قسم : الباثولوجيا .
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# أجسام ضمينيه مشابهة البلورات في كرات الدم البيضاء للكلاب

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أجرى البحث على عدد سبعة عشرة كلبا ضالا في محافظة أسيوط . أخذت عينات دم منها أجريت عليها الفحوص الاكلينيكية وكذلك افلام دموية على شرائح زجاجية تم صبغها بعديد من الصبغات وفحصت جيدا .

أخذت عينات من كبد هذه الحيوانات بعد قتلها بالصدمة الكهربائية مباشرة تم فحصها بعد تمريرها وصبغها بعديد من الصبغات .

أسفرت النتائج عن وجود أجسام ضمينيه مشابهة البلورات في سيتوبلازم كرات الدم البيضاء في عدد ثلاث كلاب ، وكذلك أجسام ضمينيه في أنوية الخلايا الكبدية لهذه الكلاب الثلاثة ،درست مواقع وأشكال وخصائص الاجسام الضمينيه ، وكذلك التغييرات الباثولوجية الموجودة بعد دراسة نتائج فحص الدم والتغييرات الباثولوجية ، وكذا الاجسام الضمينية .

نوقشت النتائج وأمكن احتمال اعزاء التغييرات الى الاصابة بمرض الالتهاب الكبدى الفيروسي في هذه الكلاب .

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# *CRYSTALLINE INCLUSIONS IN PERIPHERAL BLOOD-NEUTROPHILS FROM DOGS*

*(With One Table and 3 Figures)*

*By*
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## *SUMMARY*

Large, regular, rhomboid or crystalline inclusions were seen in peripheral blood-leucocytes from stray dogs in Assiut governorate. The location, shape, size, dimensions as well as the histochemical nature of these inclusions were studied. After the haematological and histopathological examinations, the inclusions could be probably related to infectious canine hepatitis virus infection.

## *INTRODUCTION*

In general, viral diseases are frequently characterized by either cytoplasmic or nuclear inclusions. however only rarely inclusion bodies were produced in both locations by a single virus, Habermann, WILLIAMS and FITE (1960).

Various types of inclusions were seen in the nuclei of virus infected-cells. These included viral particles, RANDALL and GAFFORD (1962), precursors of such particles, malformed viral components and also structures derived by alterations of nuclear components and latter did not contain viral particles, HIRST (1959).

Intranuclear viral particles may be solitary, in clusters, in linear arrays or crystalline formations. the intranuclear crystals which in some instances were histochemically characterized as protein have been seen in the nuclei of cells infected with, certain types of adenovirus, GIVAN and JEZEQUEL (1969), coxsakine virus, JEZEQUEL and STEINER (1966), and herpes virus, GHADIALLY, (1975). The latter author reported that herpesvirus rarely forms crystalline structures but large viral crystals are quite common with adenovirus.

Intranuclear crystals were observed in hepatic nuclei in case of human viral hepatitis, BHAGWAT, ROSS and CURRIE 61972). Reports dealing with such crystalloids in the hepatocytes of animals are few except for Canidae-wolves, foxes, jackals and non domestic dogs, WEATHERFORD and TRIMBLE (1940), and cold blooded animals and mammals, BERG (1934).

Analysis of the available literature showed no reports dealing with cytoplasmic, crystalline inclusions in leucocytes of peripheral blood of canines.

The aim of the present work is to report the occurrence of intracytoplasmic crystalline inclusion bodies in polymorphnuclear neutrophils from the peripheral blood of dogs, to illucidate the histochemical nature of these bodies and to discuss their possible significance in relation to infectious canine hepatitis.

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## MATERIALS and METHODS

This work was carried out on seventeen stray dogs obtained from Assiut governorate. Blood samples were taken from the jugular vein and blood films were made from both fresh samples as well as from 48 hours preserved others, MAHMOUD, IBRAHIM and BAYOUMI (1983). Blood smears were stained with haemtoxylin and eosin, Maximow haematoxylin- Azur II- eosin, Gimesa for inclusion bodies, Benzidine method for haemoglobin, GLICK (1949), Prussian blue reaction for haemosiderin, GOMORI (1935), and Feulgen reaction for desoxyribonucleic acid inclusions.

The animals were killed by electric current-shock and dissected within half an hour. The liver specimens were fixed in 10% neutral buffered formalin solution and processed for paraffin embedding. Section of six micron thickness were stained with the same forementioned staining procedures and examined. Blood samples were collected, total leucocytic count (WBC), packed cell volume (pcv) and differential leucocytic count were studied, COLES (1980).

## RESULTS

### I- Blood smears:

From the seventeen examined cases, only three cases showed unusual, abnormal, elongated intracellular peculiar formations whose regularity indicate, crystalloid bodies in polymorphnuclear leucocytes (Fig. 1). These bodies were located ion the cytoplasm in average of 18.5% of the neutrophilic cells. The crystalloids could not be seen neither in the nucleus of the neutrophils nor the nucleus and the cytoplasm of cells other than polymorphnuclear leucocytes. In most cases only one crystalloid body was seen in any one neutrophilic cytoplasm. Those bodies were rectangular, most of them showed rounded ends and all of them were surrounded by unstained hallow area. The nuclear membrane was never seen in contact with the crystalloid and the nuclear wall was a prominent and constant feature. The crystalloids stained lightly basophilic using haematoxylin and eosin stain, with feulgen reaction, positive but variable staining intensity was observed in all cases and within one case. The crystalloid bodies showed negative results when specific stains for bile, haemoglobin, and haemosedrin were used. But positive results wih specific inclusion stains, as Gimesa and Maximow haematoxylin azur II- eosin were obtained. In the cells in which crystalloids had been detected, no nuclear alterations could be detected.

### II- Histological findings:

On examination of the liver sections, micromorphological changes including, sinusoidal dilatations, focal hepatic necrosis and reticuloendothelial cell activation and proliferation were seen in the nine cases (Fig. 2). Intranuclear inclusions could be seen in three cases, the same dogs in which intracytoplasmic inclusions were seen in the peripheral blood leucocytes inclusions were acidophilic, round, oval, rectangular, rhomboid in shape and surrounded by unstained hallow zone (Fig. 2). The nuclear chromatin showed either regular margination or condensed at one pole of the nucleus. Their average diameter was 3.5 to 5 micron while the average diameter of the hepatocytic nuclei was 8 to 9 micron and that of the nucleoli was 1.5 to 2.5 micron. The inclusions showed negative results with bile, haemoglobin and haemosedrin reactions, but positive results were obtained with Gimesa and haematoxylin Azur II- eosin stains. The inclusions were also shiff positive hen feulgen reaction was carried out, however there was variation in the degree of intensity of the reaction.

**III- Haematological findings:**

According to our haematological findings, table (1), the dogs could be classified into two groups, firstly group one which included eight dogs and showed insignificant difference from the haematological parameters reported by COLOS (1980). Secondly, in group two which included nine dogs, the total leucocytic count was decreased and ranged from 2.200 to 10.800 with the mean value of 6.500. Packed cell volume, neutrophils and lymphocytes were decreased. Neutrophils ranged from 6% to 17% with the mean value 11.55%. Monocytes and eosinophils remained within the normal range. The dogs in which, intrahepatocytic inclusions, intraleucocytic crysalloids, and hepatomorphologic changes are in group two whic showed the haematologic alterations mentioned before.

## *DISCUSSION*

The present study was carried out on seventeen adult stray dogs obtained from different localities in Assiut Governorate. Hepatopathic alterations including sinusoidal dilatations, focal necrosis as well as reticuloendothelial cell activation and proliferation were noticed in nine animals. Haematoloically those animals showed leucopenia, lymphopenia and decreased packed cell volume. From these nine dogs three animals showed lightly basophilic intracytoplasmic crystalloid bodies in the peripheral blood polymorphnuclear leucocytes as well as acidophilic intranuclear hepatocytic inclusion bodies.

Although the sinusoidal dilatations noticed could be related to some extent to the electric shock used for sacrification, the cavernous dilatations could be assumed to the necrobiotic changes seen in the hepatic columns. Moreover such dilatations and focal hepatic necrosis were reported from the pathognomonic features in infectious canine hepatitis, JUBB & KENNEDY, (1970).

The relatively small mecrotic areas seen in our study could be probably related to the age of the animals and the chronocity of infection. The slight reticuloendothelial cell activation and proliferation detected in our findings are explained as universal reaction in viral diseases and canine hepatitis virus has an affinity for such cells, HUNT et al.,(1963) JUBB & KENNEDY (1970), SMITH, JONES and HUNT (1974).

COFFIN and CABASSO (1953), SCHALM, JAIN and CORROLL, (1975) has reported as in our findings similar leucopenia, lymphopenia and decreased cell volume in experimental and natural canine hepatitis infection. Regarding the presence of crystalloid inclusions seen in the leucocytes of three dogs manifested the forementioned heaptopathic alterations and the haematologic findings may probably strength the suggestion of a cell associated viraemia with migration of the infected circulating neutrophils into the body as the role played by lymphocytes in many other diseases, GHADIALLY, (1975). Although it has been suggested that excessive protein production is followed by crystallization, in the present work crystalloids could be detected in polymorphnuclear neutrophils which in their adult form synthesize little protein, CHEVILLE, (1976), and that perhaps refuse the suggestion that it could be considered as an host protein and suggest its viral origin as it was supposed in intrnuclear hepatocytic crystalloids by GIVAN and JEZEQUEL (1969), JEZEQUEL and STEINER (1966).

In our study both intraleucocytic crystalloids and intrhepatic inclusions seen in three animals. Both of the inclusions showed, positive inclusion body reactions, negative reactions for bile, haemosedrin, and haemoglobin and the two were surrounded by distinct hallow zones. Using haematoxylin and eosin the leucocytic crystalloids were lightly basophilic but the hepatocytic inclusions were acidophilic, this tinctorial variation could be probably related to the

stage of formation and their DNA content. Both types of inclusions were positive with feulgen reaction and all the forementioned histochemical properities were, the same described in the hepatocytic nuclei in infectious canine hepatitis, HUNT et al., (1963), and also as the crystalline intranuclear inclusions described in the hepatocytes, BAYOUMI, YOUSSEF and IBRAHIM, (1983).

In nine cases, 52.9%, the haematologic findings and the hepatopathic altrations as well as the inclusions in three of them could be probably related to infectious canine hepatitis. The relative high percent of infection could be explained that, these were adult stray dogs and it is well known that the recoverd animals from this disease considered as carriers and the persistance of the virus in the urine act as a factor in spread and dissemination of infection among canines, SEIBOLD and GREENE, (1954).

In conclusion, although the haematological findings, the hepatic alterations and the association of inclusions in both neutrophilic leucocytes and hepatoces in the same animals may suggest an infectious canine hepatitis infection, because the viral inclusions could be specifically identified with the usage of immunologic reagents such as fluorscent antibody techniques and ultrastructural examinations are needed to prove the association of leucocytic crystalloids in infectious canine hepatitis diseased animals or even only in carriers. The result will be valuable criterion for diagnosing such infected animals by means of a rabid, simple and routine blood film method instead of liver biopsy techniques and their possible dangerous side effects.

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## *TABLE (1)*

Showing the results of the haematological parameters

| Group Parameter | Group One | | Group Two | |
|---|---|---|---|---|
| | range | mean value | range | mean value |
| Pcv % | 39-51 | 45 | 34-40.44 | 38.22 |
| WBC /mm | 8.800-16 | 12.400 | 2.200-10.800 | 6.500 |
| Neutrophils % | 63-72 | 67.5 | 34-50 | 4 3 |
| Lymphocytes % | 14-32 | 21.00 | 6-17 | 11.55 |
| Monocytes % | 4-8 | 6.00 | 4-8.14 | 6.07 |
| Eosinophils % | 3.02-9.0 | 6.01 | 3.32-8.22 | 5.77 |

## *DESCRIPTION OF FIGURES*

**Fig. (1 a, b and c):** Showing large abnormal, crystalloid intracytoplasmic inclusion bodies within the polymorphnuclear leucocytes. (H. & E. X 100).

**Fig. (2)** Liver showing sinusoidal dilatation a, (H.%E. x 250), degenerative changes b, (H.&E. x 250) and focal necrosis c, (H.&E. x 160).

**Fig. (3 a and b):** Showing intranuclear inclusions in the hepatic cells (H.&E. x 1000).

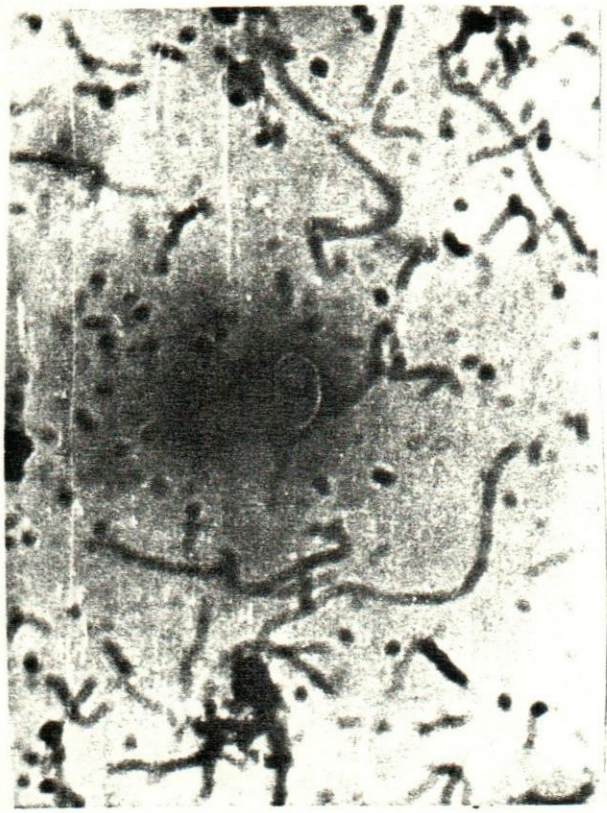

Fig. 1 a

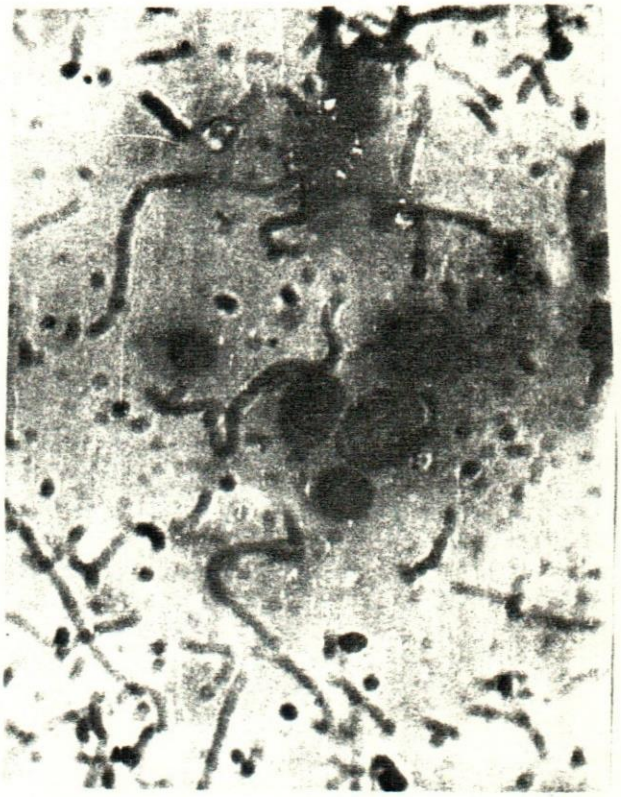

Fig. 1 b

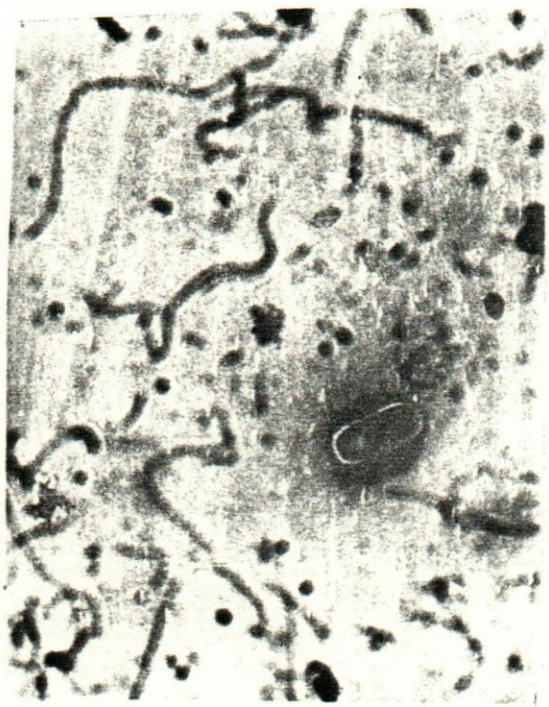

Fig. 1 c

Fig. 2 a

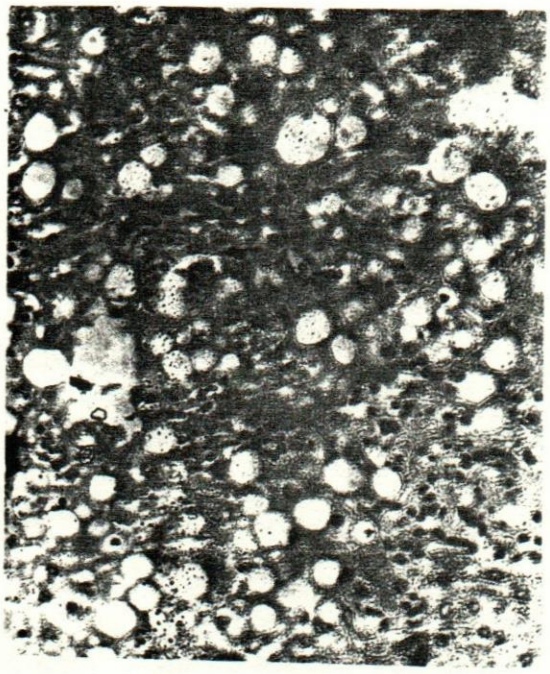

Fig. 2 b

Fig. 2 c

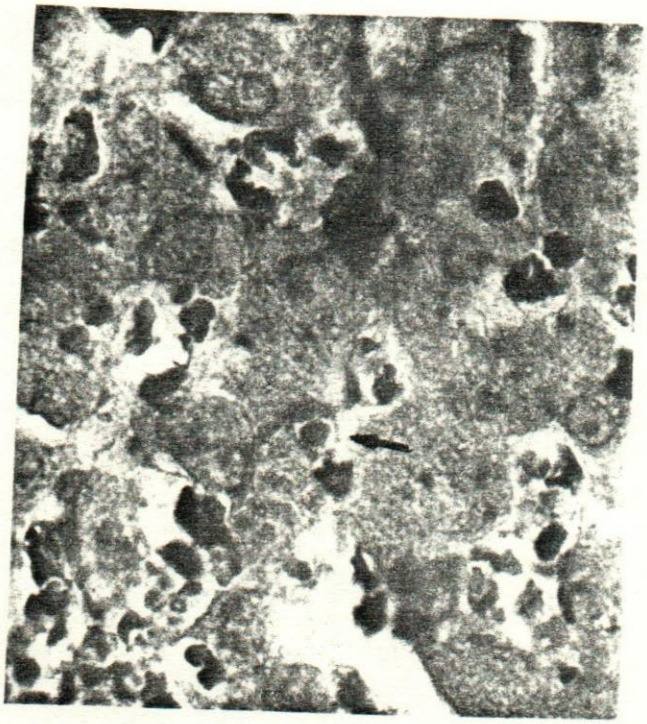

Fig. 3 a

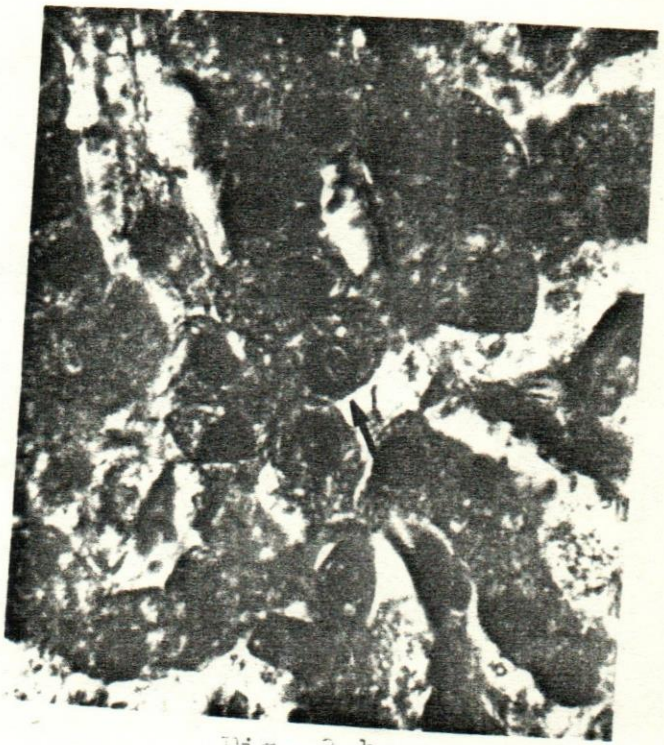

Fig. 3 b