قســـــــم : الباثولوجيا - كلية الطب البيطرى - جامعة أسيوط

رئيس القســم : أ.د / محمد ابراهيم الشرى

دراسة تجريبية على الاصابة بواسطة ميكروب الكوريـــــنى

١- التغيرات الباثولوجية فى الجاموس

عبد الرحمن خاطر ، صلاح ديب ، حمدى سالم ، عبد اللطيف بيومى ، مختار طه

أستخدمت بالبحث عترة معزولة من حالة التهاب الأوعية الليمفاوية وحقنت فى جلد عشرة عجول جاموسى وأخذت عينات من الجلد قبل الذبح بعد ٤ ، ٨ ، ١٢ ساعة من حقن الميكروب .

ذبحت الحيوانات بعد ١ ، ٢ ، ٤ ، ٧ ، ١٥ يوما من حقن الميكروب . باجراء الصفـــة التشريحية كانت أهم النتائج هى أوديما الجلد وتحت الجلد ، تنكرز الجلد والتصاقه بالأنسجة تحت الجلد كان فى الحالات المتقدمة بعد ٦ ، ١١ يوما من العدوى كانت صورة الصفراء واضحة جدا عند عمل الصفة التشريحية .

أخذت عينات للفحص المجهرى وكان التهاب الجلد وما تحته وتجلطات داخل الأوعيـــــة الليمفاوية والدموية وبعض التغيرات الطفيفة فى الكبد والكليتين من أهم النتائج .

أخذت عينات للفحص الأميونوفلورسنسى ودونت النتائج .

نوقشت النتائج جميعها وأمكن أن تعزى التغيرات الى قوة فاعلية الاكسوتوكسين الخلوية .

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STUDIES ON EXPERIMENTAL INFECTION WITH CORYNEBACTERUM PSEUDOTUBERCULOSIS (OVIS)
I. PATHOLOGICAL CHANGES IN BUFFALO-CALVES

(With 14 Figures)

By

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SUMMARY

Ten buffalo-calves were intradermally inoculated with a strain of C. peseudotuberculosis (ovis), "A Bu 77", isolated fril a case of ulcerative lymphangitis. Skin biopseis were taken at periods of 4,8 and 12 hours after infection. Two animals were slaughtered after 1,2,4,7 and 15 days. Grossly, changes consisted of oedematous swelling, which extended in some cases to the dewlap, necrosis, and in later stages adhesion of the skin with the underlying muscles. The regional lymph nodes showed congestion and necrosis. Two animals died after 6 and 11 days, the latter showed the clinical picture of jaundice. Microscopically, callulitis with infiltration of neutrophils in the area, as well as damage and thrombosis of blood and lymph vessels occurred in the skin. This was followed by proliferation of fibrous connective tissue formation. Necrotic changes were very marked both in early infiltrating neutrophils and lymphoreicular elements of the lymph nodes. Liver and kidney revealed degenerative changes. Immunofluoresceince examination revealed that the organism occurs intracellularly, most probably in macrophages in skin and lymph nodes, and that it has an affinity to endothelial linning of lymphatics in the skin. Pathological changes induced by this strain were attributed to a powerful cytotoxic effect of an exotoxin.

INTRODUCTION

Bovines, mainly cattle, are known to be susceptible to infection with C. pseudotuberculosis (ovis). HALL and FISHER (1915), in U.S.A., described an abscess over the parotid gland in a calf apparently caused by C. pseudotuberculosis. BULL (1933), in Australia, isolated the organism from a prescapular abscess in acow. HAMMERSLAND and WILKINS (1941), U.S.A., isolated C.pseudotuberculosis from an abdominal wall abscess in a cow. PURCHASE (1944) described an outbreak in the Rift Valley in Kenya caused by C. pseudotuberculosis. The affected animals usually had one or more nodular swelling which bursted in a week. In animals having more than one nodule, lesion appeared in the form of a chain in which a thickened cord was found connecting them. Gelatinous exudate poured continuously from ulcerated nodules; the organism was isolated form the exudate. RIISING and HESSELHOLT (1973), in Denmark, described a similar outbreak in which the affected animals mostly showed suppurative or granulomatous lymphadenitis of lymph nodes of the head, prescapular and precrural lymph nodes, and perilymphadenitis. Bacteriological examinations carried out by these authors indicated a corynebacterium as the aetiological factor. ADDO and DENNIS (1977), in Northern Nigeria, recorded that C. pseudotuberculosis infection in cattle is usually associated with cutenecus abscesses and lymphadenitis of the superficial lymph nodes. They mentioned, moreover, that the orgainism is found to be associated with pneumonia even in adult cattle.

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 formor or in small foci. It begins with isolated painful nodules in the skin, the nodules later increase in size and ulcerate. Secondary nodules usually follow the same fate with implication of the regional lymph nodes. SOLIMAN et al. (1963) reported two outbreaks of ulcerative lymphangitis, locally cattle "oedematous skin disease". The disease firstly appeared in 1960 affecting buffaloes and cattle. It is characterized by nodules in the skin which might burst and associated with swelling of the dewlap, side of the head and neck. Sometimes the nodules appeared in the form of a chain which right coalesco. The regional lymph

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node of the affected part were the only nodes inflammed. BARAKAT and EID (1971) described cases of ulcerative lymphangitis with nearly the same clinical picture. BOTH SOLIMAN et al. (1963) and BARAKAT and EID (1971) could isolate C. pseudotuberculosis from the lesions. FOUAD et al. (1975) made further investigations on the disease in buffalo and cattle and isolated a strain of C. pseudotuberculosis which different from that of SOLIMAN et al. (1963) by inconstant fermentation of maltose; this organism failed to reproduce the disease in experimentally infected buffaloes.

The aim of the present work is to study the pathological change in buffalo-calves intradermally inoculated with a strain (A Bu 77) of C. pseudotuberculosis isolated by BARAKAT from a buffalo showing typical lesions of ulcerative lymphangitis (oedematous skin disease).

MATERIALS and METHODS

corynebacterum pseudotuberculosis:

A strain of C. pseudotuberculosis, designated "A Bu 77", isolated from a buffalo showing typical lesions of ulcerative dermatitis in an Egyptian village in 1977 was used.

Animals:

Ten healthy buffalo-calves of about 10 months old were used in the present study. Before infection all experimental animals were examined clinically and tested for tuberlculosis, intestinal and blood parasites.

Experimental proceudres:

Each of the animals was intradermally inoculated with a total dose of 3 ml of seramized broth culture incubated for 24 hours at different sites alsong the circumference of a circle 15 cm in diameter in the left shoulder region. In the right shoulder only 0.2 ml of the same infective broth was injected intradermally at one site which was used for biopsy. One animal was injected with broth only and used as a control.

Pathological procedures:

For study of gross and histomorphological changes two animals were sacrificed 1,2,4,7, and 15 days after infection unless died during the experiment. Before slaughtering, biopsy specimens of skin (right shoulder) were taken surgically without anaesthesia at periods of 4,8 and 12 hours after inoculation of the infective material and frozen sections were prepared. As control, sections prepared from skin of an animal injected with broth only were used. Post-mortem examination was carried out on sacrificed as well as dead animals. Tissue specimens from site of injection, left prescapular lymph node, spleen, liver, kidneys, heart and lung were taken and kept in 10% neutral formalin solution. After fixation, paraffin sections were prepared and stained with haematoxylin and eosin. Specific stains for detection of bilirubin in liver (FOUCHET, 1917), and haemosiderin pigment in spleen (GOMORI, 1936) were also used. Moreover, Sudan black stain for demonstration fo fat in liver was done on fresh frozen sections.

Immunofluorescence:

Skin biopsy specimens and specimens from skin, prescapular lymph node, spleen liver, kidneys, heart and lung of sacrificed animals were used. The indirect method (WELLER and COONS, 1954) using unlabelled living antigen antiserum and conjugated antiserum at a dilution of 1:30 to unfixed forzen cyrostat sections were applied. Examinations was carried out using the fluorescence microscope Orthoplan (Leitz) provided with vertical illumination for incident light (Loempark 2.2).

RESULTS

Gross pathological findings:

At the site of injection at the left shoulder thickening of the skin with presence of gelationous straw-coloured oedema which extended to the dewlap in some animals was a consistent finding. From the 4th day of infection, adhesion between teh skin and sucutaneous tissue and muscles with the occurrence of necrotic changes was observed. The left prescapular lymph node was enlarged and oedematous. On sectioning, yellowish-white necrotic areas were found to involve a relatively great portion of the cortex at the 7th day and extdnded to

the medulla in an animal died after 11 days. The perinodal adipose and connective tissue were oedematous and gelationous in nature.

The spleen of animals died after 6 and 11 days was congested while in the rest of animals, petechial haemorrhages were occasionally observed subcapsullary. The liver and kidneys were slightly enlarged and pale in animals sacrificed after days and later on. This was accompanied with subepicradial petechial haemorrhages and a slight increase of pericardial fluid.

Features of jaundice was not prominent except in an animal died after 11 days. In this animal incteric pigmentation involved the subcutaneous tissue, werous and mucous membranes and carcass fat. The liver was enlarged and reveeled irregular yellow patches and streaks.

Other lesions occasionally found were petechial haemorrhages in urinary bladder, traches and lung.

Histopathological findings:

As revealed by skin biopsies taken from the right shoulder, histomorphological changes could be observed at the site of inoculation as early as 4 hours. In all cases the dermis was moderately but more or less diffusely infiltrated with inflammatory cells mainly neutrophils and histiocytes. In specimens taken after 12 hours the cellular reaction was remarkable in the perivascular areas and frequently revealed evidence of nuclear destruction (Fig. 1). Capillary bed and small blood vessels in the papillary layer of the dermis appeared more prominent due to swelling of endotheliel cells and the presence of perivascular cellular reaction. The epidermis revealed changes only in specimens taken after 12 hours. These changes were mostly vacuolar degeneration with the presence of minute necrotic foci which chowed nuclear fargmentation and dense cellular infilatration.

In animals sacrificed after one day the epidermis of one case revealed the formation of microvesicles while in the others there was variable degrees of necrosis with cellular disorganization (Fig. 2). In both animals changes in the dermis were the same and mostly occur in the deep reticular layer. The changes were principally related to vascular damage with increased permeability, cellular reaction and necrosis. All lymphatics were greatly dilated adn most of them revealed cellular infiltration and occasional thrombosis. This feature of lymphengitis was accompanied with marked oedema, moderate to severe neutrophilic and histiocytic infiltration and damage of most tissue elements (Fig. 3). Many blood vessels in the affected areas showed, likewise, endothelial damage and perivascular cellular reaction.

In animals sacrificed after 2 days skin changes were qualitatively the same as described but more severe in the deep dermis and began to be more prominent in the upper papillary layer (Fig. 4).

In animals sacrificed after 4 and 7 days and in that died after 6 days, necrotic foci in the epidermis were more frequent and some off them were accompanied with proliferative changes leading to marked alteration of the architure of this layer (Fig. 5). In these animals while features of oedema became less prominent, the infiltrative chenges reached its maximum and involved big area of the dermis. At this stage of the disease the main reacting cells were histiocytes while in some areas fibroblastic proliferation began to be evident. Vascular damage with presence of thrombotic phlebitis was a common finding. Necrotic changes were also more advanced and included the papillary layer and epidermal tissue (Fig. 6).

In an animal died after 11 days and in another one sacrificed at the end of the experiment, necrotic and cell infiltration were still to be observed. Fibroplasia became prominent and was mainly perivascular or marginal in distribution.

In prescapular lymph node,early changes observed 24 hours after infection was infiltration ofthe capsule and the subcapsular sinus with inflammatory cells mainly neutrophils. Destruction and disintegration of cellular elements in the lymph follicles and paracortical areas were also evident (Fig. 7). Moreover, there was an increase in cellular population in trabecular areas with changes related to vascular disroders such as hyperaemia and oedema.

After 2 days, changes were nearly the same as those observed after one day, however, necrosis and disintegration of the lymphoid elements were more advanced and proliferation and activation of reticulum cells in

the sinuses were evident. Four days after infection, besides vascular changes in the form of congestion, erythrocytic extravasation and oedema, degenerative and proliferative changes were severe and involved nearly the whole lymph node. Most sinuses showed showed increased population of their cellular elements fo histiocytic type and actively proliferating reticulum cells (Fig. 8). At the same cortical and medullary areas suffer from cell disintegration giving the picture of focal necrosis. The lymph node of an animal died after 5 days showed depletion of lymphoid elements and severe, more or less diffuse, necrosis of lymphoreticular tissue (Fig. 9). The paracortical reticular tissue appeared as a necrotic syncytium devoid of intact cells. Giant cells with evidence of active phagocytosis were frequently observed (Fig. 10). The necrotic changes after 7 days involved in whole area of the cortex and extended to a large poriton of the medulla. At the periphery of the necrotic area fibroplastic proliferation as a feature of organization occurred.

Haemosiderosis and occasional nuclear fragmentation were the main findings observed in the spleen of all animals. In the liver, congestion and disorganization of the hepatic cords were seen as early as one day after infection. After two and four days monouclear cell infiltration in the portal triads, degeneration of some hepatic cells and occasional fatty changes were found. (Fig. 11). In animals sacrificed or died after the fourth day and to the end of the experiment, toxic hepatitis was more advanced (Fig. 12). The kidneys; likewise, showed the picture of tubular nephrosis, glomerular changes and occasional interstitial mononuclear leucocytic infiltration at the fourth day of infection and later on.

Immunofluorescence:

Examination of specimens of skin from animals intradermally inoculated with infective broth revealed the presence of specific bright greenish fluorescence in individually distributed macrophage-like cells as early as 4 hours after infection. This fluorescence could be located in both superfiical and deep reticular layers of the dermis. The same picture was also observed after 8, 12 and 24 hours at which time fluorescent cellular elements were frequently distributed especially in the deep reticular of the dermis. After two days of infection fluorescent structures showed tendency to be concentrated along the wall of lymph vessels (Fig. 13). The inner cell linning of these vessels was also highly fluorescent. This picture was also observed at the fourth day and later on,however, fluorescent cellular structures were less common. In the deeper layer of the dermis, broad bundles of connective tissue and many cells interposed between them showed an intense specific fluorescencel Moreover, the connective tissue arranged to form a sheath aroung lymph spaces and vessels was highly fluorescent.

Examination of left prescapular lymph node revealed spscifically fluorescent cellular structures in the subcapsular sinus in animals sacrificed four days of infection. Similar findings were also present in cortical and medullary sinuses in animals sacrificed or died after this period (Fig. 14).

In spleen of only one animal which died after 11 days, specifically fluorescent stellate cells could be seen at the marginal zone between the white and red pulp. No free fluorescent particulates of bacterial structure could be observed in skin, lymph nodes or spleen. Likewise, no specific fluorescence of cellular or acellular nature could be detected in liver, kidneys, heart or lung of any of the examined animals.

DISCUSSION

Pathological studies in the present work revealed that the applied strain of C. pseudotuberculosis when inoculated intradermally in buffalo resulted in a quite different picture from that described by ADDO and DENNIS (1977) who reported that lesions associated with natural infection with this organism in cattle are characteristically pyogenic with suppuration and abscess formation both at the initial site of infection and regional lymph nodes. Our results showed, in contrast, that the changes were mostly related to vascular and lymphatic damage suc as oedema, lymphangitis, thrombotic phlebitis, and necrotic changes manifested by desttuction and fragmentation of cellular elements. An experimental study carried out by PURCHASE (1944) two young steers were subcutaneously inoculated with a strain of C. pseudotuberculosis (ovis) isolated from naturally infected cases, post-mortem examination of one of these two animals revealed swelling at the site of inoculation, the muscles and connective tissue in the area were saturated with lymph which exuded freely from cut

surface.

As revealed by immunofluorescence study, specific fluorescent cellular structures could be located in the superficial layer of the dermis then along lymph vessels in deep layer indicating the role of macrophages and lymphatics in spread of infection. The microorganism has been shown to have an affinity to be localized, and probably also to multiply, in the endotherlial linning of lymph vessels. HARD (1972) demonstrated that after intraperitoneal injection of C. pseudotuberculosis into mice, intracellular organisms were present viable in macrophages to the end of the 10-day study.

Histomorphological changes observed in the skin at the site of injection were mostly related to damage of lymph and blood vessels giving rise to prominent oedema, and to a cytotoxic effect mainifested by frequent cellular damage and unclear fragmentation. Increased permeability of vascular bed has been related by SOUCEK et al. (167, 1971), and CARNE and ONON (1978) directly to the action of the toxin produced by this organism on the vascular endothelial membrane. However, oedema at the site of injection in our cases were found to be not only due to this increased permeability, but also to impaired drainage through an early damaged lymphatics. Leucocidin, an exotoxin causing destruction of migrating leucocytes that has been suggested by MADDY (1953) may be responsible for necrotic changes observed in the skin and lymph nodes. Similarly, degenerative changes which were observed in the liver and kidney of our experimental animals could be related to the action of the circulating toxin. This could be ascertained by the negative results of immunofluorescence study organs.

The chemotactic nature of bacterial infection with our strain of C. pseudotuberculosis was well demonstrated in the skin by early appearance of polymorphunclear leucocytes, firstly around blood vesels then in the connective tissue of the deep dermis, and by early migration of these leuocytes to regional lymph nodes. However, it is to be noticed that lesions produced by this strain did not reveal any persistent pyogenic process. It seems, therefore, that this strain does not possess a potent pyogenic factor like that described by BULL and DICKINSON (1935) and ZAKI (1976) for other strains. This strain was also unable to induce metastatic pyogenic or necrotic lesions in organs other than regional lymph node. The presence of fluorescent cellular structures in sinuses of prescapular lymph node indicated that the organism was probably carried by macrophages from the initial site of injection to this node with the drained lymph without evidence of bacteraemia.

Thrombosis and obliteration of vessels early in infection at the site of inoculation possible play a role in preventing the organism from being migrated to other sites.

The association of jaundice with some infections with C. pseudotuberculosis has been known for a long time and repeatedly refered to in the literature. Carre and BIGOTEAU (1908) described a disease known as "Mal Rouge" or"eaux rousses" in france, while BOQUET (1912) reported a similar disease known as "el-roch" in North Africa. Both diseases were characterize by icterus and haemoglobinures, the authors believed it to be caused by C. pseudotuberculosis. Moreover, CARRE and BIGOTEAU (1908) found that an intrvascular haemolysis and haemoglobinurea or icterus occurred after sheep had been inoculated with cultures of virulent strain of C. pseudotuberculosis. ROBINSON (1928) reproduced an intense haemolytic condition which he named "bacterial icterus" by inoculation of sheep with C. pseudotuberculosis (ovis). Recovered from abscesses. Purchase (1944) found that in sheep died after subcutaneous inoculation fo broth culture, there was an intense icterus and haemolysis. Three locally isolated strains of C. pseufotuberculosis (ovis) form bovine, caprine and equine materials were compared by the author by injecting them intracutaneously into guines pigs. One of two animals infected with the bovine strain showed haemorrhage at the site of inoculation, while the whole carcass was yellow in one of these animals and pink in the other. Postmortem examination of these cases revealed, moreover haemorrhage in the epicardium, lungs and adrenals. LOVELL and ZAKI (1966) suggested that C.pseudotuberculosis (ovis) exotoxin appeared to be identical or closely related to substance producing haemolysis on blood agar. SOUCEK et al. (1967, 1971) reported that purified toxin of C. pseudotuberculosis (ovis) is a phospholipase D which splits sphingomyelin into N-acylsphingosyl (ceramide) phosphate and choline. This action occurred both when purified sphingomylein was used as substrate and when the lipid was incorporated in the lipoprotein of the cell membrane of erythrocytes. In the present experiment two animals died 6 and 11 days after infection, the latter showed faetures of icterus with an evidence fo acute intoxication. The applied strain seem, therefore, to be a powerful toxin producer.

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DESCRIPTION OF FIGURES

Fig. 1: Skin biopsy specimen 12 hours after injection showing epidermal necrosis with prominant nuclear fragmentation. H. & E. X 400.
Fig. 2: Skin 24 hours after injection showing epidermal microvesicles. E. & E. X 400.
Fig. 3: Skin 24 hours after injection showing oedema, necrosis, cellular infilteration and thrombosis in deep layer of the dermis. H. & E. X 100.
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Fig. 5: Skin 4 days after injection showing necrotic and hyperplastic changes in the epidermis.H. & E. X 100.
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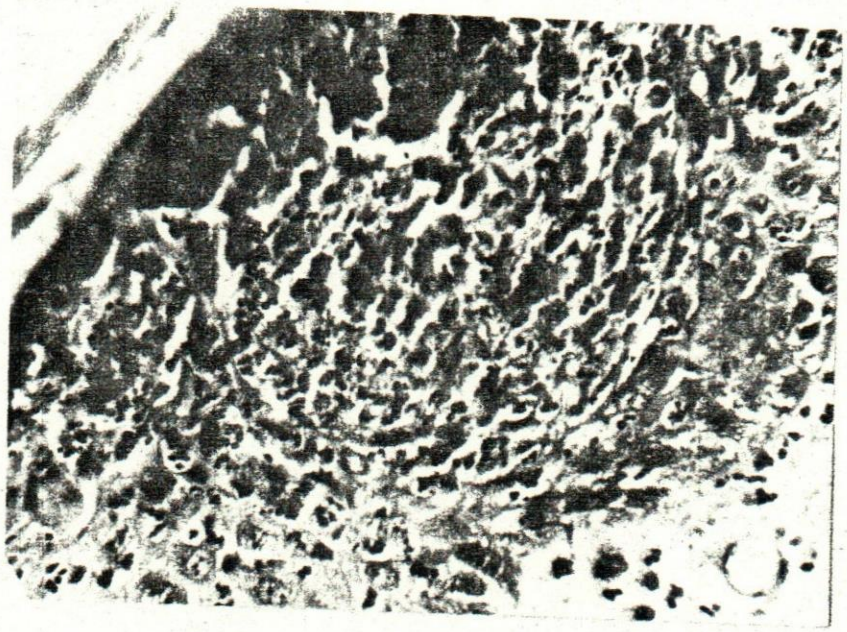
Fig. 11: Liver 4 days after injection showing fatty change. H. & E. X 400.
Fig. 12: Liver 2 days after injection showing toxic hepatitis. H. & E. X 400.
Fig. 13: Skin 2 days after injection showing fluorescent cellular structures concentrated mainly along lymph vessels. H. & E. X 400.
Fig. 14: Prescapular lymph node 5 days after injection showing fluorscent cellular structures in cortical sinus. H. & E. X 400.



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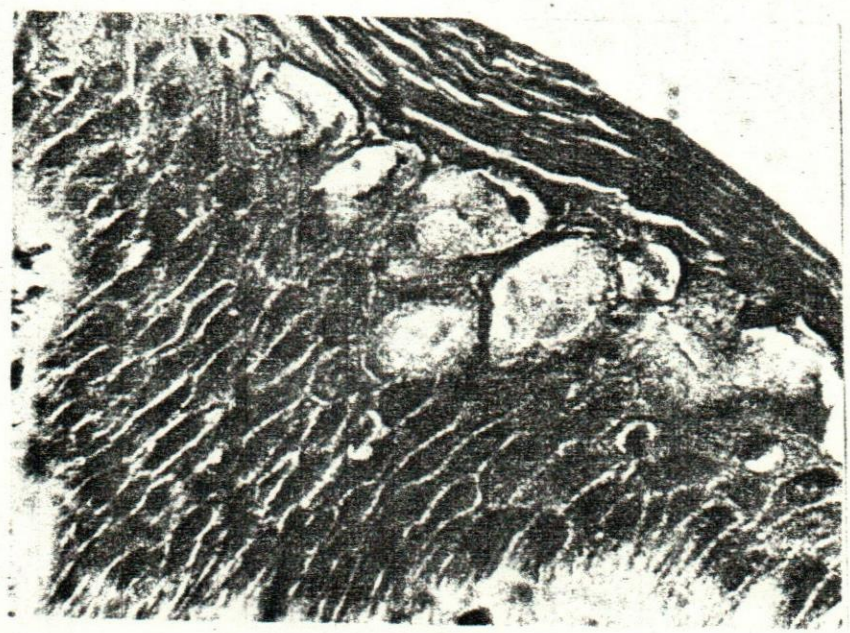

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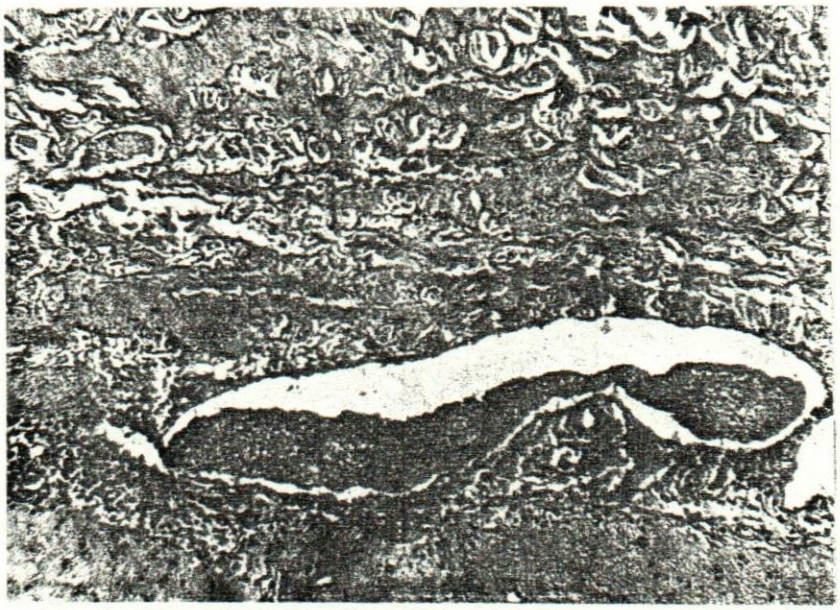

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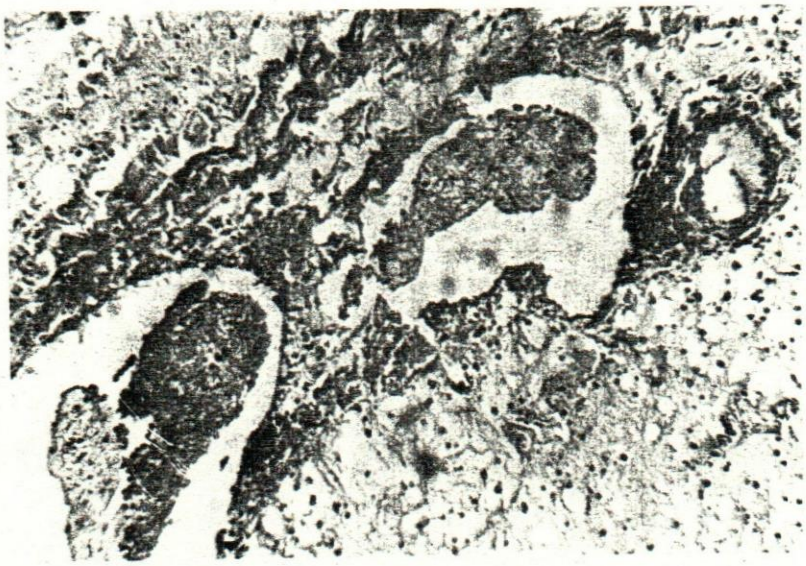


Fig.: (4)

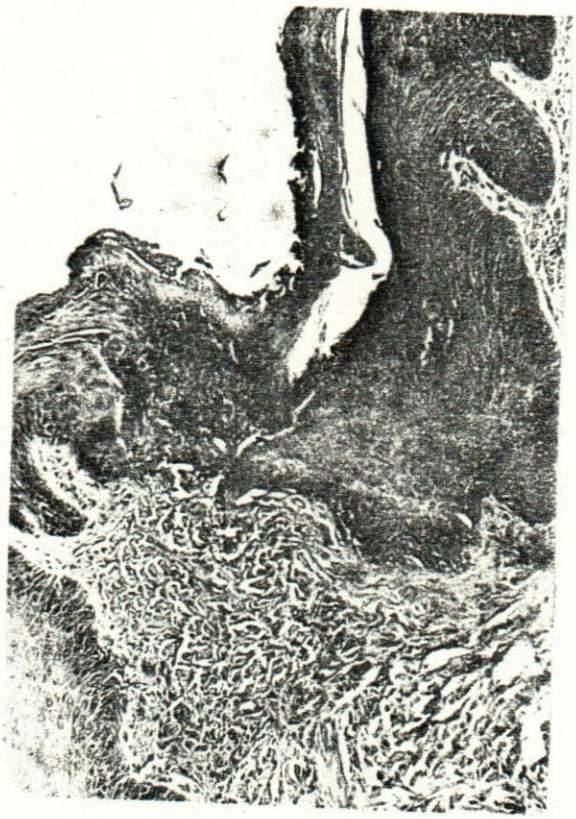
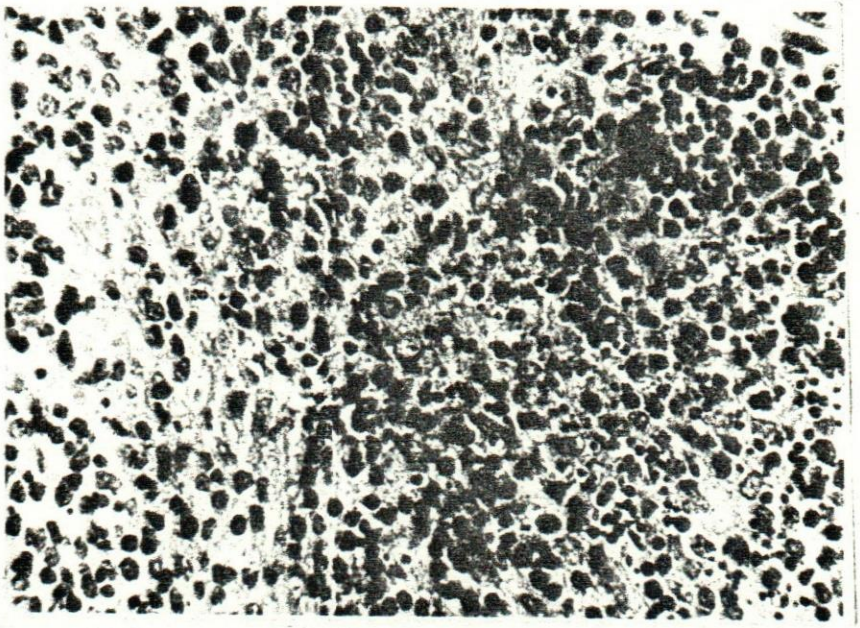


Fig.: (5)

Fig.: (6)



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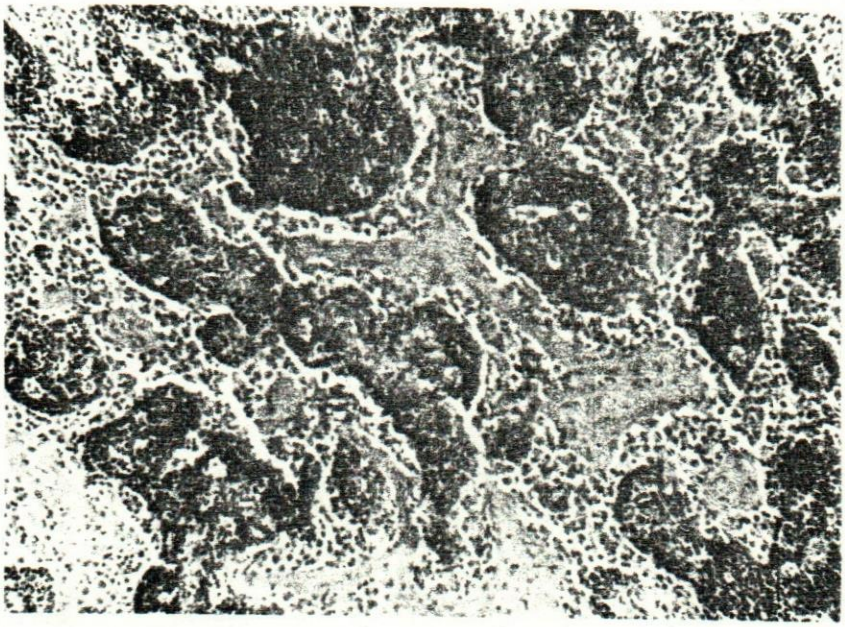

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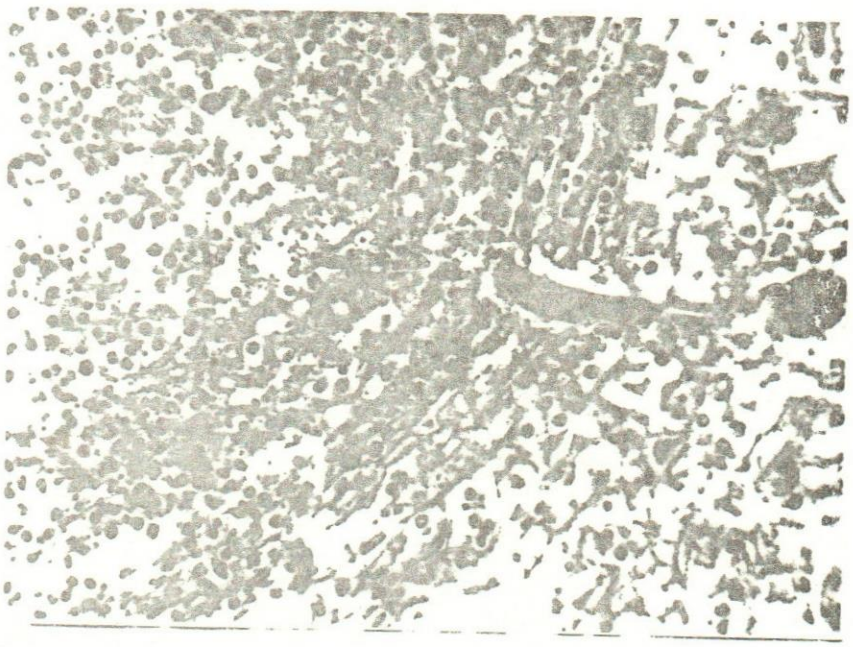


Fig (9)

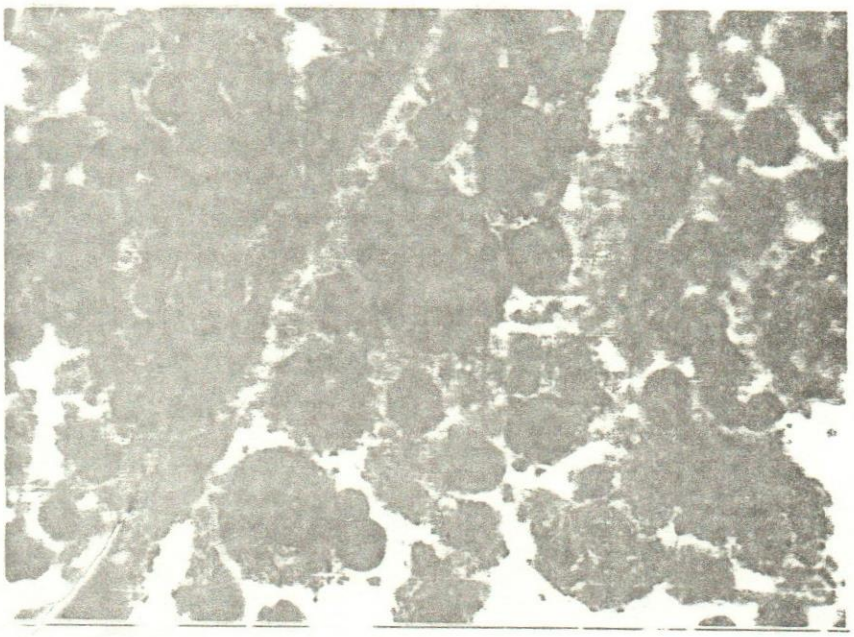
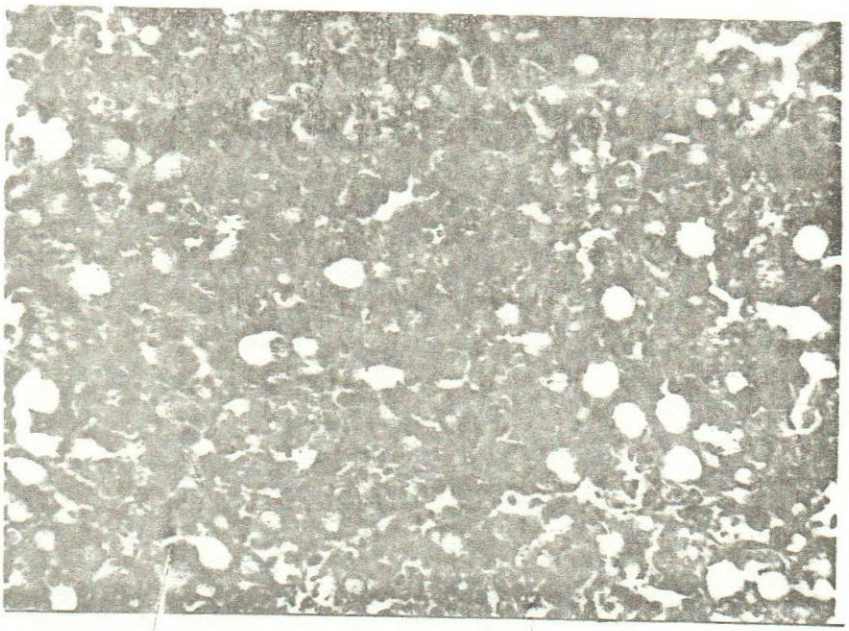


Fig. (10)



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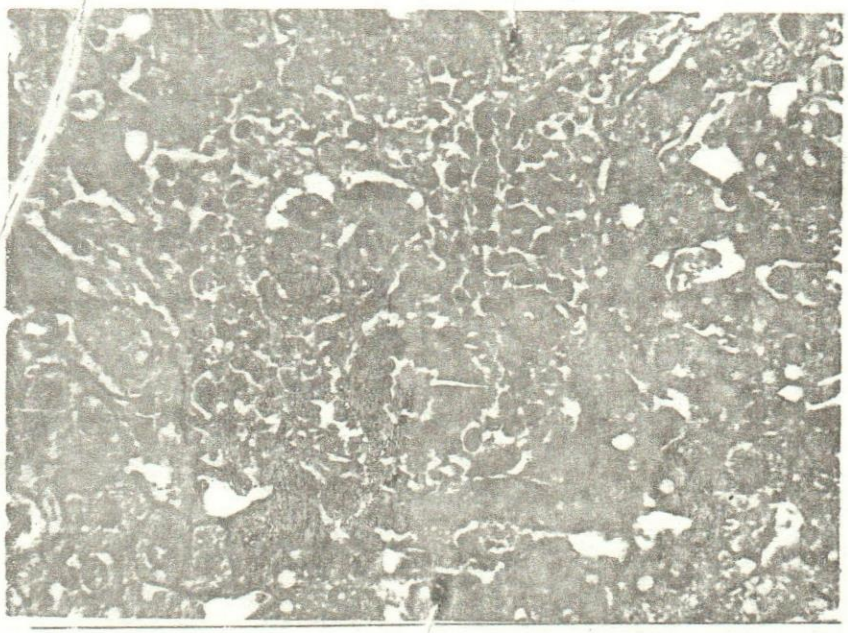
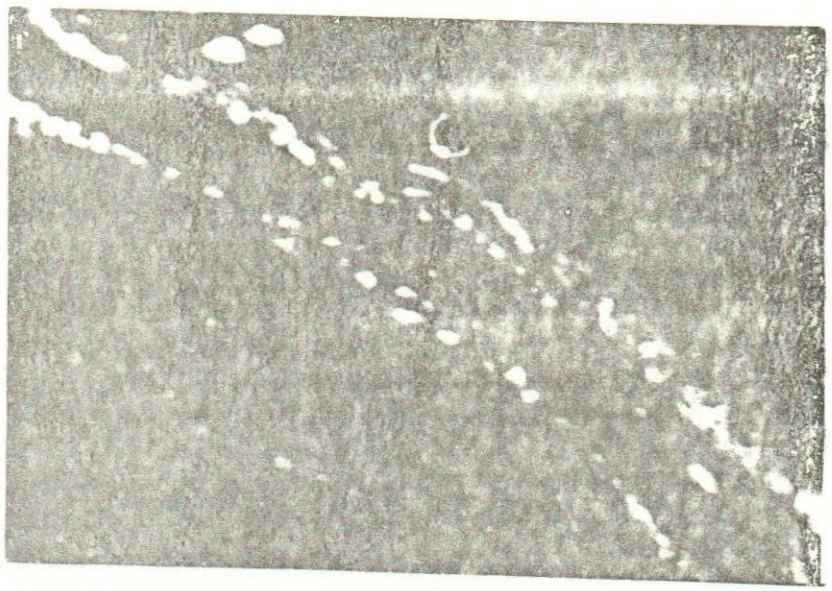


Fig.. 12)



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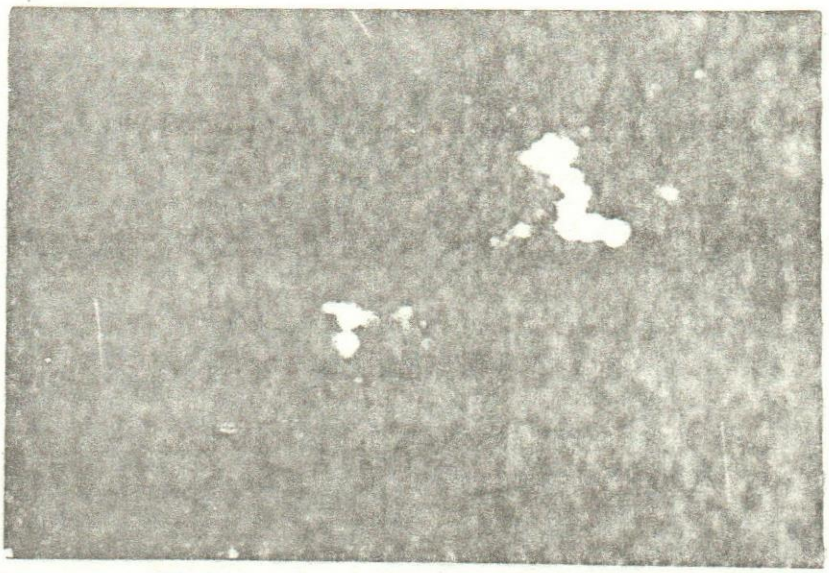

Fig.: (14)