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د راسة ميكروسكوبية لمسحات من الانسجة والدم للطيور  
المصابة بمرض الالتهاب الكيسى

عبد اللطيف بيومى ، صلاح موسى ، خيرى ابراهيم ، صلاح ديب

تمت د راسة ميكروسكوبية للتغيرات الباثولوجية فى مسحات من كيس مصلى ، الطحال ، الكلية  
الكبد ، الدم . أخذت المسحات من الكتاكيت اللجهورن بعد أن أعدت فى العين بعشرة  
ضاربة لمرض الالتهاب الكيسى .

كانت أهم التغيرات هى نشوء تجاويف أو حويصلات بالأنبوية وكذلك انحلال الكروماتين  
والسيتوبلازم الخاص بالكريات الليمفاوية ، الطلتهمة بالسد الشبكية فى كل من مصلى والطحال  
وكذلك الكريات الليمفاوية والطلتهمة بالدم كما لوحظ نشوء تجاويف بالسيتوبلازم الخاص بالخلايا  
الطلائية للكلية ولم تلاحظ أى تغيرات بالخلايا الكبدية .

تمت مناقشة النتائج وأوضحت النتائج أهمية مسحات الانسجة والدم فى التشخيص بالتفريق  
لمرض الالتهاب الكيسى بالكتاكيت اللجهورن .

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## MICROSCOPICAL STUDIES OF SMEARS FROM TISSUE AND BLOOD OF BIRDS AFFECTED WITH INFECTIOUS BURSITIS

(With 9 Figures)

By

A. BAYOUMI, S. MOUSA\*, M.K. IBRAHIM and S. DEEP

(Received at 23/3/1982)

### SUMMARY

Changes in bursa, spleen, liver and blood following intraocular application of highly pathogenic strain of infectious bursal disease, strain Cu-1, were studied in smears from infected Leghorn chicks. These changes consisted of nuclear vacuolation, and chromatolysis associated with cytoplasmolysis in lymphocytes, macrophages and reticular cells of bursa and spleen, lymphocytes and macrophages in the blood and cytoplasmic vacuolation in the epithelial cells of the kidney, while no changes were detected in the liver. It was suggested that, by intraocular application the virus may be transmitted to predilection seats associated with the lymphocytes. Smears from tissues and blood of infected birds seems to be helpful in diagnosis of the disease.

### INTRODUCTION

Pathological changes in infectious bursal disease (IBD) are known to occur mainly in lymphoid structures and kidneys. The disease, however, may be confused with other infections; bursal atrophy was recorded by JAKOWSKI *et al.* (1969) in Marek's disease. Water deprivation may be associated with atrophied bursae and nephrosis (HOFSTAD *et al.*, 1978). The latter condition was also observed in outbreaks due to certain strains of infectious bronchitis (WINTERFIELD and HITCHNER, 1962). Studies carried out were based mainly upon histopathological examinations (CHEVILLE, 1967; MANDELLI *et al.*, 1967; PETERS, 1967; LUNGER and MADDUX, 1972; MANDELLI and VALERI, 1972 and DONGANKAR *et al.*, 1979). Electron-microscopic studies on the disease were reported by KAEUFER and WEISS (1976), these studies dealt mainly with changes in the bursa.

Cytopathological investigation using the smear technique not only of the bursa but also of other affected organs and blood, seem therefore necessary to elucidate the cellular reaction in the disease which may be helpful in differential diagnosis.

### MATERIAL and METHODS

**Virus:** The Cu-1 strain of Gumboro disease was used in the present study. Infected chorioallantoic membranes (CAM) were homogenized, suspended 1:1 in normal saline, centrifuged and the supernate was stored as stock at - 20 °C. The 50% embryo infective dose (EID<sub>50</sub>) was determined by the method of REED and MUENCH (1938) by CAM inoculation in groups of 5 chicken embryos per virus dilution.

**Agar-gel precipitin test:** The microtechnique was carried out after CULLEN and WYETH (1975), to detect precipitinogen in bursae of experimental chicks.

**Chicks:** Two groups, one-day old and four-weeks old leghorn chicks were supplied by the farm of the Faculty of Agriculture, Assiut University. Birds were checked for precipitins to IBD virus before being inoculated. Each bird was infected intraocularly with a virus dose of 10<sup>4</sup> EID<sub>50</sub>. Birds were sacrificed six days postinoculation (pi) and the bursae were subjected to agar-gel precipitin test and for smear technique.

Negative controls were made parallel to all experiments. Cytopathological examinations: Smears from bursa, spleen, liver, kidney and blood were made, fixed in ether-alcohol 1:1 and stained with Harris haematoxylin and eosin.

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## RESULTS

Chicks most severely affected were those of four weeks old. In the bursae of these chicks, lymphoblasts, large and medium sized lymphocytes were the cells most specifically affected. Different degrees of severity were observed in different birds. Changes began with nuclear vacuolation of the affected cells. In lymphocytes, the vacuoles were usually eccentric, having a well defined borders, of variable diameters and occurred either single or multiple in the nucleus. Minute or relatively large vacuoles were usually empty, while those of medium size occasionally showed a homogenous acidophilic substance in its lumen, (Fig 1). Similar nuclear vacuolations were also observed in macrophages and reticular cells (Fig. 2 & 3). Other areas showed nuclear chromatolysis of lymphocytes, the chromatin content appeared peripherally condensed and highly refied at the center of the nucleus (Fig. 4 & 5). These nuclear changes were usually associated with cytoplasmolysis or complete disappearance of the cell. More severely affected bursae showed only remains consisting of few pyknotic, darkly-stained nuclei possibly of small lymphocytes, (Fig. 6). Intact polymorphnuclear leucocytes were not seen at any stage and plasma cells were more or less normal.

Changes in the spleen were principally the same as in the bursa (Fig. 7). The main difference from the latter seems to be that they occurred more or less focal indistribution and milder in degree.

In the kidney, cellular changes appeared to be restricted to the epithelial lining of convoluted tubules. The cytoplasm of many of these cells showed large number of tinny empty vacuoles, while there was no detectable changes in the nucleus, (Fig. 8). Microscopical fields were filled with an eosinophilic finely granular substance probably originated from degenerated and disintegrated epithelial cells, many of which were deformed and occurred in the affected areas. No changes were observed in the liver.

Blood smears from affected birds showed, likewise, the occurrence of many abnormal lymphocytes. These cells showed nuclear chromatolysis, stained lightly basophilic and was irregular in shape, while the cytoplasm either appeared ragged due to the presence of many minute vacuoles or showed lysis, (Fig. 9). Totally deformed cells, considered to be lymphocytes, was a constant finding in blood smears of all infected birds. Other types of leucocytes, erythrocytes and blood platelets were not affected while many macrophages (monocytes) were destroyed.

Pursae of infected birds reacted positive in agar-gel precipitin test.

Changes demonstrated in one-day old chicks, either in the bursa, spleen or blood were mild compared to those described above.

No changes could be observed in control non-infected birds.

## DISCUSSION

In bursa of infected birds ASDRUBALI and MUGHETTI (1972) found virus particles in many lymphoid cells and only in very few macrophages. MANDELLI and VALLERI (1972) described virus particles in macrophages only, but stated that the virus replicates mainly in these cells as well as in lymphocytes. KAEUFER and WEISS (1976) demonstrated virus particles in the cytoplasm of lymphoid cells and macrophages early as 6 hours p.i. and in reticular cells latter on. They considered lymphoid cells and macrophages to represent the main areas of virus multiplication and added that the virion, however, can also replicate in other cells like heterophils, reticular cells and reticular epithelioid cells.

In our experimental study changes were found in cells identified as lymphocytes, macrophages and reticular cells. These changes consisted mainly of cytoplasmolysis and characteristic vacuolation and lysis of the nucleus.

Nuclear changes mainly in the form of margination of the chromatin, pyknosis and karyorhexis in viral containing cells were described in ultramicroscopic studies by KAEUFER and WEISS (1976). In the present study, intranuclear acidophilic material found in some cells may represent an altered nucleolus. The presence of intracytoplasmic inclusion-like materials recorded by DONGANOKAR *et al.* (1979) to be found in epithelioid cells as well as in the reticular cells three days after infection were not demonstrated in our materials.



## INFECTIOUS BURSITIS

The fact that the virus replicates in the cytoplasm and in the nucleus of the affected cells indicate that these nuclear changes are mainly secondary degenerative changes. These changes were clearly and easily demonstrated in our materials and are recommended as a rapid, simple and practical diagnostic indices for the disease. Of importance were also changes which occurred in blood of infected birds. It can be suggested, therefore, that by intraocular application of the virus, infection may be transmitted to predilection seats, namely, the lymphoreticular tissue, by lymphocyte virus-associated cells which are known continuously to recirculate to and from the peripheral blood.

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## DESCRIPTION OF FIGURES

- Fig. (1): Bursa showing nuclear vacuolation; one of the nuclei showed an acidophilic material inside a vacuole. H. & E. (x 1000).
- Fig. (2): Bursa showing remains of vacuolated cytoplasm. H. & E. (x 1000).
- Fig. (3): Bursa showing nuclear vacuolation. H. & E. (x 1000).
- Fig. (4): Bursa     a) Control. H. & E. (x 400 )  
                  b) Infected showing nuclear chromatolysis H. & E. (x 400 ).
- Fig. (5): Bursa showing nuclear chromatolysis. H. & E. (x 1000 ).
- Fig. (6): Bursa showing pyknotic nuclei and fragmented cells. H. & E. (x 400 ).
- Fig. (7): Spleen     a) Control H. & E. (x 1000 ).  
                  b) Showing cytoplasmic vacuolation, H. & E. (x 1000 ).  
                  c) Showing nuclear vacuolation, H. & E. (x 1000 ).
- Fig. (8): Kidney showing cytoplasmic vacuolation. H. & E. (x 1000 ).
- Fig. (9): Lymphocytes in blood smear, their nuclei suffer from chromatolysis, stained lightly basophilic and irregular in shape. H. & E. (x 1000 ).

REPORT





Fig. (1)

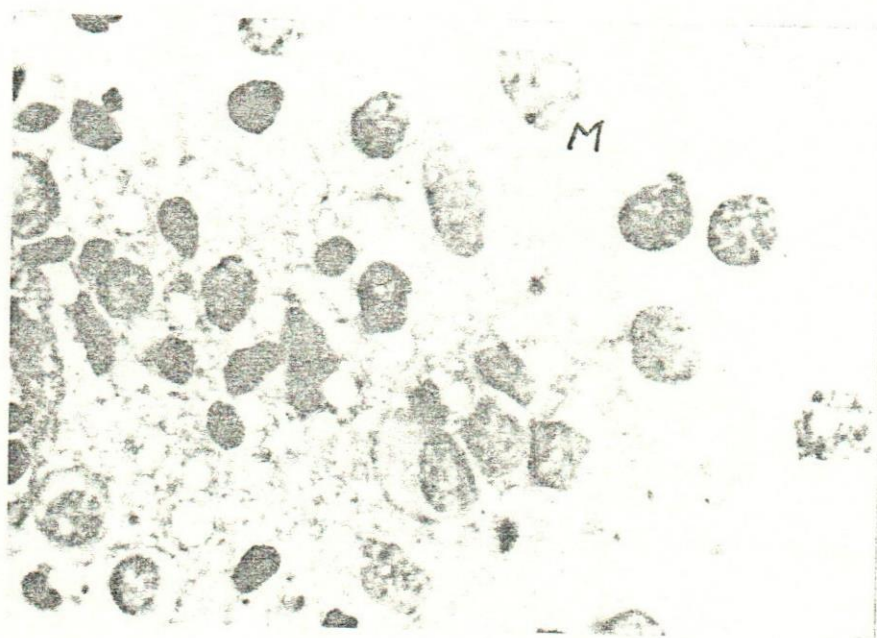


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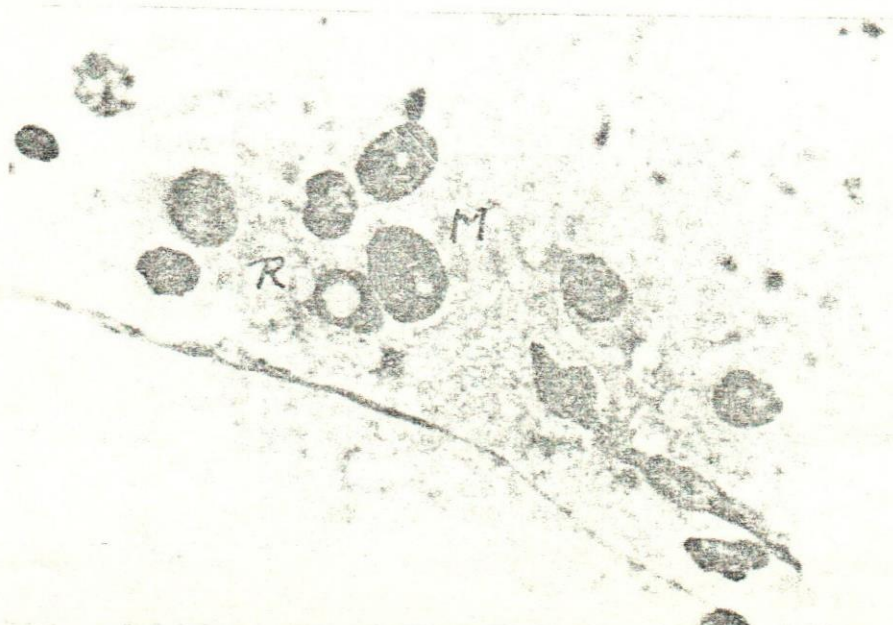
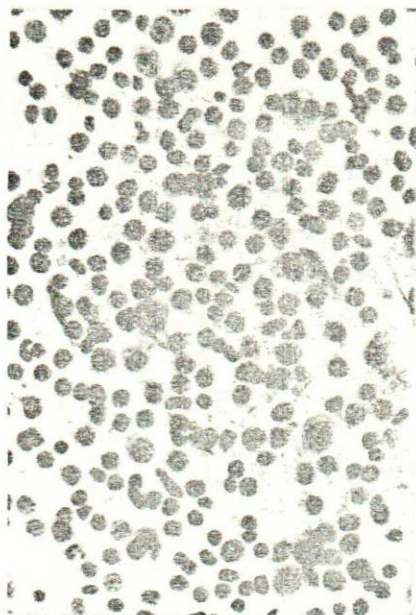


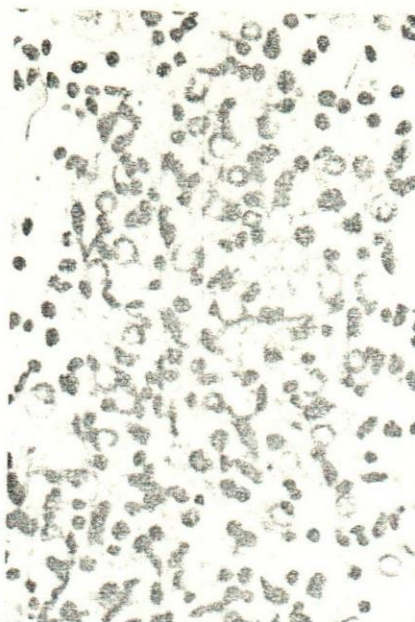
Fig. (3)







(a)



(b)

Fig. (4)

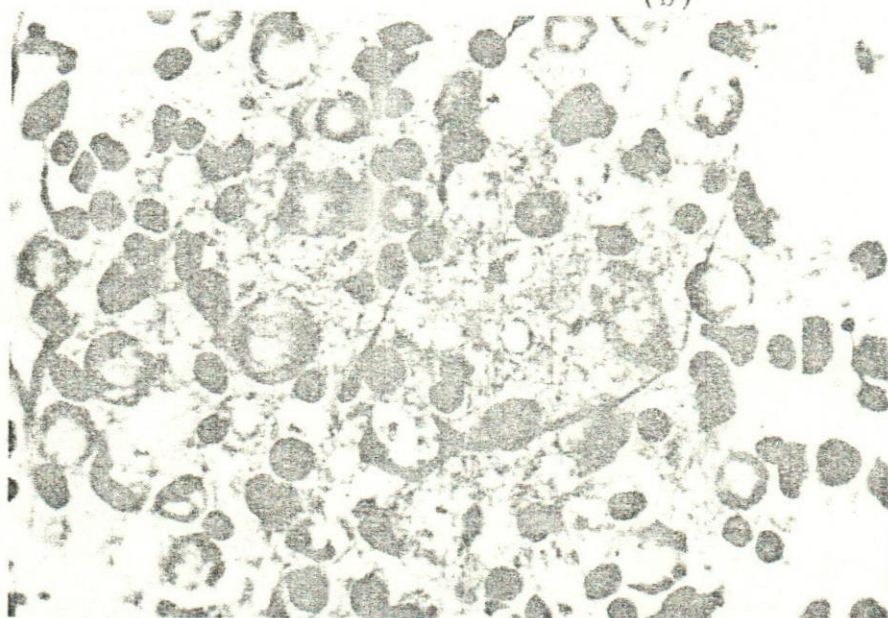


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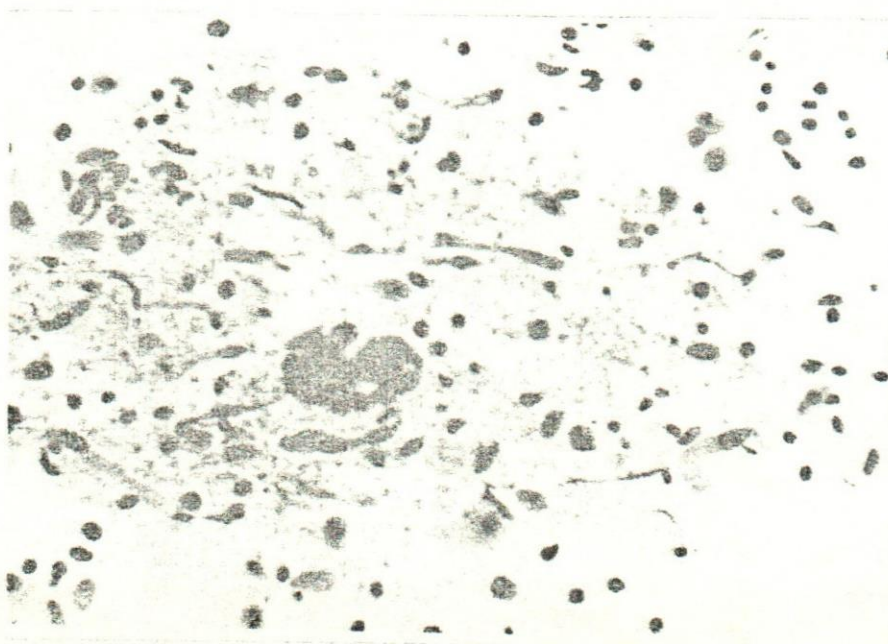


Fig. (6)





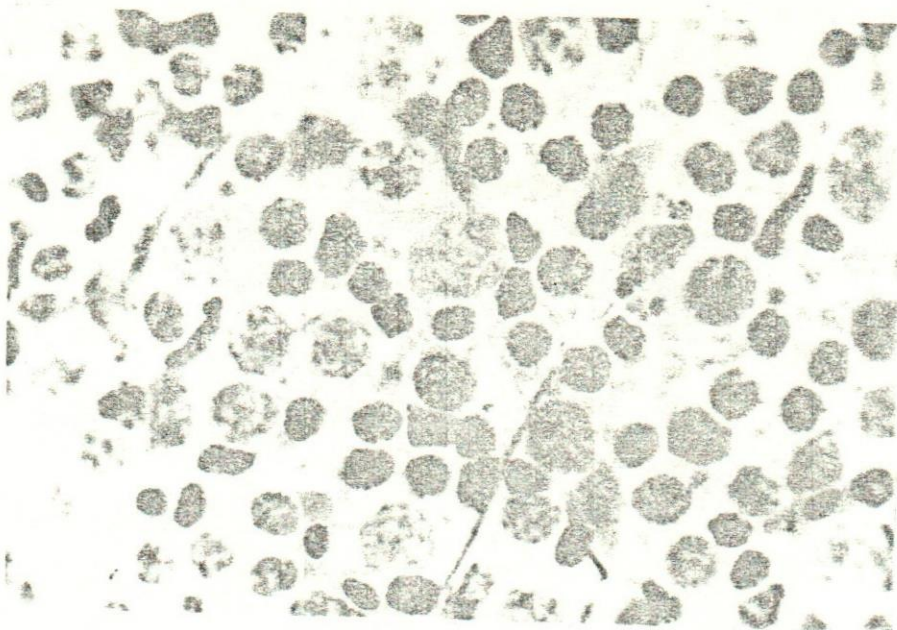


Fig. (7a)

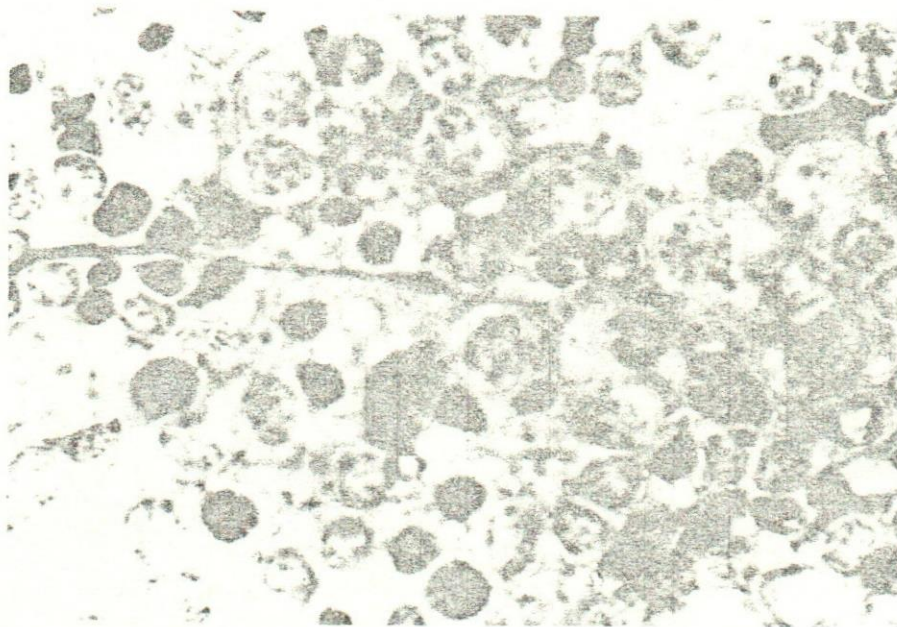


Fig. (7b)

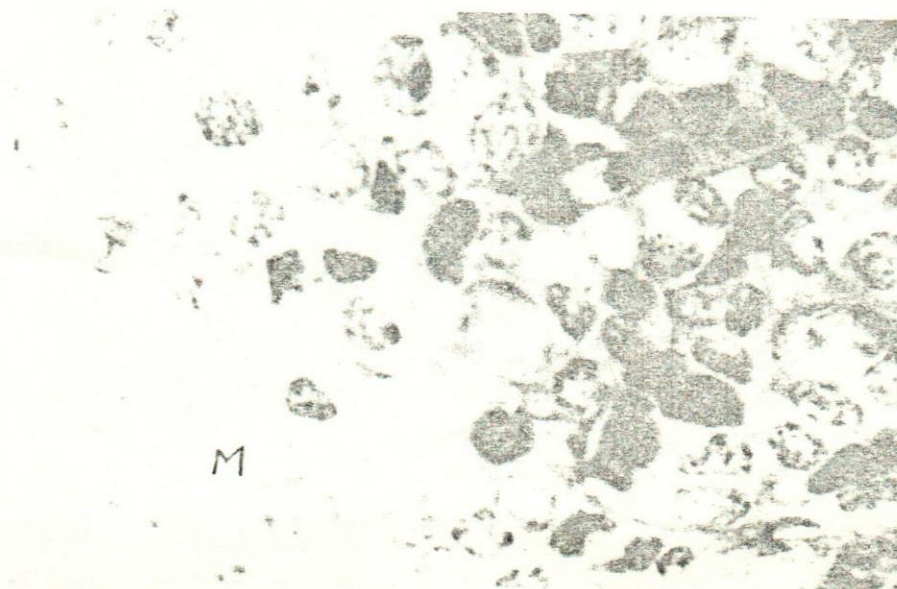


Fig. (7c)





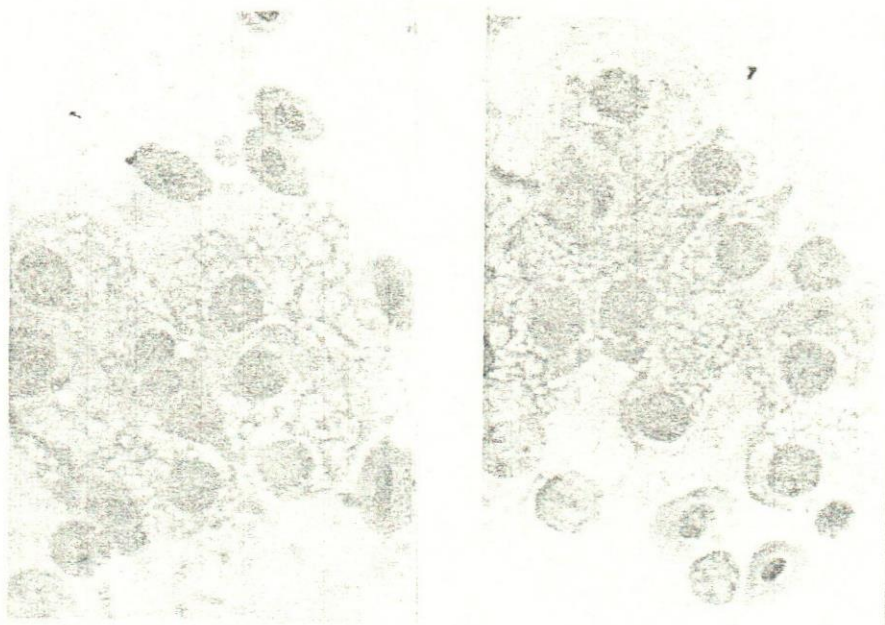


Fig. (8)

(a)

(b)

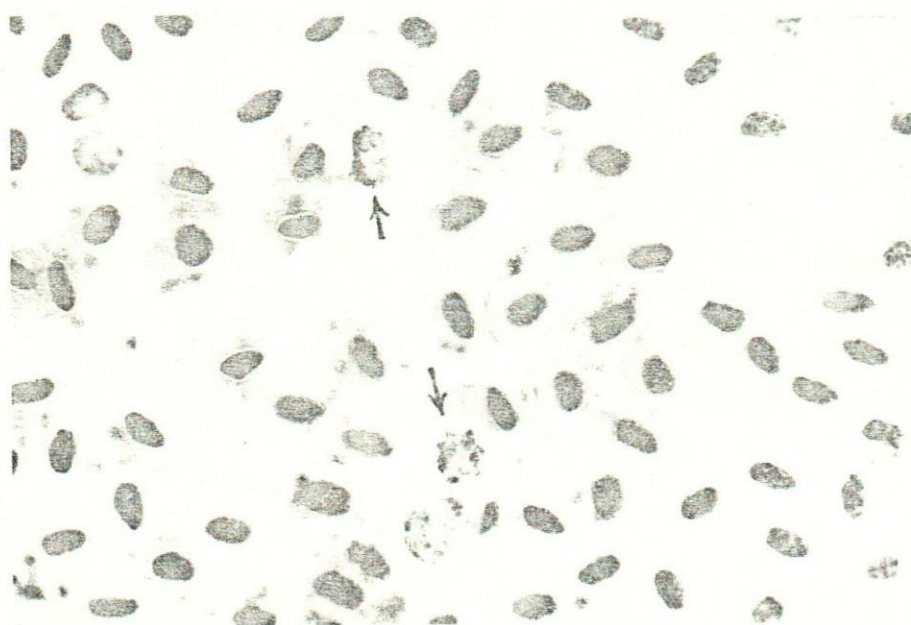


Fig. (9)

