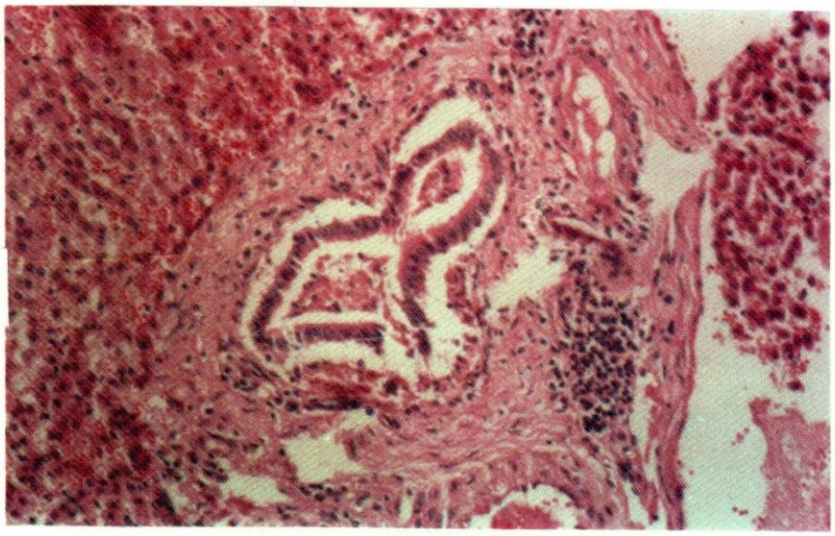

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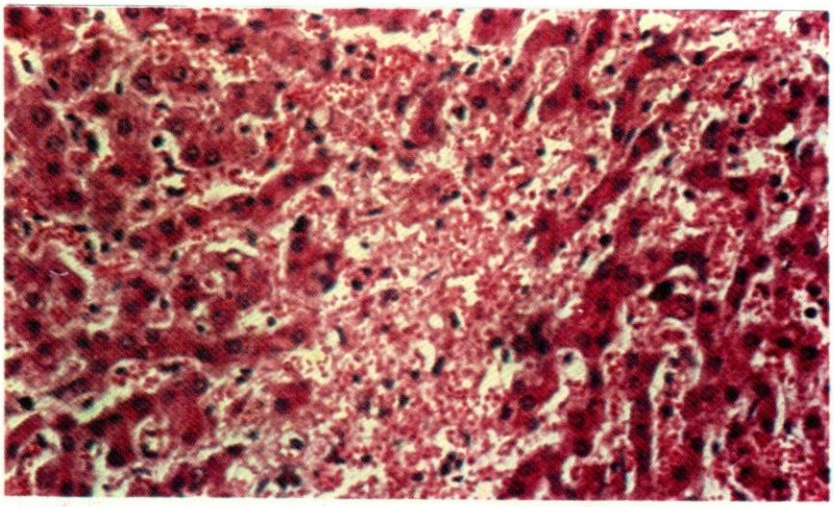


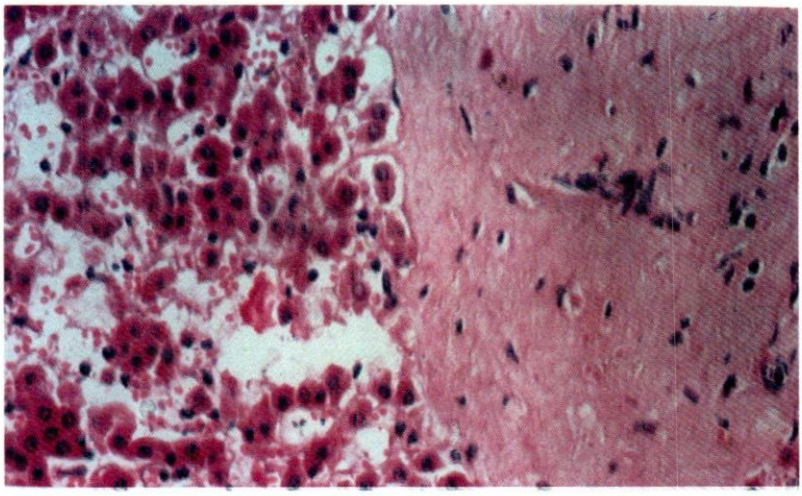


Fig. 9

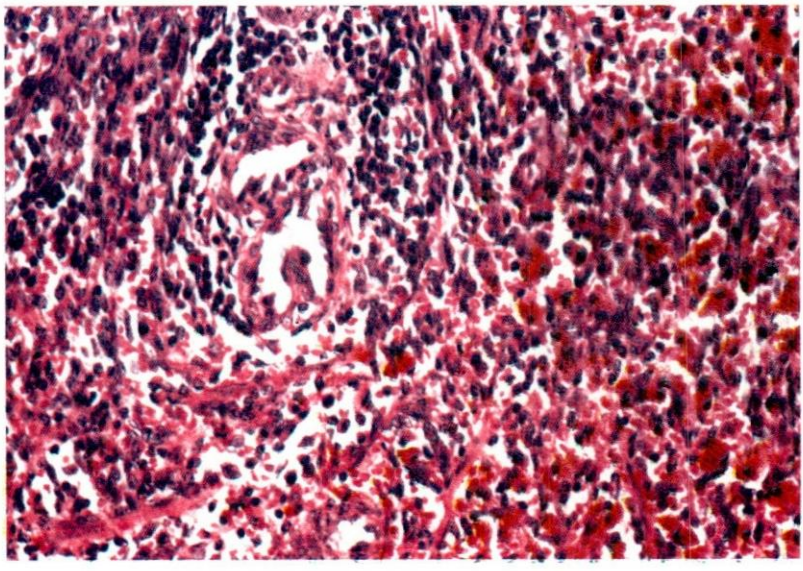
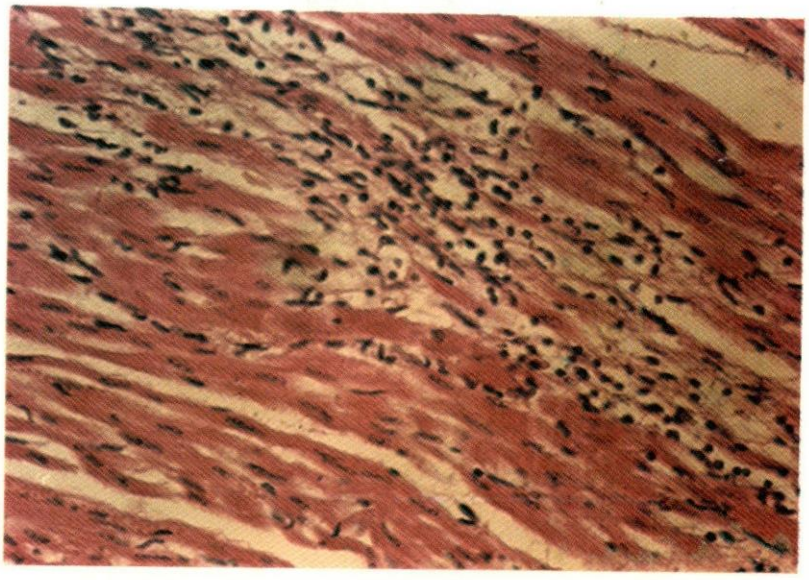


Fig. 10



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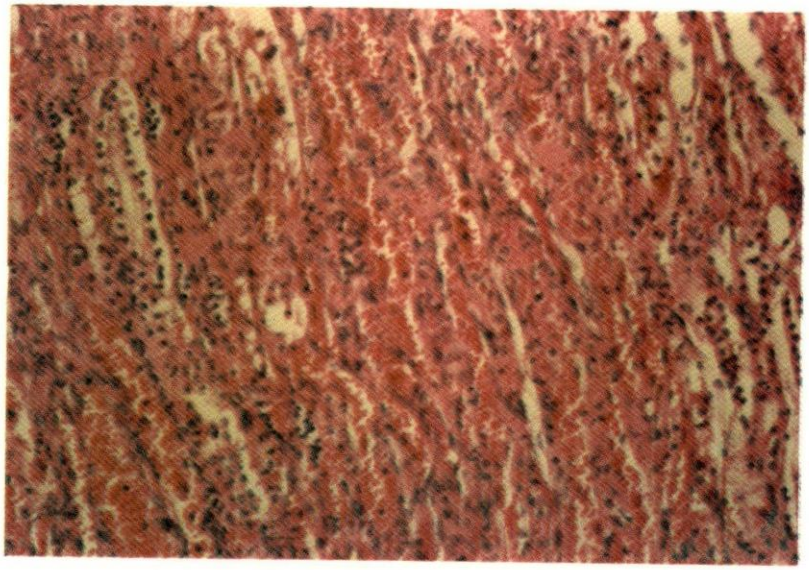

Fig. 12