قســـــــم : الباثولوجيا - كلية الطب البيطرى - جامعة أسيوط.
رئيس القسم : أ.د / محمد ابراهيم الشـــرى .

دراسة تجريبية على الاصابة بواسطة ميكروب الكوريـــنى
٢- التغيرات البلثولوجية فى الماشية

عبد الرحمن خاطر ، صـــلاح ديب ، عبد اللطيف سيومى ، حمدى سالم

تم حقن عترة معزولة من ميكروب الكورينى فى جلد خمسة عجول بقرى تراوحت أعمارهم من عمر سنة الى عمر ستة ونصف. أخذت عينات من الجلد قبل الذبح بعد ٤ ، ٨ ، ١٢ ساعه مـــن العدوى. ذبحت الحيوانات بعد ١ ، ٢ ، ٤ ، ٧ ، ١٥ يوما من الحقن وأجريت الصفـــة التشريحية ودونت النتائج وكذلك أخذت العينات للفحص المجهرى .

تمت دراسة مستفيضة ومفصلة لتبيان خصائص الميكروب وتأثيره على أنسجه الجسم المختلفـــة ودونت النتائج .

Dept. of Pathology,
Faculty of Vet. Med., Assiut University,
Head of Dept. Prof. Dr. M.I. El-Sherry.

STUDIES ON EXPERIMENTAL INFECTION WITH CORYNEBACTERIUM PSEUDOTUBERCULOSIS (OVIS)
II. PATHOLOGICAL CHANGES IN CATTLE

(With 22 Figures)

By

A.R. KHATER, S. DEEB, A.H. BAYOUMI and H. SALEM

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SUMMARY

Five calves, 1 to 1½ years old, were intradermally inoculated with a lovally isolated strain of C.pseudotuberculosis. Skin biopsies were taken 4, 8 & 12 hours of infection. The animals slaughtered after 1, 2, 4, 7 and 15 days. Grossly, inflammation and slight oedema of the skin at the sits of injection, congestion and necrotic changes in regional lymph nodes, toxic hepatitis and nephrosis. Microscopically, the most pronounced changes in the skin consisted of suppurative inflammation. This was accompanied with destruction and thrombosis of blood and lymph vessels. The regional lymph nodes showed destruction and fragmentation of lymphoreticular elements and infiltrating leuocytes. In parenchymatous organs changes were mainly degenerative. Using immunofluorescence, it has been demonstrated that the organism when intradermally injected it spreads from the site of inoculation only to regional lymph nodes.

INTRODUCTION

In Egypt, CARPANO (1934) was the first who described ulcerative dermatitis of ruminants as a particular disease mainly affecting skin of cattle, buffaloes and sheep caused by diphtheria-like organism. The disease occurred in a sporadic form or in a small foci. It begins with isolated painful nodules in the skin, these later increase in size and ulcerate. Secondary nodules usually follow the same fate with implication of the regional lymph nodes. SOLIMAN et al. (1963) also described two outbreaks of ulcerative lymphangitis locally known as "oedematous skin disease". The disease dirstly appeared in 1960 affecting buffaloes and cattle. It is characterized by nodules in the skin which might burst, may be associated with swelling of the dewlap, side of the abdomen, shoulder, or side of the head and neck. Sometimes, the nodules appeared in the form a chain which might coalesce. Unopened nodules usually contain oedematous fluid and in some cases pus. The regional lymph nodes of the affected part are the only nodes inflammed. Postmortem examination of discased animals revealed, moreover, inflammation of the intestine and lung and slight increase of pericardial fluid. Further investigation of this disease was carried out by AWAD (1966), BARAKAT and EID (1971) and FOUAD et al. (1972). However, while some authors considered C.pseudotuberculosis as the main causative agent, (SOLIMAN et al., 1963; BARAKAT and EID, 1971), others believed that this organism is a secondary invador to primary virus or parasitic infection (AWAD, 1966; FOUAD et al., 1972). KHATER et al. (to be published) could reproduce the disease by intradermal inoculation of a locally isolated strain of C.pseudotuberculosis in buffaloes. The aim of the present study was to determine the pathological changes in cattle experimentally infected with the same strain.

MATERIAL and METHODS

Culture:

The reference strain (A Bu 77) was isolated from a buffalo showing typical lesions of ulcerative lymphanigitis in Asneet village, Dakahlia provinve in 1977.

Experimental procedures:

Five clinically healthy calves with an average age of 1 to 1½ year were used. The animals were proved to be free from internal and blood parasites and tuberclin tested. Each animal was inoculated intradermally with 3 ml of seramized 24 hours broth culture of " A Bu 77 " strain of C.pseudotuberculosis at different sites along the circumference of a circle of 15 cm in diameter in the left shoulder region. On the right shoulder, only 0.2 ml of the same inoculum was intradermally injected at one site which was used for biopsy. Animal number 5 was injected in the right shoulder region with broth only and used as a control.

Biopsy sampling from the skin of right shoulder was taken surfically without anaesthesis at periods of 4, 8 and 12 hours. Frozen cryostat sections of these specumens were prepared for immunoflourescence and histochemical studies. A control sections prepared from the skin of the animal injected with broth only were used. Specimens from the skin, prescapular lymph nodes, spleen, liber, kidneys, heart and lung of slaughtered animals were also included in the immunofluorescence study. The indirect method (WELLER and COONS, 1954) using unlabelled living antigen antiserum and conjugated antirabbit antiserum at a dilution of 1 : 10 was applied. Specimens were examined under the fluorescence microscope (Orthoplan, Leitz).

For study of gross and histopathological changes, one animal was slaughtered 1, 2, 4, 7 and 15 days after infection. Postmortem examination was carried out on animals and tissue specimens from site of infection, left prescapular lymph node, spleen, liver, kidney, heart and lung were fixed in 10 neutral formalin solution. After embedding, paraffin sections were cut and stained with haematoxylin and eosin, and with specific stains for detection of bilirubin in liver (FOUCHET,1917) and haemosiderin in spleen (GOMORI, 1936). Moreover, Sudan black stain for demonstration of fat in liver was done on fresh frozen sections.

RESULTS

Gross pathological findings

In animals slaughtered one and two days after infection, the skin and subcutaneous tissue were thickened, oedematous and adherednt with each others and with the underlying muscles; these changes were much more pronounced at the 7th and 15th day of infection. Changes in left prescapular lymph node in animals slaughtered at one and two days consisted mainly of oedema and congestion especially in the meduallary area. One third to one half of the lymph node was found in animals slaughtered 15 days of infection, these foci were discreted in the other animals.

Visible lesions in other organs consisted of congestion of spleen and kidneys,and presence of haemorrhagic foci subendocardially in animal slaughtered at the fourth day. A slight increase of pericardial fluid was also found in infected animals.

Histopathological findings:

No changes could be demonstrated in skin biopsies taken from the right shoulder 4 hours of infection. At 8 hours,epithelial cells of the epidermis at the site of inoculation were totally destroyed with focal accumuls of polymorphnuclear leucocytes in the area and surrounding tissue of the underlying dormis. In other areas, sore cells of the Malpighian layer showed degenerative changes characterized by increased acidophilia of the cytoplasm and nuclear pyknosis with detechment from neighbouring cells and sometimes fragmentation Fig. 1. The connective tissue of subepidermal papillary layer was moderately infiltrated with polymporphnuclear leuoctes, their distribution was related to blood vessels (Fig. 2). Similar picture was also observed after 12 hours in which narrow oedematous areas with swelling and separation of collagne bundles occurred deep in the dermis. Also, sweat glands and ducts located subepidermally were widely dilated (Fig. 3).

One day after infection, epidermal changes consisted fo coagulative necrosis in cells of the Malpighian layer associated with infiltration of few number of polymorphs. The subepidermal papillary layer was, likewise, infiltrated with inflammatory cells. The adventitia of superficial and deep vascular plexuses was activated. Diffurse oedema and infiltration of large number of polymorphnuclear leucocytes occurred in the deep dermis and subcutis at this stage (Fig. 4).

Two days after infection some cells of stratum granuloeum and stratum spinosum were disorganized, swollen and undergo vacuolation. Intercellular oedema in these areas resulted in the development of microvesicels (Fig. 5). Massive aggregation of polymorphnuclear leucocytes, many of which were disintegreated,and histiocytic reaction occurred in deep dermis and subctis (Fig. 6). The collagen and reticular fibers in both of these two layers were degenerating. Vasculitis and necrosis of the wall of many lymphatics and occlusion of their lumens by masses of infiltrating cells could be observed at this stage. After four days, polymprphnuclear leucocytes diffusely infiltrated the superficial layer of the dermis.The epithelial linning of widely dilated sweat glands

showed degeneration and desquamation, their lumens occasionally contained inflammatory cells (Fig. 7). Fragmentation of infiltrating leucocytes was more extensive leading to the formation of wide necrotic areas in which basophilic staining chromatin particles were widely distributed. Oedema of the deep dermis and subcutis subsided at this stage to a great extent. Many of subdermal lymph and blood vessels were thrombosed and occluded (Fig. 8). Seven days after infection necrotic changes of infiltrating and structural elements of the dermis were widespread and extensive. Young proliferating connective tissue made its appearance between the collagen bundles (Fig. 9). After 15 days, areas of hyperkeratosic and parakeratosis were observed throughout the epidermis (Fig. 10). In the deep dermis maturation of connective tissue and its extension to deep cutis lead to adhesion and fixation of the skin. Generally, at all stages of infection some lymph and blood vessels both of the superficial and deep plexuses remained patent (Fig. 11)

In Lymph nodes, one day after infection slight oedema, congestion and extravasation of erythrocytes occurred in the parenchyma especially in the medullary area. The marginal and cortical sinuses were infiltrated with a moderate number of polymprphnuclear leucocytes (Figs.12, 13). Indicidual cells of the latter were also observed in lymphoid tissue. In germinal centers of the lymphoid follicles fragmented cells and chromatin particles were found. In medullary lymphoid cords and sinuses infiltrating leucocytes also occurred. Two days after infection catarrh of the marginal and trabecular sinuses of lymph nodes occurred in the form of masses of polymorphnuclear leucocytes and, therfore, their obliteration. Polymorphs also diffusely infiltrated the lymphoid tissue of cortical and paracortical areas. Necrotic chenges, affecting nuch more the lymphoblastic tissue in germinal centers of some lymphoid follicles, were observed (Fig. 14, 15). The reticular network was exposed and appeared microscopically as a hyalinized highly acidophilic syncytium. These necrotic changes were predominent in the medallary tissue compared to the cortex. After four days necrotic changes were wide-spread involving larger areas of the cortical lymphoid tissue. The endothelial and reticular cells in the medullary area undergo highly active proliferation forming masses of cells filling the medullary sinuses, many of these cells showed phagocytic activity. In later stages of infection, i.e., in animals slaughtered at the 7th days necrosis was more advanced with coaleascence of affected cortical areas into wide zones. The latter was surrounded by a capsule of actively proliferating fibrous connective tissue. The medullary tissue was atrophied (Fig. 16).

In the lung, changes consisted of peribronchial lymphoid hyperplasia (Fig. 17) and degnerative changes of pummonary blood vessels (Fig 18) were occasionally observed. In the liver, minute foci of infiltrating cells, mainly lymphocytes, in the hepatic parenchyma was a consistent finding at all stages of infection (Fig. 19). The cellular elements at the portal triads were more dense partly due to the presence of infiltrating polymorphnuclear leucocytes. The latter were also found infiltrating the red pulp of the spleen (Fig. 20), but rarely the lymphoid tissue After 15 days this organ showed an increased number of phagocytic macrophages which were laden haevily with golden-yellow haemosiderin pigment. The heart of all animals showed activation of interstitial mesenchymal elements (Fig 21) In teh kidney, only congestion and extravasation of erythrocytes of cortical and medullary vessels were visible. Animals slaughtered after 7 and 15 days of infection showed areas of non-suppurative interstitial nephritis (Fig. 21)

Immunofluorescence findings:

In skin, both the biopsy samples taken after 4, 8 and 12 hours as well as specimens taken from slaughtered animals after 24 hours fo infection, specific greenish fluorescence was shown by a moderate number of star-shaped, macrophage-like cellular structures; the whole cell fluor esced. These cells,they were found throughout all layers of the dermis, were slightly more in number in the papillary layer after 12 hours. In animals slaughtered 2 days postinfection specific fluorescenee could be demonstrated in cellular and fibrous elements forming the wall of blood vessels and lymphatics in the deep dermis (Fig. 23). In the latter, moreover, many relatively thin collagen bundles also showed specific fluorescence. Elastic fibers running everywhere in deep dermis appeared yellowish green. Similar findings were found in animals slaughtered at latter stages, i.e., after, 7 and 15 days.

In prescapular lymph nodes, bright greenish fluorescente cells were observed in subcapuslar sinus as early as 24 hours, then in cortical and medullary sinuses in latter stages. Negative results were found in internal organs and no specifically fluorescent substances could be detected.

DISCUSSION

Corynebacterium psudotuberculosis infection in cattle is usually associated with abscess in skin and superficial lymph nodes (HALL and FISHER, 1915; BULL, 1933; HAMMERSLAND and WILKINS, 1941; RIISING and HESSELHOLT, 1973; ADDO and DENNIS,1977), and the organism may be involved in lesions in other organs such as the lung (ADDO and KENNIS, 1977). In contrast, results of the present study revealed that, although lesions in the skin and lymph nodes induced by intradermal injection of this locally isolated staain (A Bu 77) in cattle consistated of early infiltration of neutrophils, however, cytotoxicity manifested by cellular destruction and nuclear fragmentation both of infiltrating and fixed tissue cells rapidly ensues. Other changes which are characetristic for this type of infection included lymphangitis, vascular damage,oedema and thrombosis of lymphatics and blood vessels. Similar changes were also found in buffaloes intradermally infected with the same strain (KHATER et al., 1981) and have been attributed to the effect of an exotoxin. The latter was, most probably, also responsible for degenerative changes affecting parenchymatous organs of our experimental animals. As indicated by immunofluorescence studies, our earlier observation (KHATER et al., 1981) that the organism occurs intracellularly, probably in macrophages in both skin and lymph nodes,and that it has an affinity to endothelill linning of lymph vessels were emphasized in the present work. Morever, it has been shown that the organism when intradermally injected remain localized at the site of injection in the skin and may spread to regional lymph nodes but not to other organs.

Pathological changes resulting from infection with the strain of C.pseudotuberculosis under investigation revealed, however, a slight species difference between buffalo and cattle. In the latter animal species, oedema and swelling of the skin was less extensive,localized only to a narrow fim at sites of inoculation and subsided rapidly. Fissuring of the skin and swelling of the dewlap seen in some cases of buffaloes were not found in cattle. Microscopically, there was less severe inflammatory reaction in superficial layer of the dermis, either in the form of inflammatory cell infiltration, oedema or haemorrhage in cattle. Compared to buffalo, skin of cattle is characterized by a relatively thin epidermis and dermal layer, and sweat and sepaceous glands in the dermis occupy a large area subepidermally. Moreover, the callular elements interposed between collagen bundles were relatively less, and the vessels of both superficial and deep plexuses have a more thickened walls especially the latter. Therefore,our earlier suggestion (KHATER et al.,1981) that the great thickness of the dermis particularly in the shoulder region and the posterior aspect of the thigh with presence of high vascularity and plenty of macrophages may create a favourable media for establishment of infection with C.pseudotuberculosis in buffalo seems to be valid. Other factors which may be related to formal pathogenesis of this type of infection in intradermally inoculated cattle consist of that the organism may pass rapidly to subcutaneous tissue. Presence of relatively thick-walled blood vessels, some of which together with lymphatics remained patent in the affected areas even at latter stages of infection, have the consequences on one hand that these vessels are not easily injured and on the other maintainance of sufficient frainage that prevents the development of tremendous oedema as it is the case in buffalo. This is parallel to our early conclusion that oedema at the site of inoculation was not only due to an increase in permeability of blood vessels, but also the impaired drainage through early damaged lymphatics is an important factor.

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DESCRIPTION OF FIGURES

Fig. (1): Malpighian cells of skin epidermis showing degenerative changes and fragmentation. H.&E. X 400.

Fig. (2): Skin. Subepidermal layer showing infilteration with polymorphnuclear leucocytes connective tissue of papillary layer, with periarteritis. H.&E. X 400.

Fig. (3): Skin. Subepidermal layer showing dilated sweat glands and ducts. H.&E. X

Fig. (4): Deep dermis and subcutis showing oedema and infilteration with polymprphnuclear leucocytes. H. & E.X 100.

Fig. (5): Stratum spinosum showing microvesicles. H. & E. X 400.

Fig. (6): Deep dermis and subcutis showing massive aggregation of disintegrated polymorphnuclear anucocytes and histiocytic reaction. H. & E. X 160.

Fig. (7): Dilated sweat gland with periglandular inflammatory reaction. H. & E. X

Fig. (8): Subepidermal lymph vessel occluded with a thrombus. H. & E. X 250.

Fig. (9): Dermis showing young proliferating connective tissue. H. & E. X 400.

Fig. (10): Epidermis showing hyper- and para-keratosis. H. & E X 400.

Fig. (11): Showing patent a- lymph b- blood vessels H. & E. X 100.

Fig. (12): Lymph node. Cortical sinuses are infilterated with moderate number of polymorphnuclear leucocytes. H. & E. X 400.

Fig. (13): Prescapular lymph node showing neerotic changes. H. & E. X 100.

Fig. (14): Prescapular lymph node showing atrophy of the medulary tissue. H. & E. X 400.

Fig. (15): Lymph node showing atrophy of the lymphoid elements. H. & E. X 400.

Fig. (16): Lung showing peribronchial lymphoid hyperplasia. H. & E. X 160.

Fig. (17): Pulmonary blood vessel showing degenerative changes. H. & E. X 160.

Fig. (18): Liver showing mononuclear cell infilteration. H. & E. X 160.

Fig. (19): Spleen showing polymprphnuclear leucocytic infilteration. H. & E. X 400.

Fig. (20): Heart showing activation of the mesenchymal elements. H. & E. 400.

Fig. (21): Kidney showing areas of non suppurative interstitial nephritis. H. & E. X 400.

Fig. (22): Deep dermis showing specific fluorescence in the cellular elements of blood vessels and lymphatics in the deep X 100.

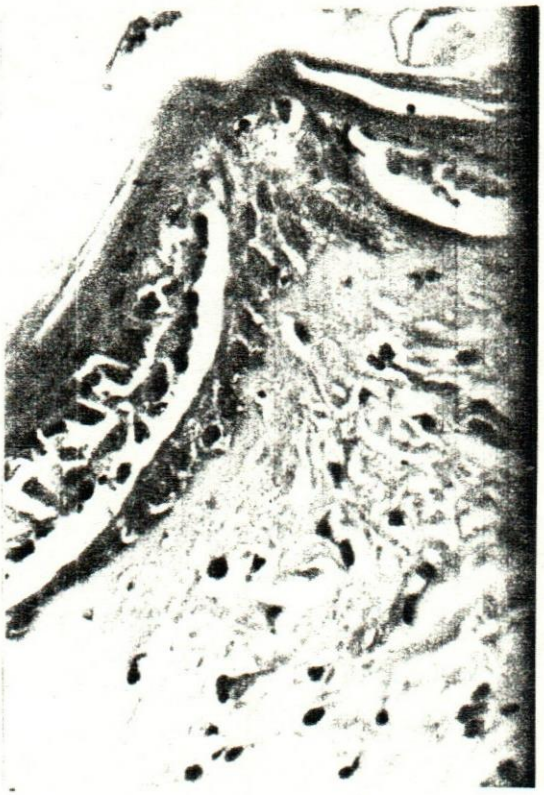
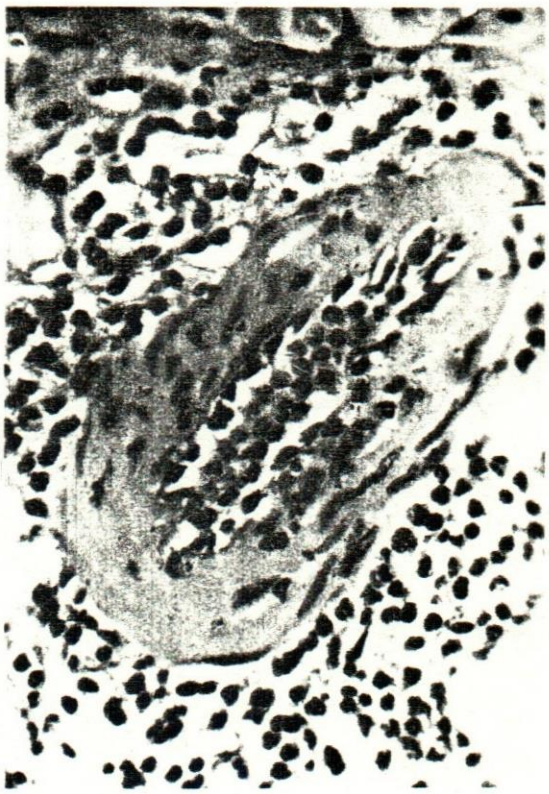


Fig. 1



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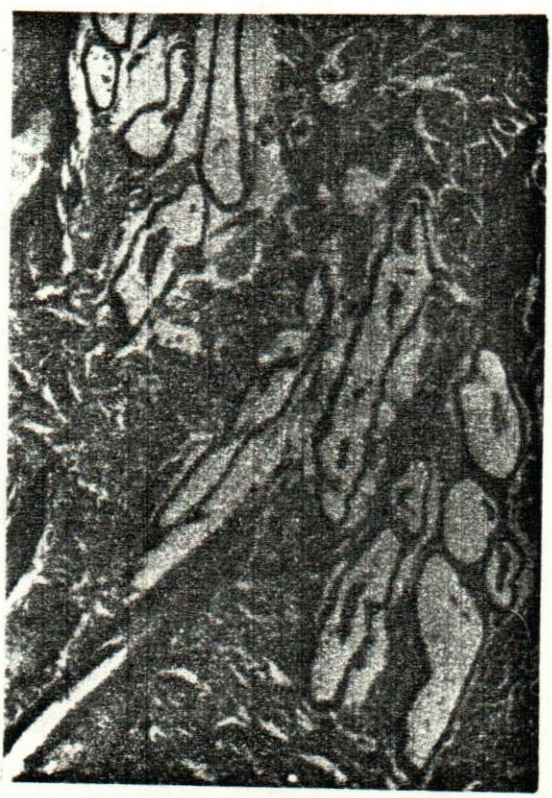

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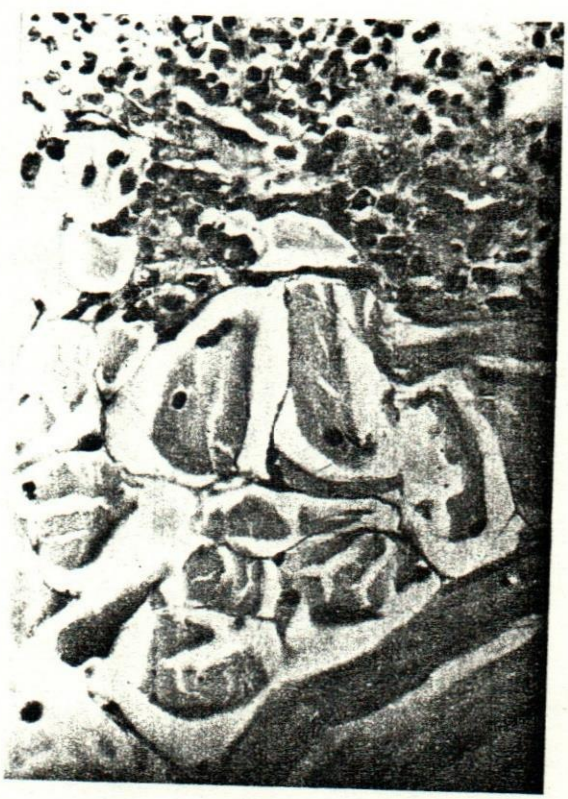

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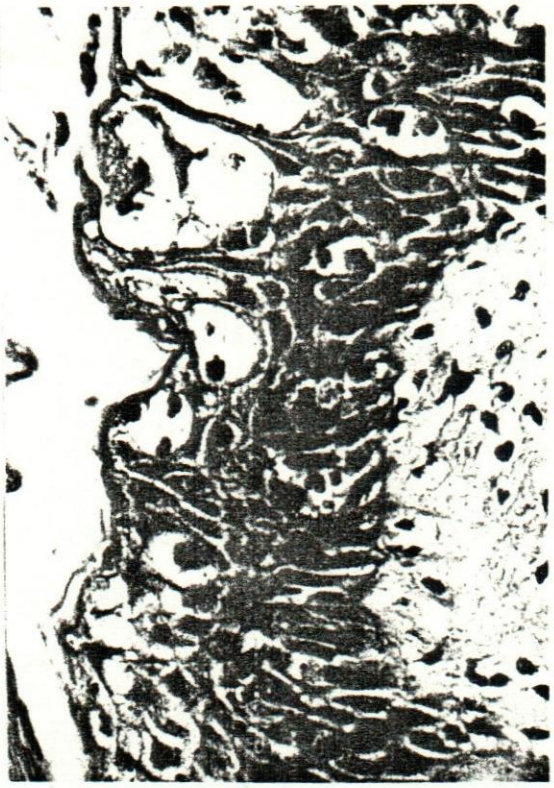
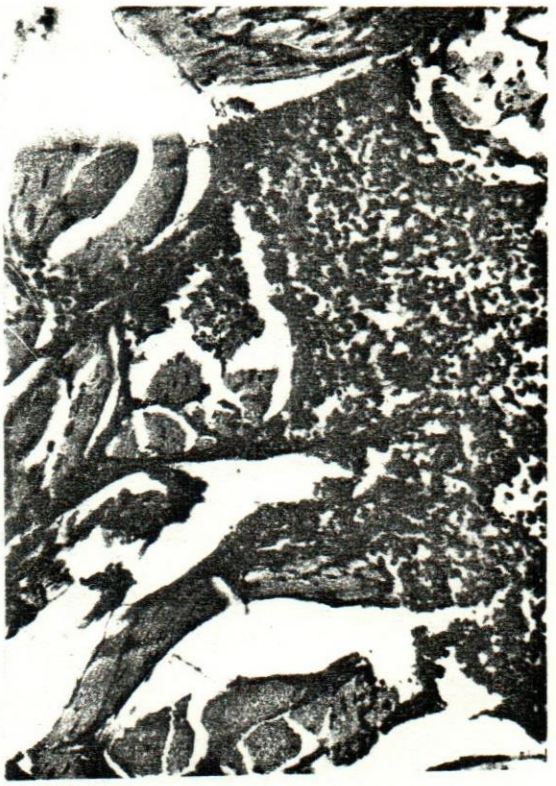


Fig. 5



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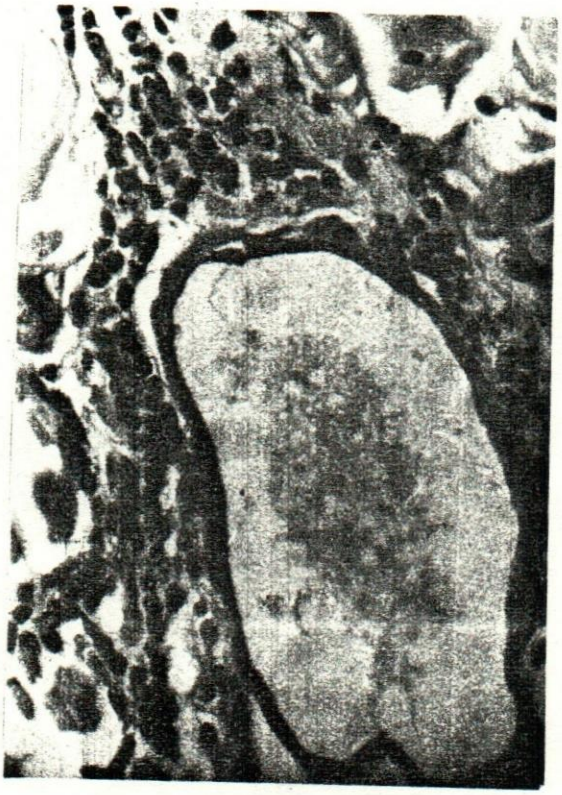


Fig. 7

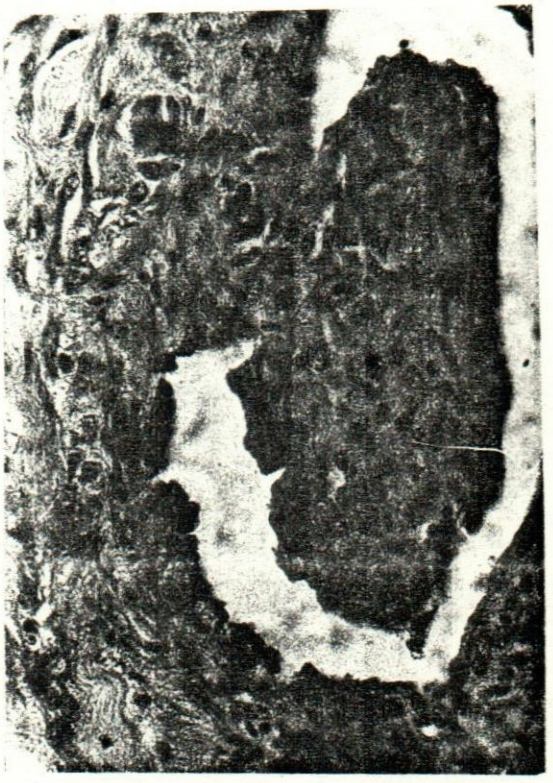


Fig. 8

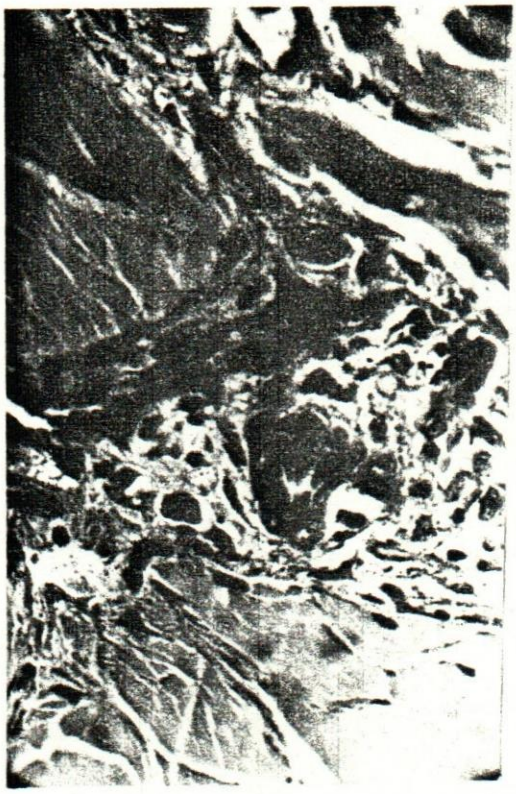


Fig. 9

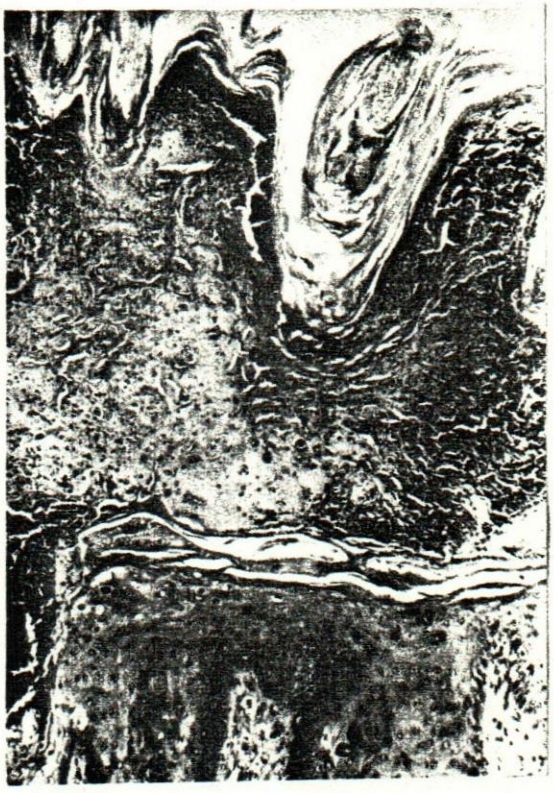


Fig. 10

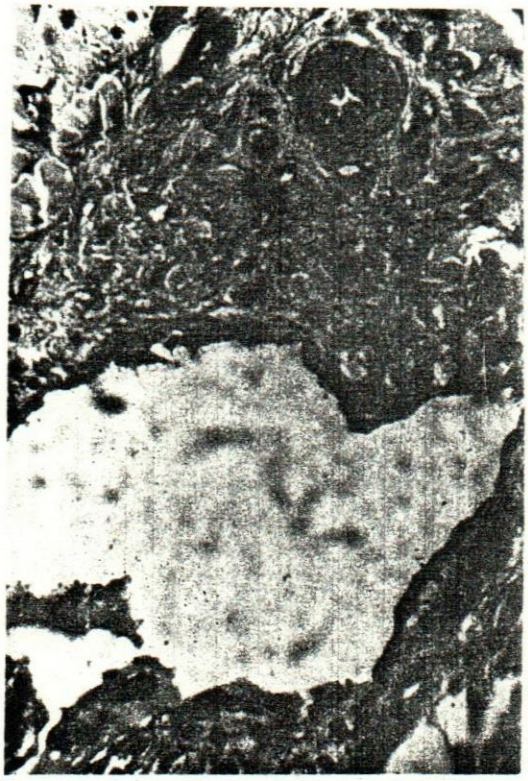
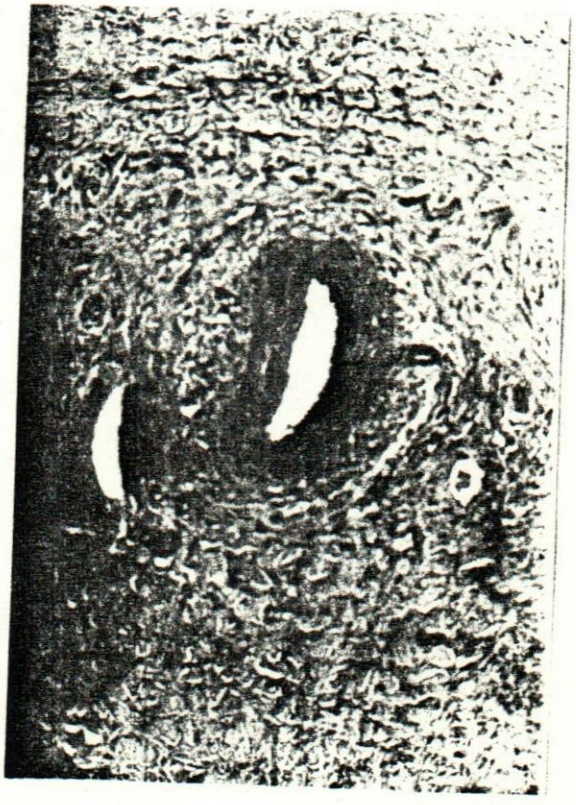


Fig. 11 a



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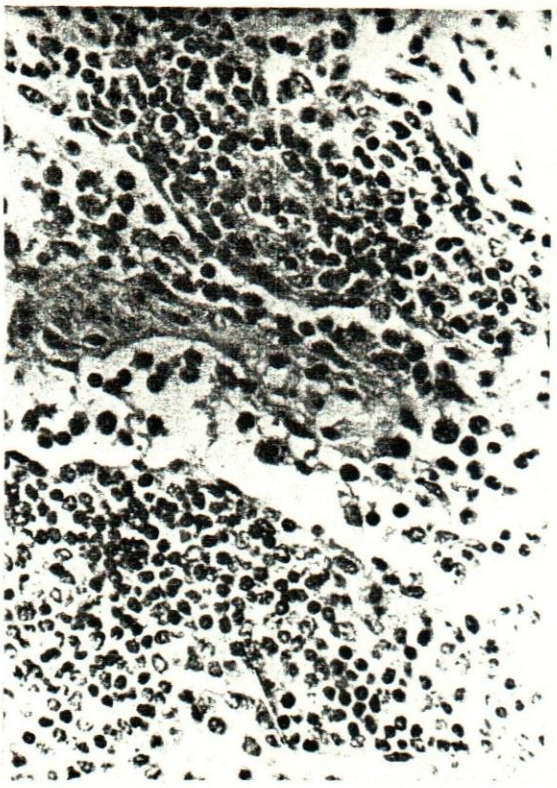


Fig. 12

Fig. 13

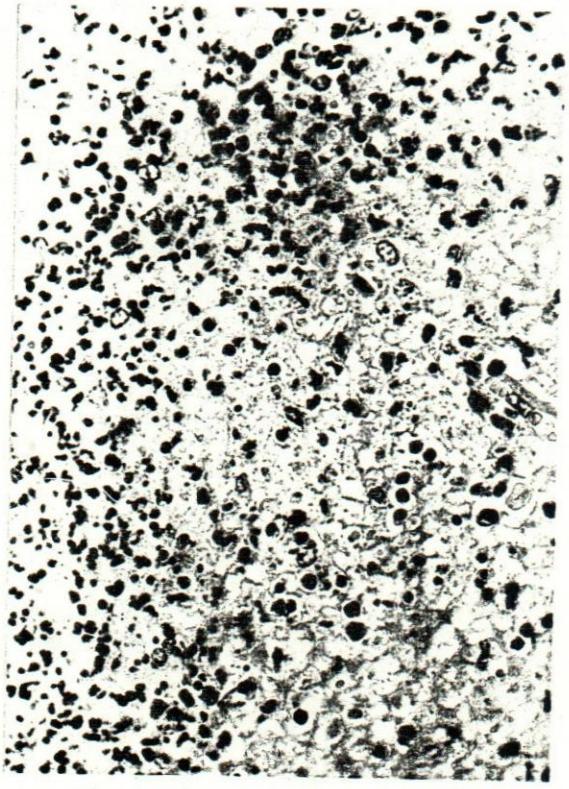
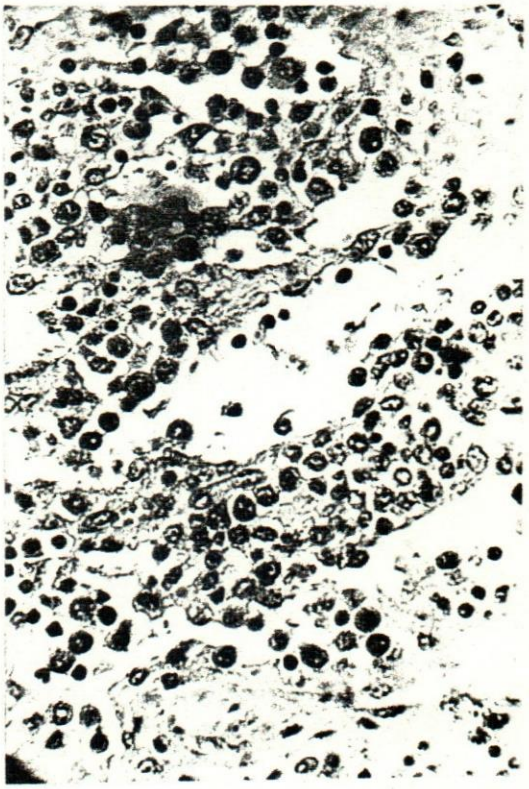


Fig. 14



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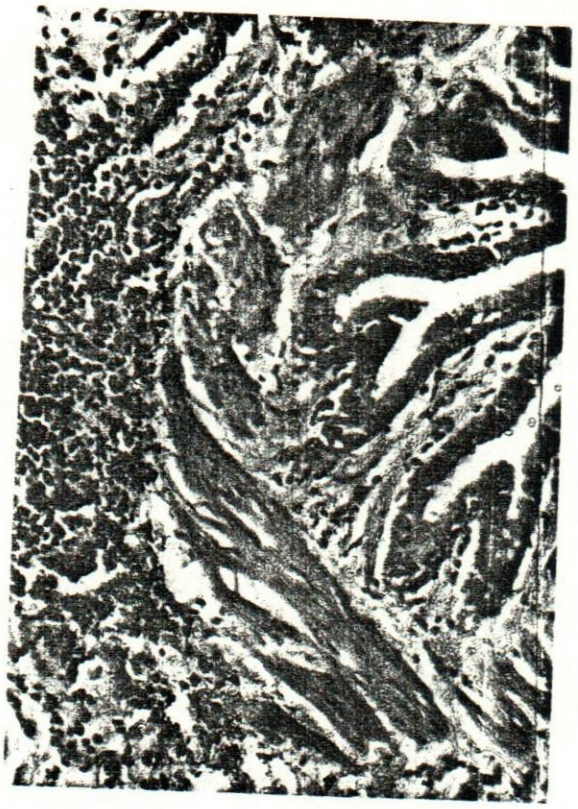

Fig. 16

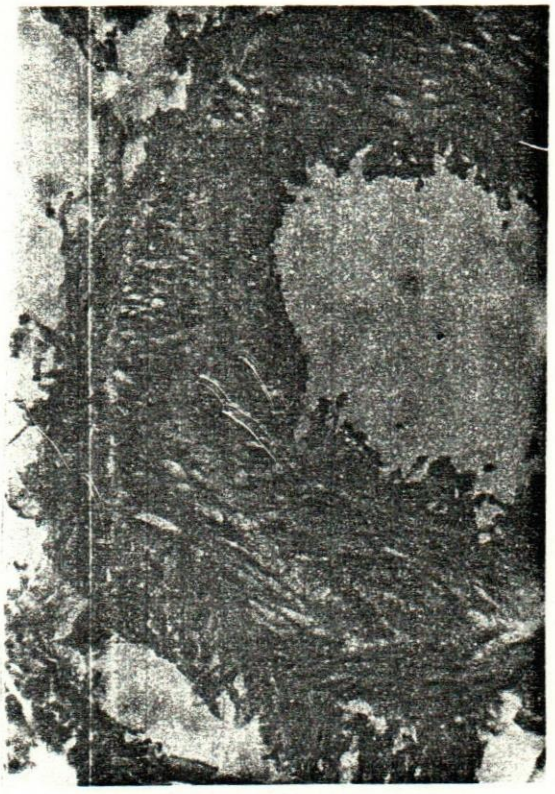

Fig. 17

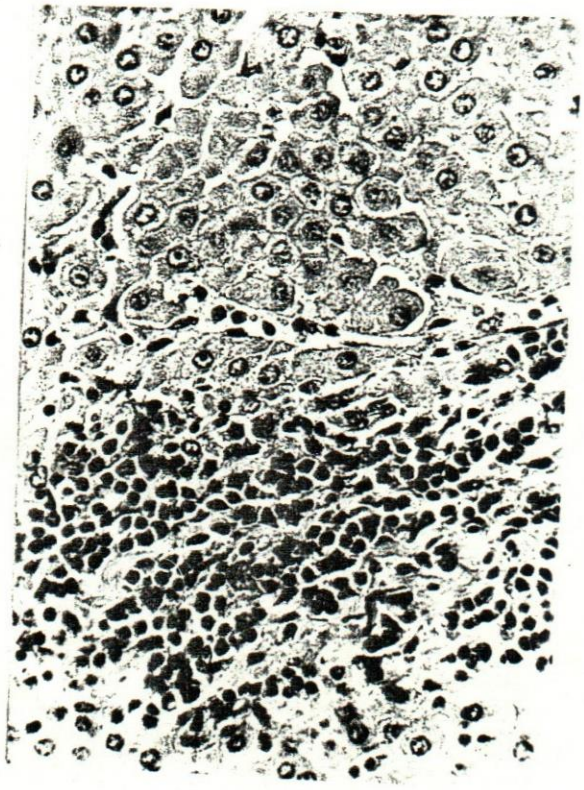

Fig. 18

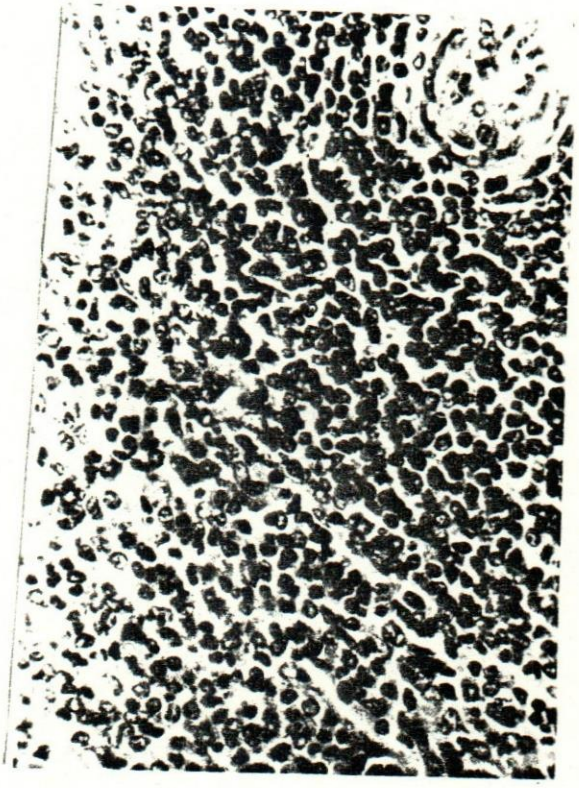

Fig. 19

Fig. 20

Fig. 21

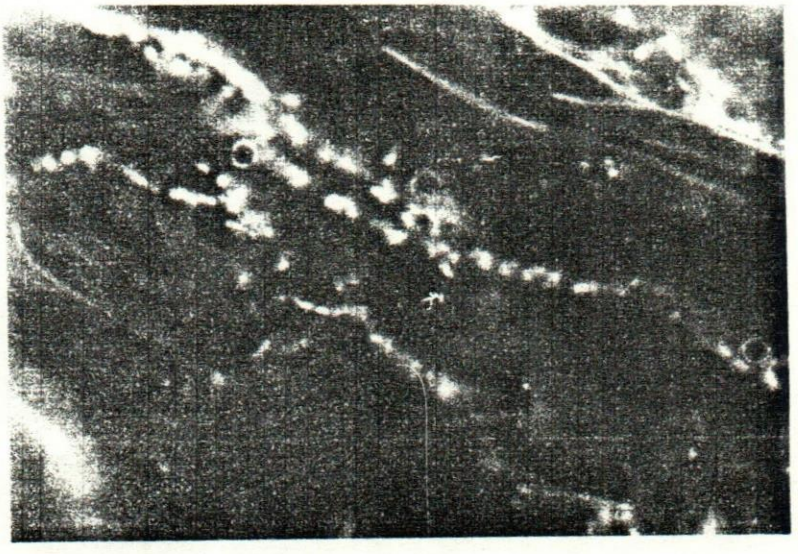

Fig. 22