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DETECTION OF THEILERIA-CARRIER ANIMALS BY LYMPH NODE BIOPSY AND FURTHER CONFIRMATION BY MORPHO PATHOLOGY OF THE AFFECTED NODES

(With 13 Figures)

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إكتشاف الحيوانات الحاملة لطفيل الثيليريا بإستخدام خزعات من الغدد الليمفاوية وتأكيدها بالفحص الباثولوجي للغدد الليمفاوية المصابة

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من إجمالي ١٥٠ (مائة وخمسون) رأس جاموس في مزرعة المنيا الحكومية كان هناك عدد ٢٢ (أثنان وعشرون) حيوان لوحظ عليهم أرتفاع في درجة حرارة الجسم (٤٠ درجة مئوية) وفقدان الشهية وتضخم في الغدد الليمفاوية السطحية. تم إكتشاف طفيل الثيليريا في دم هذه الحيوانات . بعد إعطاء العلاج المتاح إختفى طفيل الثيليريا من الدم ولكن الحالات التي تم علاجها ظلت في حالة صحية رديئة وبعد ذلك سجل نفوق عدد ٨ (ثمانية) حيوانات. بفحص الخزعات المأخوذة من الغدد الليمفاوية السطحية المتضخمة تم إكتشاف أطوار اطفيل في الخلايا الليمفاوية مما يعني وجود حالات حاملة المرض. تم التأكيد من الحالات الحاملة للمرض بإستخدام الفحص الباثولوجي لنسيج الغدد الليمفاوية. النتائج المتحصل عليها في هذه الدراسة أشارت إلى أن طريقة أخذ خزعات من الغدد الليمفاوية السطحية هي طريقة يمكن الأعتماد عليها في إكتشاف الحيوانات الحاملة لطفيل الثيليريا.

SUMMARY

A total of 22 buffaloes (22 / 150) at El-Minia governmental farm manifested fever (40 C), anorexia and enlargement of the superficial lymph nodes (precrural and prescapular nodes). Theileria parasite was detected in the blood films prepared from these animals. After application of the convenient treatment, parasitaemia disappeared but the

relevant cases remained debilitated and thereafter 8 cases died. Examination of the biopsy samples taken from the superficial lymph nodes of the treated animals revealed schizont-infected lymphoblasts. This indicated a carrier state of theileriasis. The condition was confirmed by the pathomorphology of the affected lymph nodes. The pathological picture was that of the late stage of theileria infection. The obtained results indicated that the method of lymph node biopsy is a reliable mean for the detection of theileria-carrier animals.

Key words: *Theileriasis- Lymph node- Biopsy- Pathology.*

INTRODUCTION

Tropical theileriasis is a tick-borne disease of cattle caused by the protozoan parasite, *Theileria annulata* (Barnett et al., 1961; Krier et al., 1962; Valli and Party, 1993). The disease is widespread and causes morbidity and mortality in indigenous and imported breeds of cattle (Sharma and Grautam, 1971; Gill et al., 1977; Purnell, 1978; Jones et al., 1997). In Egypt, the infection percentage was found 59.36% and 23% in cattle and buffaloes, respectively (Rizkalla, 1991). Infection rate among cows and buffaloes reached 10% in Assiut locality (Fahmy, 1980). This means that *Theileria annulata* is the most important blood parasite among animals in our localities.

Incomplete recovery leads to the presence of carrier animals which manifest debilitation and loss of productivity (Srivastava and Sharma,1981). Carrier animals are undoubetdly represent a great risk for dissemination of the disease and their detection and treatment are important for the control of theileriasis.

Various molecular biological methods were used by some workers (Pipano&Cahana, 1969; Bishop et al., 1992; D'Olivera et al.,1995) for detection of theileria-carrier animals. However, these methods require special laboratory procedures which are extremely difficult to be applied in the field. As far as we know, there are no other reported practical methods which can be used in the field conditions to detect theileria-carrier animals.

Therefore, the present work was undertaken to determine whether the method of biopsy sampling from the superficial lymph nodes is a reliable and practical mean for the detection of theileria-

carrier animals. Also, the pathomorphology of affected lymph nodes was studied for further confirmation of diagnosis.

MATERIALS and METHODS

Case history:

Out of 150 buffaloes at El-Minia governmental farm, 22 animals manifested fever (40°C), anorexia and recumbency. Microscopic examination of blood films prepared from these animals revealed theileria infection. After application of the convenient treatment, body temperature returned normal but treated animals remained debilitated and had enlarged superficial lymph nodes (prescapular and precrural nodes). Thereafter, 8 cases died (8/22) after manifesting a bad health condition.

Blood films:

Blood samples were obtained regularly from the suspected 22 animals (before and after treatment) for detection of parasitaemia . Blood was obtained from ear veins and thin blood films were made and stained with Giemsa's stain.

Biopsy samples and smears:

Biopsy samples were obtained from all 22 animals which showed enlargement of one or more of the superficial lymph nodes, such as prescapular and precrural nodes. Samples from these nodes were taken by using the proper biopsy needles. Impression smears were prepared by just apposing the tissue samples to the surface of glass slides. The nodal fluid drained with the biopsy samples was also smeared. Smears were air-dried, fixed in methanol for 3 minutes and air-dried again before staining with Giemsa's stain.

Histopathology:

Tissue samples were collected from the spontaneously dead animals (8 cases) which had enlarged superficial lymph nodes. In addition to the nodal samples, all other organs and tissues including liver, lungs, kidneys, spleen, heart, and adrenals, were represented. Tissue specimens were fixed in 10% neutral buffered formalin and processed routinely for paraffin embedding technique. Embedded tissues were sectioned at 3 u and stained with haematoxylin and eosin (HE) (Bancroft and Stevens, 1982).

Tissue imprints:

Imprints from liver, lungs, kidneys, spleen, heart and adrenals, obtained from the freshly dead animals, were also prepared and stained with Giemsa's stain.

Electron - microscopy:

Proper samples (1 mm cubes) prepared from nodal biopsies were fixed by immersion in 3% buffered glutaraldehyde and then post-fixed in 1% osmium-tetroxide. Tissue samples were then dehydrated in ascending grades of ethanol and embedded in Epon 812. Toluidine blue-stained semi-thin sections were prepared for tissue orientation. Subsequently, thin sections were made and counterstained with lead citrate and uranyl acetate and examined under transmission electron-microscope (JEOL, CX II 100) operated at 80 kv.

RESULTS

Blood films:

Examination of blood films prepared from the suspected cases before treatment revealed the presence of theileria infection (Fig.1 a&b). Schizonts were detected in lymphoid cells. Parasitaemia disappeared after treatment of infected animals.

Smears and imprints:

The examined nodal smears revealed large number of schizont-infected lymphoblasts. Schizonts in the infected lymphoblasts appeared as tiny cytoplasmic bodies of bluish tint (Fig. 2 a&). In many cells, the schizonts tended to locate at the cytoplasmic margin. Schizont-infected lymphoblasts were also detected in the splenic imprints.

Gross pahological findings:

Lymph nodes of the dead animals (prescapular and precrural nodes) were enlarged and edematous and their capsules were turgid and showed whitish gray coloration. Some nodes had haemorrhagic areas. There were splenic enlargement and pulmonary congestion. Kidneys and liver were pale.

Histopathology:

Histological changes of lymph nodes, specially those of prescapular nodes, were the most remarkable. Capsules of these nodes were edematous and infiltrated with lymphocytes. The affected lymph nodes (grossly enlarged) in general were hyperplastic having many lymphoblasts and expanded paracortical areas. Paracortex contained

numerous lymphoblasts and showed large nubmer of mitotic figures (Fig. 3). Lymphoid follicles had small-sized germinal centers (inactive germinal centers with decreased number of mitotic figures) and thickened mantle zone. Considerable number of germinal centers were necrotized and this was evidenced by the presence of hyalinized material (necrotic debris) and debris-filled macrophages (Fig. 4). In some nodes, the paracortex was expanded but cell density was low. Some paracortical zones contained large number of vacuolated reticular phagocytes associated with the presence of degenerated blast cells. Medullary cords had increased number of lymphoblasts but less number of plasma cells. In association with lymphoblasts, medullary cords contained large nuumber of vacuolated reticulum cells (Fig.5). The nodal sinuses (subcapsular, intermediate and medullary sinuses) contained many lymphoblasts (mitotically active) and degenerated cells (Fig. 6). The perisinusal areas contained plasma cells. Trabeculae of affected nodes were infiltrated by lymphoblasts and small lymphocytes.

Spleen showed increased number of lymphoblasts in the white pulps but lymphoid follicles were decreased in size. Liver disclosed congestive edema and vacuolar degeneration of hepatocytes (Fig.7). Haemorrhages were scattered throughout the hepatic tissue. The hepatic peri-portal areas had increased number of lymphocytes. Focal hepatic necrosis was also noticed. Lungs had thickened alveolar septa and this thickening was due to edema and infiltration by lymphoblasts (Fig. 8). Dense peri-bronchiolar lymphoid cell aggregates were frequent (Fig. 9). Kidneys revealed tubular necrobiotic changes and enlarged glomeruli which had distended capillaries and moderately proliferated mesangial cells. Myocardial tissues showed perivascular and inter-myofiber edema and numerous extravasated erythrocytes (Fig. 10). Adrenals displayed cortical degenerative changes and widespread haemorrhages (Fig. 11).

Electron - microscopy:

The examined nodal biopsies reaveled the presence of numerous lymphoblasts. Nuclei of blast cells had large prominent nucleoli, much euchromatin and condensed heterochromatin. Cytoplasm of parasitized lymphoblasts contained schizonts which appeared as varied-sized electron-dense bodies. Some blast cells contained, in addition to schizonts, merozoites which had varied numbers of nuclei (Fig.12 a&b). Parasitized cells, in general, had copious cytophsm, numerous polyribosomes, few mitochondria, dilated granular ER and small Golgi apparatus. The cytoplasm of many cells was vacuolated and some had

multi-vesicular bodies (Fig. 13). Some parasitized lymphoblasts had indented nuclei. Many degenerated lymphoblasts were detected and these cells were evidenced by their condensed cytoplasm and shrunken nucleus. The recognized phagocytic reticulum cells contained many lysosomal structures and phagosomes.

DISCUSSION

In the present study, biopsy samples from lymph nodes of suspected animals were employed to detect theileria - carrier animals. For confirmation of the carrier state, pathological features of the nodal tissues obtained from some suspected spontaneously dead animals were also studied. Parasitaemia in the present cases disappeared after treatment. Thereafter, some of the treated animals died and the survivors were debilitated and parasitized cells were detected in the superficial lymph nodes. This indicates the development of a carrier state in the surviving animals. The presently demonstrated pathological picture of lymph node included expansion of paracortical zones by many proliferating lymphoblasts, necrosis of germinal centers and infiltration of nodal sinuses by lymphoblasts. Similar picture was described in the late hyperplastic stage of theileriasis (De Martini and Moulton, 1973) which may also points to a carrier state. Presence of many blast cells in the nodal tissues substantiates the conclusion that theileria parasite causes transformation of lymphoid cells (De Martini and Moulton, 1973 and Ole-Moi Yoi, 1989). This conclusion was based upon the morphological similarity between the parasitized lymphoblasts and the phytohaemaglutinin (PHA)-stimulated lymphoblasts.

Presence of large number of vacuolated reticulum cells in the affected nodes may reflect the contribution of these cells in removal of the parasitized degenerated cells. None of the presently observed parasitized cells contained lysosomal or phagosomal structures which indicates that these cells are not phagocytic cells. In a recent work, Forsyth et al. (1999) found that many of the theileria-parasitized cells are phagocytic mononuclear cells by using monoclonal antibodies (Mabs) against some characteristic soluble products (cytokines) of these cells. Therefore, the point of what the target and disseminating cells of theileria parasite will be the focus of our future study.

The observed necrosis in the nodal germinal centers is probably attributed to some sort of immune reaction to parasitic antigens. Forsyth

et al. (1999) attributed the tissue damage in theileria-infected nodes to the harmful effect of some products, such as tumor necrosis factor (TNF) and interferon (IFN-) which released from parasitized cells. The immune response in the present parasitized nodal tissues seems to be mainly of cellular nature as evidenced from the blastogenic changes.

Other organ and tissue changes described here are probably ascribed to infiltration by parasitized lymphoblasts or to the damaging effect of the parasitic antigens. The haemorrhagic lesions in the hepatic and adrenal tissues may confirm the concept of antigenic vascular damage.

Here we employed a quick and easy method (nodal biopsy) to detect theileria-carrier animals. There are other methods which employ molecular biological techniques such as fluorescent antibody test and polymerase chain reaction (PCR) (Pipano and Cahana, 1969; Bishop et al., 1992 and D'Oliveira et al., 1995) for detection of theileria infection. However, these methods are expensive, time-consuming and practically unapllicable in the field conditions.

Conclusively, the present results indicate that the method of nodal biopsy may be considered as a beneficial mean for detection of theileria- carrier animals. This method was further confirmed by the morphopathology of the affected nodes. Thus, carrier animals can be treated or discarded to overcome the risk of parasite transmission.

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LEGENDS FOR FIGURES

Fig. 1. Blood films from parasitaemic animals. Schizonts (arrows) in (A) are located at the cytoplasmic margin while in (B) located near the lymphoblast nucleus. Giemsa's stain. X 1000.

Fig.2a&b Impression smears from precrural lymph node showing parasitized lymphoblasts. Schizonts (arrows) appear as cytoplasmic tiny bodies of bluish tint. Giemsa's stain. X 1000.

Fig. 3. Expanded paracortical area containing numerous lymphoblasts. Nuclei of blast cells are pleomorphic. Mitotic figures (arrows) can be also seen. Precrural lymph node. HE. X 200.

Fig. 4. Lymphoid follicle in the precrural lymph node showing necrotized germinal center (G) which contains lyalinized material, degenerated lymphoid cells and vacuolated phagocytes (arrow). Mantle zone (M) of the follicle is thickened. HE. X 320.

Fig. 5. Medullary cords containing large number of vacuolated reticulum cells (arrow). The area is also infiltrated with lymphoblasts. Precrural lymph node. HE. X 320.

Fig. 6. Subscapular sinus (*) of precrural lymph node containing many lymphoblasts. HE. X 320.

Fig. 7. Liver shwoing vacuolar degeneration of hepatocytes which are swollen and of irregular outlines. Haemorrhages are also seen. HE X 320.

Fig. 8. Lung showing thickened alveolar septa (S). Thickening is due to congestion and edema. HE. X 320.

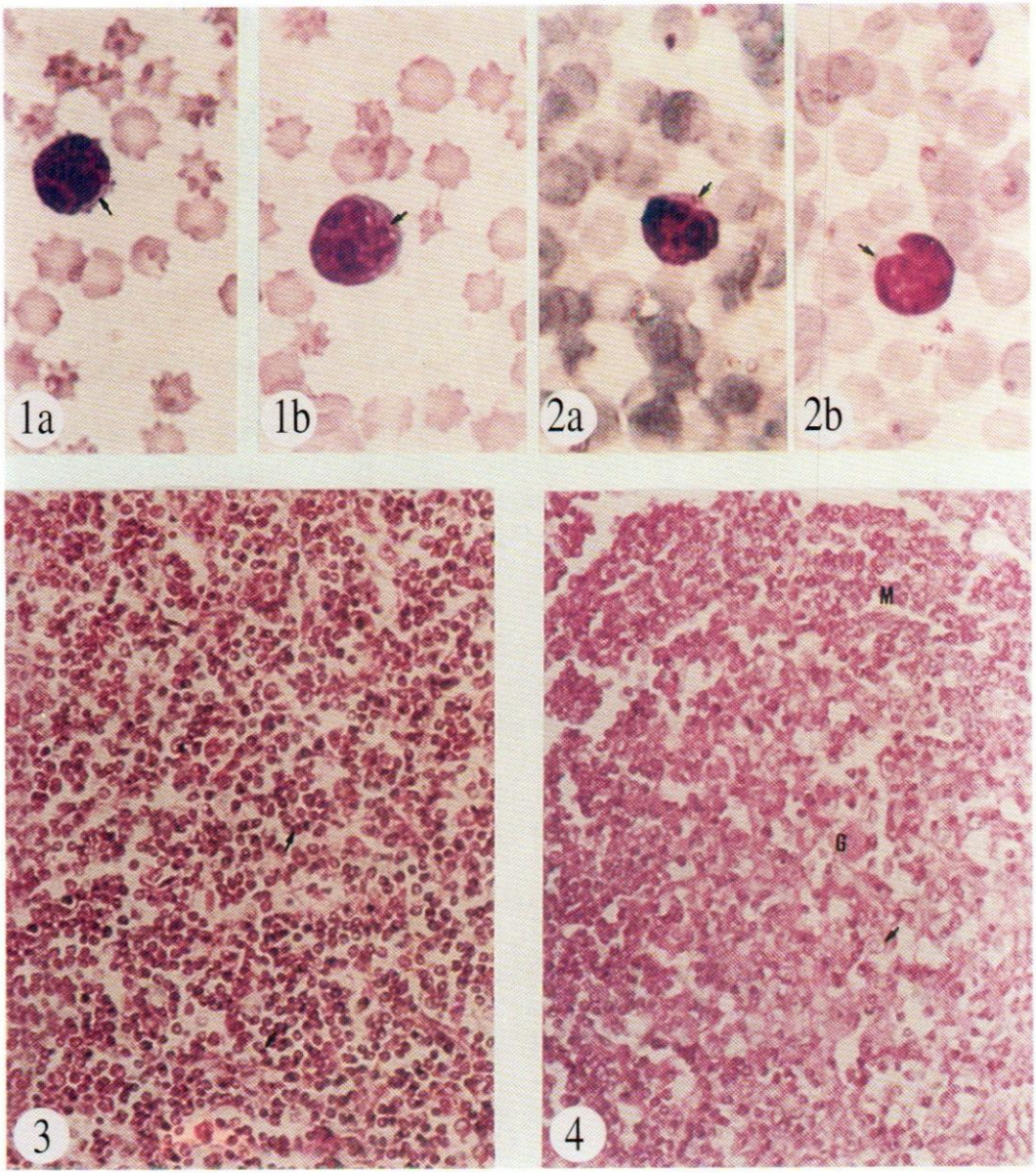
Fig. 9. Dense peri-bronchiolar lymphiod cell aggregate (L) in the lung of an infected animal. The bronchiolar epithelium (arrow) is hyperplastic. HE. X 320.

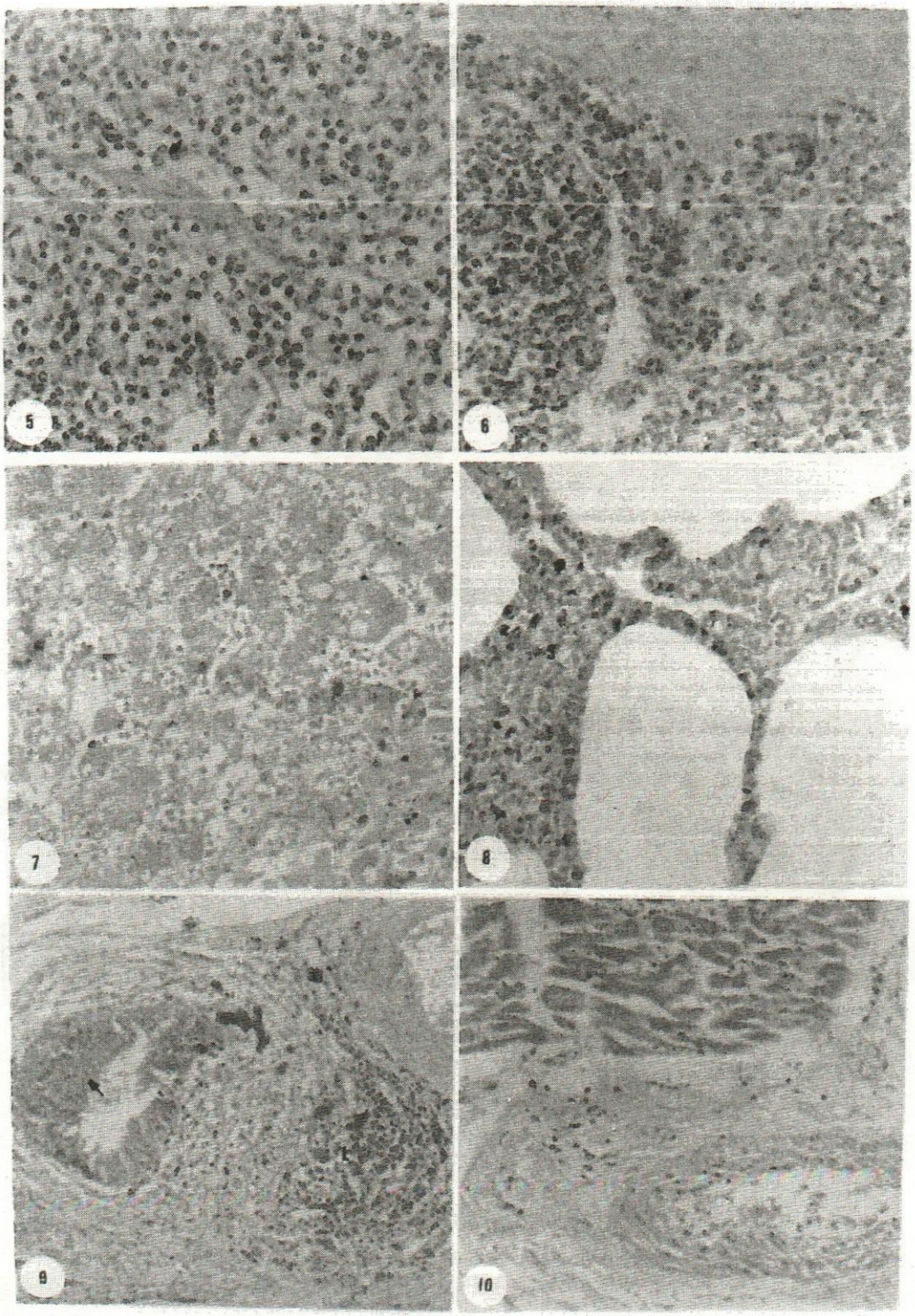
Fig. 10. Heart showing intermyofiber and perivascular edema. Many extravasated erythrocytes are seen between myofibers. HE. X 200.

Fig. 11. Adrenal cortex showing widespread haemorrhages and degeneration of cortical cells. HE. X 200.

Fig.12a&b. Transmission-electron micrographs showing merozoites (a) (arrow) and schizonts (b) (double arrows) in the cytoplasm of lymphoblasts. Nuclei of parasitized lymphoblasts are relatively shrunken. Precrural lymph node. X 7500.

Fig.13. Lymphoblasts showing cytoplasmic vacuolation due to mitochondrial and endoplasmic reticulum dilatation. Precrural lymph node. Transmission electron-micrograph. X 6000.

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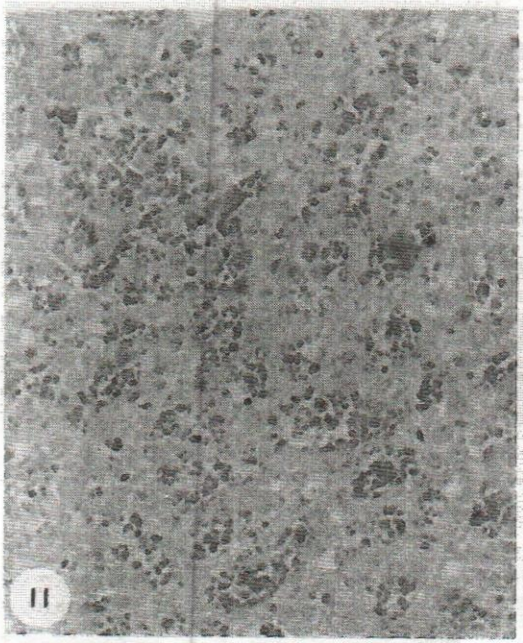
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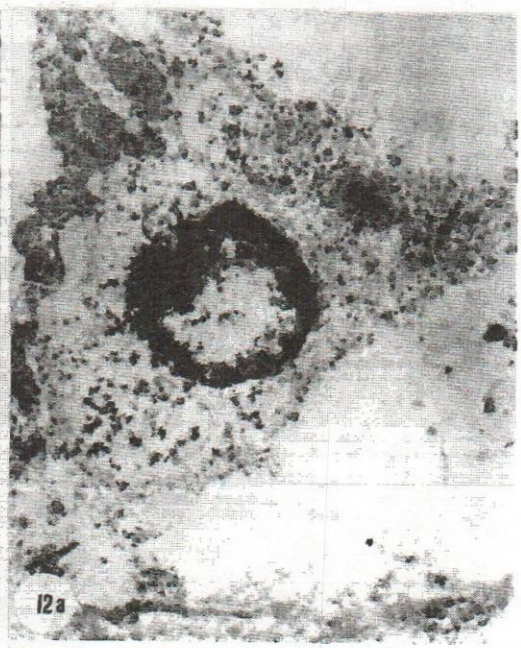
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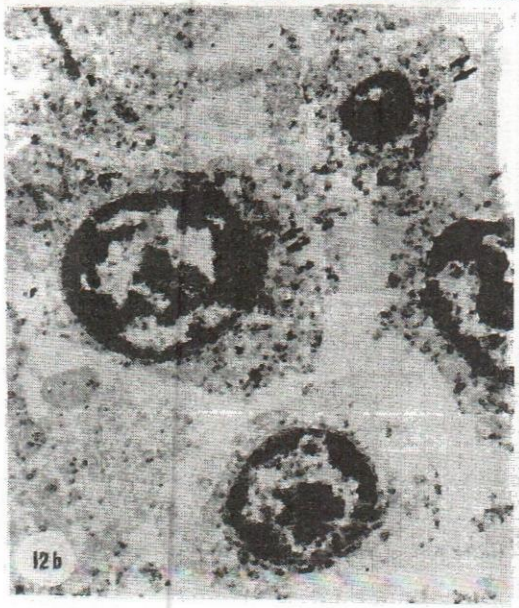
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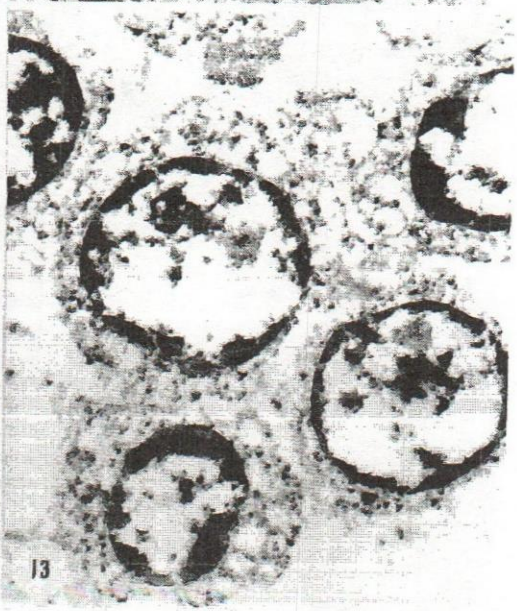
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