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ميكروب الكلوستريديا في النسيج الكبدي

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أجريت الدراسة على عدد خمسون كلب تم قتلهم بالصدمة الكهربائية . أخذت عينات من الكبد وصبغت بصبغات مختلفة .

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**INDIGENOUS MICROFLORA CLOSTRIDIA LIKE ORGANISM
IN THE LIVER TISSUE**
(With One Figure))

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SUMMARY

A histopathological study was carried out on fifty dogs liver killed by electric shock to determine the incidence, severity of infection and tissue reaction in animals harbouring clostridia without apparent clinical disease. For this purpose, Paraffin sections from the liver were stained with Heigert haematoxylin and eosin as well as four bacterial stains. The results revealed the occurrence of clostridia in eight cases (16%). The microorganisms appeared in the form of sporulated bacilli that occurred either single or in pairs but never in chains. They were mostly located in the connective tissue of the portal triads between the hepatic columns, and in the lumen of the blood vessels. These microorganisms were never seen intracellular. Changes in the hepatic tissue were in the form of degeneration of variable degree, fibrinoid changes of the portal connective tissue, and necrosis of the endothelial lining of the portal and central veins. It has been suggested that clostridia were not merely passing through the liver but this microorganism was actually colonise this organ. Harris haematoxylin and eosin and methyl green-pyronin stains were recommended for demonstration of clostridia in the liver tissue.

INTRODUCTION

It is well known that, in addition to the internal nares, the oropharynx, liver intestinal tract and urogenital tract, deep tissue and visceral organs such as the liver, lung, spleen and kidneys are colonized with microorganisms (JUBB and KENNEDY, 1963, DEJONGH *et al.*, 1968, DALAN *et al.*, 1971, KONEMAN *et al.*, 1971, KONEMAN and DAVIS, 1974, NAHRING *et al.*, 1971, WILSON *et al.*, 1972, WMITH *et al.*, 1974). These microorganisms may play a part as a source of clinical infection particularly in animals or individuals with reduced resistance (KONEMAN and DAVIS, 1974). In animals, the liver of many healthy cattle, sheep as well as dogs harbour latent infection with clostridia (SMITH *et al.*, 1974) and fascioliasis may predispose for the occurrence of clinical disease in these animals, e.g., black disease in sheep and bacillary haemoglobinuria in cattle (JUBB and KENNEDY, 1963).

In the present study, a survey was carried out on dogs liver to determine the incidence, severity of infection as well as tissue reaction in animals harbouring clostridia without apparent clinical disease. Moreover, the staining characteristics of this organism in tissue were studied using two formula of haematoxylin and four bacterial stains to determine the most proper one for staining bacteria in tissue.

MATERIAL and METHODS

The material consisted of fifty stray dogs sent alive to the Departement of Pathology, Faculty of Veterinary Medicine, Assiut University. The animals were killed by an electric shock and were eviscerated within 1/4 hour after killing. Postmortem examination was carried out and samples of the liver were fixed in 10% formalin solution. The Materials were embedded in paraffin. Sections, 5-7 μ , were stained by Harris haematoxylin and eosin without treatment with acid-alcohol, Weigerts haematoxylin and eosin with differentiation in 1% acid-alcohol, crystal violet (HUMASON, 1961), alkaline methylene blue (MERCHANT, 1971), Hansens method using carbol fuchsin (MERCHANT, 1971) and methyl green-pyronin (SAATHOF, 1905). No trials was made for culturing or typing of the microorganisms from the tissue were carried out.

RESULTS

In the liver of eight dogs, clostridia was demonstrated clearly by all stains used except Weigerts haematoxylin and Hansens method. The bacilli appeared rather long with unstained spores located toward the center. They were usually found single or were arranged in pairs but never in chains. The organisms were distributed between the hepatic columns, in the portal triads, large number of microorganisms were found in the connective tissue and intravascular in the portal and central veins. The number of bacilli was sometimes so many that they resulted in occlusion of the vessel. In the hepatic parenchyma, peripheral areas of the lobes were the most severely affected ones. The organism did not occur intracellular neither in hepatic nor kuffer cells, endothelial lining of blood vessels or epithelium of the intrahepatic bile ducts and canaliculi.

Congestion and severe oedema without extravasation of erthrocytes were consistent findings. The hepatic columns were disoriented and individual cells were widely seperated from each others. The reaction of the hepatic tissue to the presence of bacilli generally coincided with its density in the tissue. The hepatic cells in the affected areas showed mild degenerative changes varying from cloudy swelling to hydropic degeneration. Hepatic cells with vacuolated cytoplasm and characteristically large, vesicular, pale-stained nuclei in which the chromatin particles were margined and the nucleoli disappeared were frequently found. Nuclear pyknosis or necrotic changes in the hepatic cells were observed. The kupffer cells were slightly activated. The connective tissue in the portal triads was disorganized and have under gone fibrinoid necrosis. The endothelial cells of the portal and central veins were mostly necrotic. The epithelial cells of the intrahepatic bile ducts were swollen and many of which had nuclei that have undergone karyolysis.

Infiltrations with sporadic neutrophil cells were observed mainly in some zones of the hepatic tissue suffering coagulative necrosis. Giant cells or active phagocytes were not found. Lesions related to parasitic infection or eosinophilic infiltration were not demonstrated in our material.

In the present study, although the microorganisms could be demonstrated by most of the stains used, they were easily decolorized by treatment with acid-alcohol for differentiation after Weigerts haematoxylin. As well, the bacilli were rapidly decolorized using 5% actic acid after Hansens method. Clostridia showed a great affinity to be stained with crystal violet and alkaline methylene blue. Using methyl green-pyronin stain, the bacteria stained lightly green and failed to stain with pyronin. Harris haematoxylin gave a quite satisfactory results for demonstration bacteria.

INDIGENOUS MICROFLORA

DISCUSSION

In the present study, congestion and oedema of the liver appeared to be mostly related to the shock induced by the electric current. An incidence of 16% of livers infected with clostridia was found. In the affected organs the reaction to the presence of bacteria was localized and consisted of necrotic changes and diffuse leucocytic infiltration mainly of mononuclears even in livers harbouring a large number of the organism.

Currently, it is not known whether organs such as the liver harbouring bacteria are acting merely in the clearance of these microorganisms periodically seeds from distant focus of contamination, or that the organs are actually colonized, i.e., the microorganisms undergo reproduction in situ. Necrotic changes of the hepatic cells and infiltration of inflammatory cells, associated with the presence of bacteria in binary fission may support the point of view that the liver is actually colonized by clostridia like organism. Little phagocytic activity of the reticuloendothelial cells, mainly Kupffer cells, in the affected liver in contrast to the presence of large masses of bacteria may indicate that this tissue plays a minimal role in clearing the liver of these large bacteria. The suggestion that some members of clostridia, e.g., *Clostridium novyi*, remains as latent infection in the reticuloendothelial cells of the liver of many healthy sheep, cattle and dogs (JUBB and KENNEDY, 1963) could not be emphasized by the present work as bacilli were never seen intracellular in these cells. However, experimental studies have evidenced that importance of local organ environment in the defence against bacterial invasion. GREEN and KASS (1964) have demonstrated that mice previously exposed to starvation, hypoxia or ethanol were unable to clear aerosol clouds of airborne bacteria from their lungs presumably from a decline in the phagocytic activity and ability of the alveolar macrophages. RETLIFF *et al.*, (1970) and WILSON *et al.*, (1971) have demonstrated ultrastructural damage in pulmonary leucocytes and alveolar macrophages of the dogs following shock injury to the lungs. The influence of similar factors on the reticuloendothelial system in the liver of stray dogs examined in the present work and their relationship to reduced phagocytic activity could not be determined.

As shown in the present study, examination of tissue for the presence of bacteria may be a useful aid to make a correlation between a specific microorganism and a certain type of disease or a clinical disturbance. Harris haematoxylin and eosin without treatment of the sections with acid alcohol was a quite satisfactory method for demonstration of these Gram-positive bacilli and for histological study. In addition to this routine work stain, methyl green-pyronin method after SAATHOF (1905) is to be recommended as a bacterial stain.

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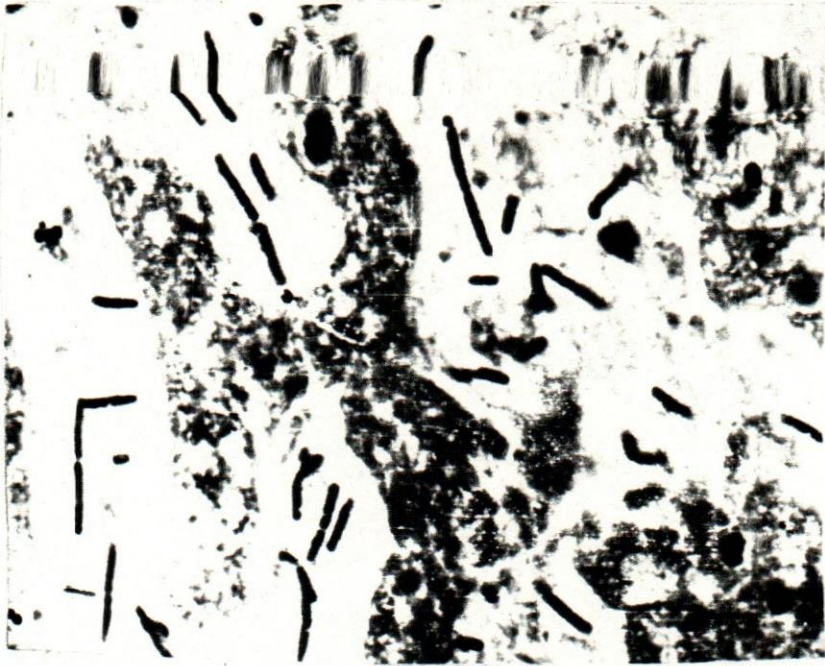


Fig 1 (a)

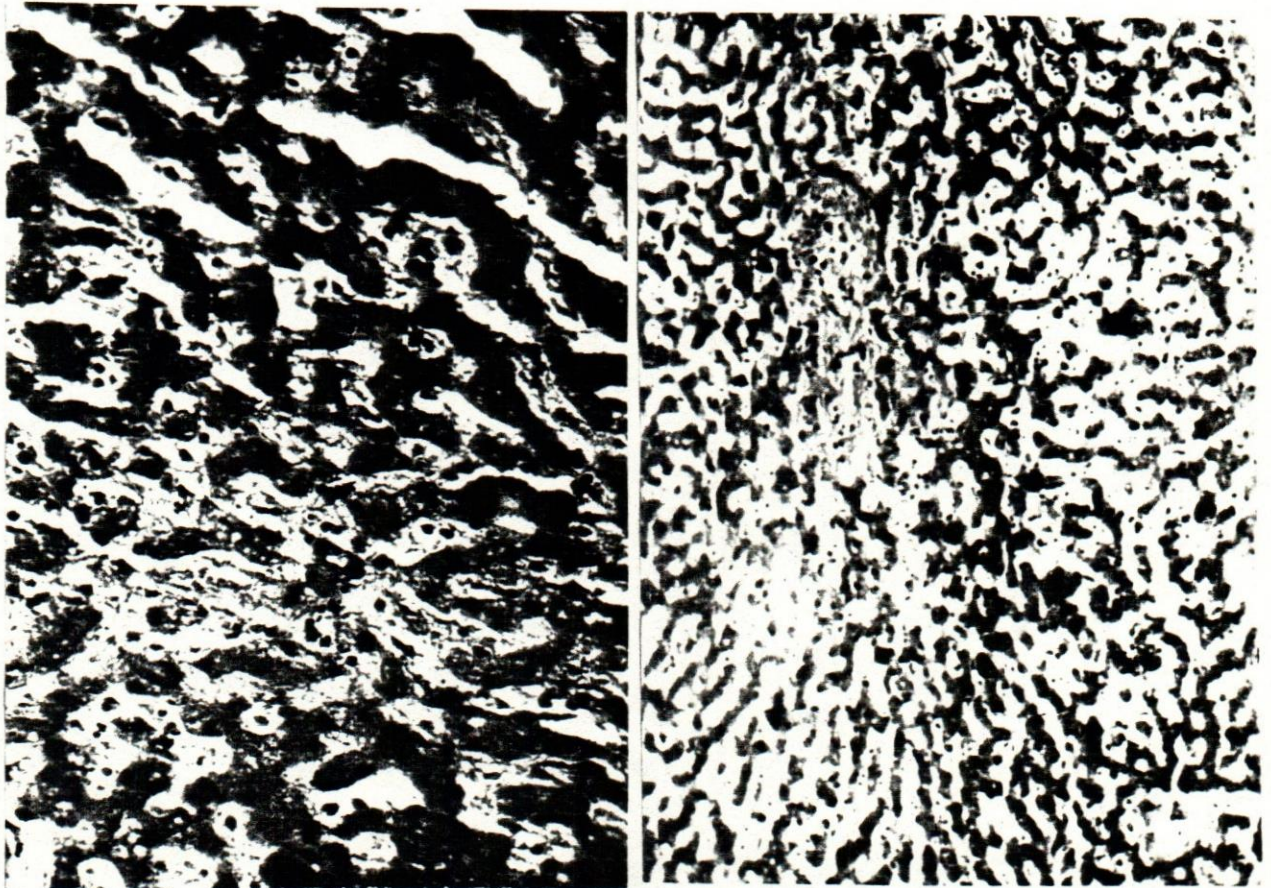


Fig. 1 (B & C)

Fig. 1: Showing a - the araganisms distributed inbetween the hepatic columns, b & c : hepatic cell necrobiosis and necrosis with neutrephilic cell infiltration.

