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بللورات داخل الأنويه في الخلايا الكبدية

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درست طبيعة بللورات وجد تداخل الأنويه في الخلايا الكبدية للكلاب وقد استخدمت في البحث عديد من الصبغات لمعرفة طبيعة تكوينها كما نوقشت النتائج .

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CRYSTALLINE INTRANUCLEAR INCLUSIONS IN HEPATOCYTES
(With Two Figs.)

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SUMMARY

In the liver of dogs postmortally examined in the Faculty of Veterinary Medicine, Assiut University, intranuclear inclusions of characterstic appearance were incidently found microscopically. The nature of these inclusions was studied and it was suggested that these crystalline inclusions were related to permenant cell injury after viral infection.

INTRODUCTION

Various types of inclusions were seen in the nuclei of hepatocytes of dogs. Intranuclear crystalline inclusions beside those related to viral infections such as canine hepatitis (HABBERMANN et al. 1960, SMITH and JONES, 1961) and canine distemper (McMONBREUN, 1937, SMADEL, 1959, HABBERMANN et al. 1960), were described in the liver of dogs (BROWICZ, 1902, NICOLAN and KOPCIOWSKA, 1936, WEATHERFORD and TRIMBLE, 1940, BLOOM, 1954, THOMPSON et al. 1959 a,b, RICHTER et al. 1965). Moreover, intranuclear crystals have been reported to occur in the liver cells of apparently normal dogs and has been suggested to be an indication of latent viral infection (JEZEQUEL and STEINER, 1966, GIVAN and JEZEQUEL, 1969). The toxicity of lead was also been found to be associated with intranuclear inclusions in the dogs liver (FINNER and CALVERY, 1939).

In the livers of dogs postmortally examined in the Faculty of Veterinary Medicine, Assiut University, intranuclear inclusions of characteristic appearance were incidently found microscopically. Since inclusion bodies are considered a pathognomic lesion of many viral diseases, it was the aim of the present work to illucidate the nature of these bodies and to study the tissue reaction associated with their occurrence.

MATERIAL and METHODS

The material consisted of fifty dogs collected from different localities of Assiut governorate and sent to the Faculty of Veterinary Medicine for post-mortum examination. The animals were killed by electric current-shock and dissected within 1/2 hour. The liver and other organs, were fixed in 10% formalin solution and processed for paraffin embedding. Sections of 6 micron in thickness were stained with haematoxylin and eosin, Maximow haematoxylin-azur II-cosin for inclusion bodies, Benizidine method for haemoglobin (GLICK, 1949), Iodine stain for bile pigment (GLICK, 1949), Prussian blue reaction for haemosiderin (GOMORI, 1936) and Feulgen reaction for DNA.

RESULTS

The liver of four dogs showed the occurrence of intranuclear inclusion bodies. In sections stained with haematoxylin and eosin, the nucleus of the affected cells revealed the presence

of homogenous eosinophilic bodies of sharp-cut ends. These intranuclear bodies were found only single in the cell. They were either cubic or rectangular in shape (Fig. 1,2). The bodies may be small in size and was surrounded by a regular nuclear membrane or they may be increased in size, elongated to the extent that the nuclear membrane was tightly stretched around it (Fig. 2). A clear, unstained narrow rim always lie between the material of the body and the nuclear membrane. The chromatin particles were mostly absent in nuclei having large bodies inside, while they were basophilic and condensed along the nuclear wall in most cells. Similar bodies were not found outside the nucleus in the cytoplasm or interstitial cells. Most of the affected cells had an intact cytoplasmic membrane and the cytoplasm was lightly stained. The affected liver cells showed no specific lobular distribution. The bodies did not occur in tissue elements other than hepatocytes, i.e., the kupffer cells, connective tissue cells of the portal triads, or the epithelial cells lining the bile ducts were free from these bodies. Degenerative changes in the form of cloudy swelling or hydropic degeneration were observed in the liver of the affected animals. Diffuse infiltration of dispersed number of mononuclear cells, and in one animal focal aggregation of these cells were found in the hepatic parenchyma. The bodies showed negative reaction for DNA and haemoglobin. Liver of affected dogs did not reveal features of haemorrhage or abnormal accumulation of bile bigment as indicated by Prussian blue reaction and iodine stain.

Characteristically, the bodies intensely stained with Maximow haematoxylin-azur II-eosin appearing magenta blue in contrast to the blue colour of th nucleus. Using this stain, fine granules having the same staining affinity as that in the intranuclear bodies were observed in the cytoplasm of many cells with apparently normal nuclei.

DISCUSSION

As shown in the present study, the intranuclear inclusion bodies found in the hepatocytes of dogs were not composed of nucleoprotein, haemoglobin and lipids. These results are in parallel to those of THOMPSON et al. (1959 a) who further found that they did not react to cholesterol, mucin, mucopolysaccharides, glycoproteins or glycolipids. THOMPSON et al. (1959 b) indicated that these inclusions contained reactive groups normally associated with the presence of proteins. The suggestion of BROWICZ (1902) that these inclusions were haemoglobin or haemoglobin deriviatives could not be emphasized in the present work. Our results revealed that, morphologically, these intranuclear inclusions were different from that bodies found to associate lead toxicity and described in the liver (WACHSTEIN, 1949, GUEFT and MOLNAR, 1961).

Intranuclear crystal formation was seen in quite a variety of virus infection. It is not clear whether the crystalline protein is host or viral protein, but it has been suggested that excessive protein production probably occurs in the nucleus, and that when a critical concentration is reached crystallization happens (CHADAILLY, 1975). NICOLAN and KOPCIOWSKA (1936) suggested that these inclusions were caused by saprophytic virus. Due to the absence of nucleic acids, THOMPSON et al. (1959 a,b) has ruled out that they were viral in origin. RICHTER et al. (1965), did not found any resemblance of these bodies to structures of known viruses or virus crystals. However, two viral diseases are known to be associated with inclusion body formation in hepatocytes, these are canine hepatitis and distemper. While the inclusion bodies related to the first disease are known to be Feulgen positive, i.e., contain DNA, the inclusions related to the second viral disease did not contain DNA (RONALD et al. 1963). In the present study, the intranuclear crystalline bodies were highly positive to Maximows haematoxylin-azur II-eosin, a stain which is used for demonstration of inclusion bodies of diseases as rabies and pox. Moreover, mild degree of mononuclear cell infiltration were found in the parenchyma of affected livers. Accordingly, it may be suggested that the crystalline inclusion bodies demonstrated in our materials were related to permenant cell injury after viral infection.

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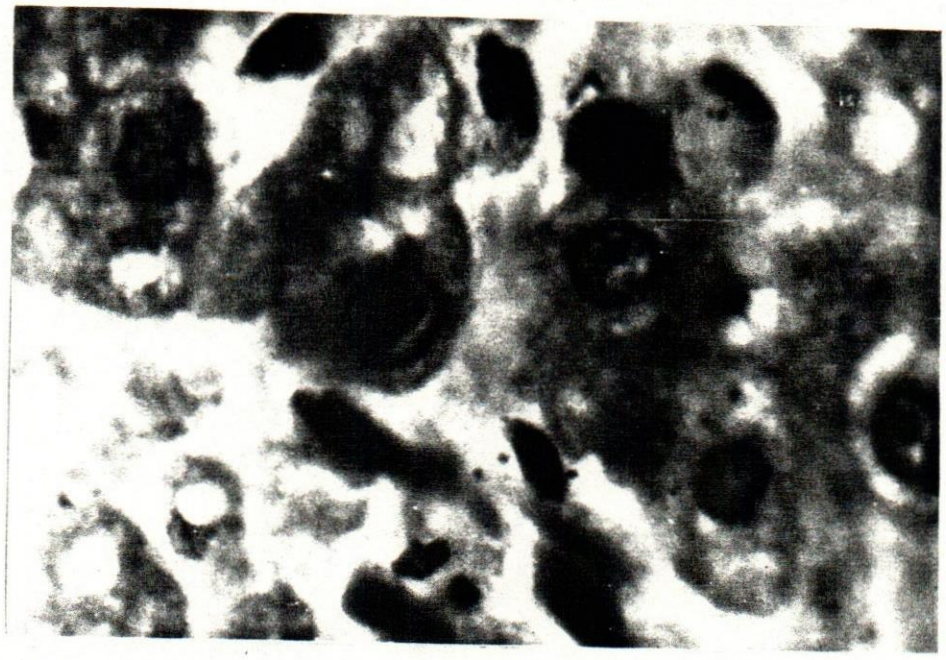

Fig. (1): Intranuclear inclsion body, cubic form, in the liver cell of dog. Hae:atoxylin-azur II- eosin stain. X 1000.

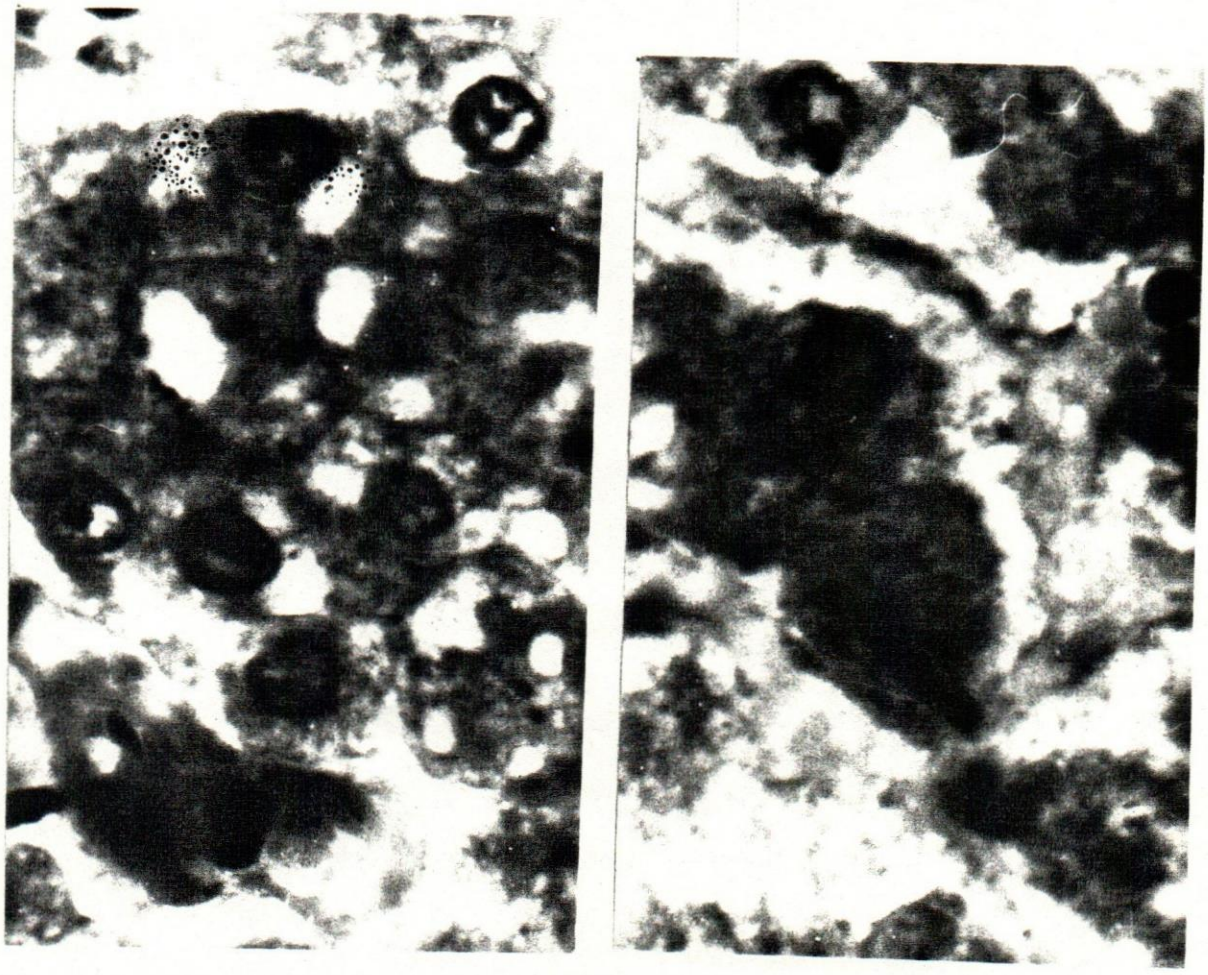

Fig. (2 a,b): Intranuclear inclusion body, a rectangular form in which the nuclear membrane is stretched around the body. Haematoxylin-azur. II- eosin. X 1000.