قســـــم : البائولوجيا ـ كلية الطب البيطرى ـ جامعة أسيوط.
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بعض الدراسات عن الأصابة التجريبية بعترتين من ميكروب الكوربـــــنى بكتريم فى الأغنام

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أجرى هذا البحث لمعرفة مدى وكيفية وكذلك باثولوجية تأثير عترتين من ميكروب الكـــــوربـــنى بكـــتريم فى الأغنام. أعديت الأغنام تحت الجلد وفى الجلد واجريت الصفة التشريحية بعد ٢ ، ٤ ٧ ، ١٤ يومـــا من الحقن وأخذت العينات من مكان الحقن والغدة الليمفاوية الموضعية وكذلـــك الأعضـــاء الداخلية والغدد الليمفاوية.

بعد فحص العينات نوقشت النتائج المقارنة بين العترتين المستعملتين فى البحث.

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STUDIES ON EXPERIMENTAL INFECTION WITH CORYNEBACTERIUM PSEUDOTUBERCULOSIS (OVIS)
III- COMPARATIVE STUDIES ON THE EFFECT OF TWO STRAINS IN SHEEP

(With 7 Figures)

By
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SUMMARY

In the present experiment, a total number of adult apparently normal sheep were used. Animals were divided into four groups, two of these groups were infected with a strain of C. pseudotuberculosis (ovis) by subcutaneous and intradermal inoculation respectively. The other two groups were similarily inoculated using A Bu 77 strain. Animals were slaughtered 2,4,7,14 days after inoculation.

Postmortem examination was carried out and samples from skin at the site of injection, regional lymph nodes, heart, lung, liver, spleen, kidneys, gastrointestinal tract, testicles in males as well as internal lymph nodes were taken.

The primary local lesion, lesions in lymph nodes and those observed in internal organs were described and discussed. Our results using A Bu 77 strain emphasized our earlier observations in buffalo and cattle that this strian is potent toxin producer; however, it has a weak invasive power.

INTRODUCTION

Corynebacterium pseudotuberculosis was recorded by many investigators to be the essential cause of caseous lymphadenitis in sheep and goat. clinical as well as pathological manifestations of the disease in sheep were described by SHIRLOW and ASHFORD (1962) JUBB and KENNEDY (1963) CHORS (1966) SMITH et al. (1972) and AYERS (1977). The organism could be isolated from cases with suppurative bronchopneumonia and others with mastitis, ADDO and DENNIS (1977). Reproductive disorders in the disease process were described by KHALIMBKOV et al. (1961), CHORS (1966) and KHATER, et al. (1975).

The aim of the present work was to study the pathogenesis and disease process of infection with "A Bu 77" strain and a strain of corynebacterium pseudotuberculosis (ovis) isolated from sheep.

MATERIAL and METHODS

A total number of 13 adult, apparently normal sheep (4 males, 9 females) were used. The animals were divided into groups, the first group, A, included only one animal while the other 3 groups, B,C and D consisted of 4 animals each. Two of these groups, A, and B, were infected with a strain of C. pseudotuberculosis (ovis) by subcutaneous and intradermal inoculation respectively. The other two groups, C and D, were similarily inoculated through one of the two routes using A Bu 77 strain. The animal in group A was killed after two days of infection while one animal in the other 3 groups, B,C and D was slaughtered after 2,4,7 and 14 days. Infected animals were given a single injection of the infecting microorganism at the right side intradermally in the skin of the neck and subcutaneously in the flank region.

Postmortem examination was carried out and samples from skin at the site of injection, regional lymph nodes, heart, lung, liver, spleen, kidneys, gastrointestinal tract, testicles in males as well as internal lymph nodes were taken and preserved in 10% neutral formalin solution. Tissue specimens were processed for paraffin section which were stained by conventional methods.

RESULTS

Gross pathological findings:

Generally, local skin lesions were found at the site of injection in all animals infected by any of the two routes. These lesions consisted of suppurative foci involving the epidermis and dermis at early stages of infection and penetrated deep to subcutis and subcutaneous muscles later on. All animals subcutaneously infected with A Bu 77 strain of C. pseudotuberculosis showed subcutaneous accumulation of gelatinous strew-coloured fluid, this picture was not observed in animals infected with conventional strain except one. The later was also inoculated subcutaneously.

The prescapular lymph nodes of the right side in 3 animals inoculated intradermally and prefemoral lymph node in one animal inoculated subcutaneously with conventional strain of C. pseudotuberculosis showed areas of caseous necrosis. Animals infected with A Bu 77 strain through intradermal inoculation showed no lesions in the prescapular lymph nodes. An animal inoculated subcutaneously with this strain and killed after 14 days of infection showed necrotic area in the prefemoral lymph node.

Lesions in other organs consisted of caseous necrosis in muscles of the neck of an animal 14 days after intradermal inoculation with conventional strain of C. pseudotuberculosis, isolated areas of pneumonia in the lung of two animals killed 4 and 14 days after intradermal inoculation with A Bu 77 strain, and petechial haemorrhages in the spleen of an animal 7 days postinfection using A Bu 77 strain.

Histopathological findings:

Examination of the skin in animals intradermally inoculated with the conventional strain of C. pseudotuberculosis revealed the occurrence of raised areas of necrosis and parakeratosis along the length of the epidermis already two days after infection (Fig. 1). Directly at the site of inoculation, both the epidermis and dermal tissue were totally destroyed with focal accumulation of neutrophils. These inflammatory cells moreover, diffusely infiltrated the surrounding area, more frequent at the papillary layer of the dermis and infilterating the wall and accumulated in the lumens of sweat glands. Oedema and vascular congestion were not so prominent in the dermis except at its deeper layer. In the latter, lymph vessels were widely dilated and their lumens occasionally contained infilterating cells as a result of necrosis and destruction of their walls (Fig. 2). Four days after infection, fragmentation of infilterating neutrophils and caseous necrosis associated with central lysis was clearly demonstrated in areas of focal aggregation. Also at this stage and latter on that both diffuse and focal infiltration of inflammatory cells occurred in adipose subcutaneous tissue (Fig. 3). Oedema which was mild, totally subsided at the 7th day and the blood vessels were patent with no tendancy for thrombus formation. Fibroplasia and histiocytic reaction were observed at this stage and increased in activity at the 14th day. At this stage, foci of neutrophils were partly surrounded by a capsule of mature connective tissue. Plasma cells in few numbers make their appearance especially around blood vessels.

The reaction of the skin following subcutaneous inoculation of this strain consisted of more or less diffuse infiltration of neutrophils in subcutis and deep dermis after two days. Infiltrating cells accumulated more around blood vessels while they were sparsely distributed in the superficial layer of the dermis. Both superficial and deep lymphatic plexuses were dilated and filled with an inflammatory exudate. There was an increased proliferation and activity of adventitial tissue of blood vessels in the area. The epidermis was intact with no apparent lesions.

Changes occurred in regional lymph nodes after infection with this strain through intradermal inoculation during the first four days consisted of increased infilteration of neutrophils and eosinophils in the marginal and cortical sinuses. These cells as well as lymphocytic elements of the lymph node undergo caseous necrosis. Prescapular lymph nodes in animals killed after 7 and 14 days showed large confluent areas of caseous necrosis. The borders of these areas of necrosis consisted of fragmented neutrophils and lymphocytes, histiocytes, fibroblasts and fibrocytes admixed with epithelioid cells and mature fibrous connective tissue seperating them from surrounding non-affected tissue. Followin subcutaneous inoculation, the prefemoral lymph nodes at the affected site showed similar lesions.

In animals intradermally infected by A Bu 77 strain, the skin generally appeared oedematous and highly necrotic during the first 4 days of infection. Epidermal changes directly at the site of injection showed a similar picture as that following intradermal inoculation of the conventional strain. However, necrosis and fragmentation of infilterating leucocytes were more extensive. Infilterating neutrophils invaded also the glandular structures in the dermis. The collagenous connective tissue of the dermis was disorganized and undergo degeneration. Lymphatics were obliterated by masses on inflammatory leucocytes (Fig. 4). After 7 and 14 days of infection active proliferation of fibrous connective tissue and histiocytic reaction was similar to that seen in animals intradermally inoculated with the conventional strain.

Epidermal changes of the same features as above werer also observed in animals infected subcutaneously by this strain especially along the needle-track. At the 7th day of infection, uncapsulated multiple foci of neutrophils occurred in subcutaneous tissue imparting a pressure form below upward leading to occlusion of many lymphatics. Fibroplasia in deep dermis and subcutis was clearly demonstrated at this stage associated with active histiocytic reaction. The intermuscular connective tissue in direct contact with the subcutis was also invaded with inflammatory cells. The muscle fibers lost their nuclei, were swollen and pale eosinophilic (Fig. 5). Deposition of calcium in necrotic muscular areas was observed at the 14th day of infection.

The subcapsular, cortical and trabecular sinuses of the medulla in all lymph nodes in animals inoculated inoculated intradermally and subcutaneously with A Bu 77 strain were filled with polyhedral reticulum cells which showed high phagocytic activity. Lymph follicles as well as in the paracortical areas, groups of cells undergo necrosis and were replaced by a pink, eosinophilic mass. In the medulla, trabecular connective tissue showed degenerative changes. Degeneration and necrosis could also be observed in the wall of intertrabecular blood vessels. Occassionally, leucocytic margination and formation of microthrombi were found in afferent blood vessels in the capsule (Fig. 6). The regional lymph node of an animal killed after 14 days after subcutaneous infection showed a large necrotic area of structureless, eosinophilic reticulum and disperesed chromatin fragments.

Degenerative and necrobiotic changes of hepatic cells and blood vessels were demonstrated in all animals, however these changes were more mild in those animals inoculated intradermally. In animals inoculated intradermally and killed 7 days after infection and later on necrosis of the wall of the portal veins was observed, the groups of the hepatic cells around were necrosed and detached with the occurrence on many of them in the lumen of the vein (Fig. 7).

In the intradermally inoculated animals, kidney showed glomerulo-and tubulonephrosis. Acute focal non-suppurative nephritis with accumulation of mononuclear cells in the interlobular connective tissue were demonstrated in the first 7 days of infection. The kidneys of the animal killed after 14 days showed increased focal proliferation of connective tissue. Changes of the kidneys in these animals infected through the subcutaneous route were restricted to nephrosis both in the glmeruli and tubules.

Suppurative bronchopneumonia, haemosiderosis in the spleen and testicular degeneration could be observed in animals infected through the intradermal route. Other organs appeared normal.

DISCUSSION

As revealed by the present work, primary local lesion following either intradermal or subcutaneous inoculation of conventional strain of C. pseudotuberculosis isolated from sheep always occurred in the skin. Lesions in lymph nodes appeared to be metastatic and depend upon the site of inoculation, i.e., these usually affected are the regional lymph nodes. CAMERON et al. (1972) stated that when sheep have been used as experimental animals it has proved difficult to reproduce pathogenic effect in the peripheral lymph nodes, most abscesses being located in the skin following intracutaneous infection, or lungs following intravenous injection. In contrast pyogenic foci were found in regional lymph nodes in four of the five animals infected with this strain either intradermally or subcutaneously in the present study.

Although generalized infection with C. pseudotuberculosis and the occurrence of abscesses and pyogenic foci in many organs was recorded in sheep (ADDO and DENNIS, 1977; PALIWAL et al., 1974), this condition was however rare. No generalization following experimental infection was observed in our animals.

DAVIES et al. (1960) stated that although C. pseudotuberculosis produces an exotoxin, the pathogenicity of the organism in relation to animal disease appears to be linked up with the bacterial bodies rather than with the toxin, and that the organism behaves more as a pyogenic organism than a toxigenic one, this may be true only for the conventional strain used in our experiment. It has been found that C. pseudotuberculosis has a high percentage of easily extractable and persumably surface lipids (JOLLY, 1966). Electron microscopic studies have shown that existance of an electrondense floccular layer exterior to the cell wall, a lipid layer (HARD 1969). Suspensions of C. pseudotuberculosis subjected to physical and chemical treatment faiked to kill mice but were able to produce sterile pyogenic foci (ZAKI, 1976). Exotoxin has been suggested to be a product other than the pyogenic factor and the later is likely to be associated with a heat stable substance present in the body of the bacillus (BULL and DICKINSON, 1935; ZAKI, 1976).

Lesions in the skin induced by the conventional strain differed from that induced by A Bu 77 strain in that oedema was less conspicuous and rapidly disappeared, and pyogenic foci either in the skin or regional lymph nodes showed a central area of lysis, and a much less necrotizing effect on connective tissue of the skin.

Our results using A Bu 77 strain in sheep emphasized our earlier observations in buffalo and cattle that this strain is potent toxinproducer, however, it has a weak invasive power, Toxicity was indicated in the present study by the occurrence of degenerative changes in small blood vessels and parenchymatous organs and abscence of any pyogenic foci in regioal lymph nodes or organs (suppurative bronchopneumonia found in two experimental animals are thought to be secondary and mainly associated with weakness of tissue resistance).

JOLLY (1966) reported, however, that surface lipids provide the organism with a structural barrier against antibacterial mechanism of the host phagocytic cells. It seems that the severity and nature of infection do not depend only on the strain used but also together with the infective dose. CARNE (1940) and ABDEL-HAMID (1973) showed that experimental inoculation of sheep and goats with broth culture of C. pseudotuberculosis was followed by death within 48 hrs and the symptoms and the lesions were due to acute intoxication. No deaths occurred in animals of the present study and the lesions were not severe enough.

Generally, our results are in parallel to those of many investigators (CARRE, 1910; DAINES and AUSTIN, 1932; LOVELL and ZAKI, 1966) who showed that not all strain produce a toxin and a non-toxogenic strains may be present.

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DESCRIPTION OF FIGURES

Fig. (1): Skin showing raised area of necrosis and parakeratosis along the length of the epidermis. (H.E. X 100).

Fig. (2): Dilated lymph vessels contained infilterating cells. (H.E. X 100).

Fig. (3): Adipose subcutaneous tissue showing focal and diffuse inflammatory cellular infilteration. (H.E. X 100).

Fig. (4): Lymphatics obliterated by massos of inflammatory leucocytes (H.E. X 100).

Fig. (5): Muscle fibers showing degenerative changes. (H.E. X 250).

Fig. (6): Microthrombi in afferent blood vessels. (H.E. X 400).

Fig. (7): Hepatic cells in the lumen of the vein. (H.E. X 400).

FIG. (1)

FIG. (2)

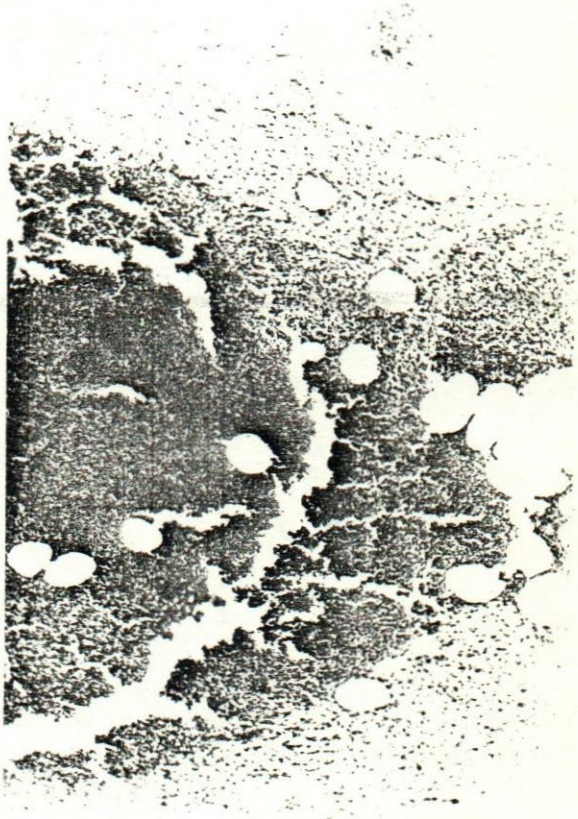

FIG. (3)

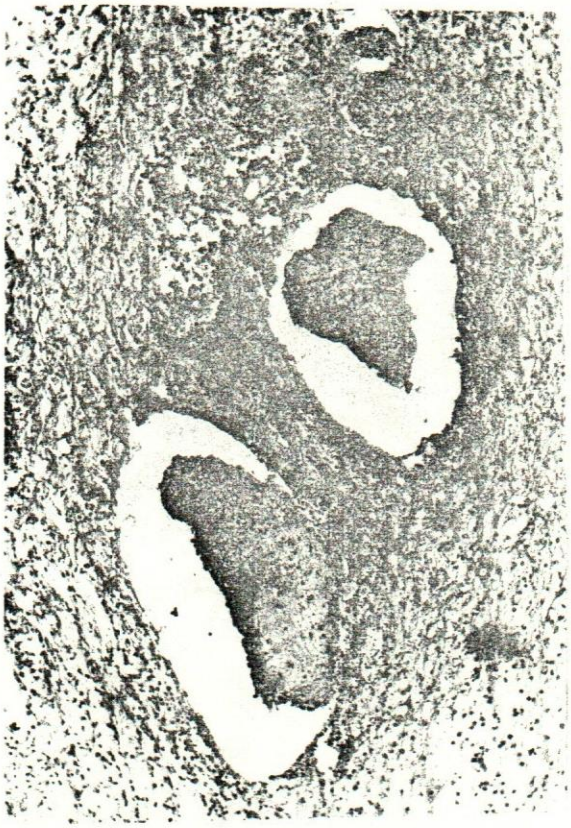

FIG. (4)

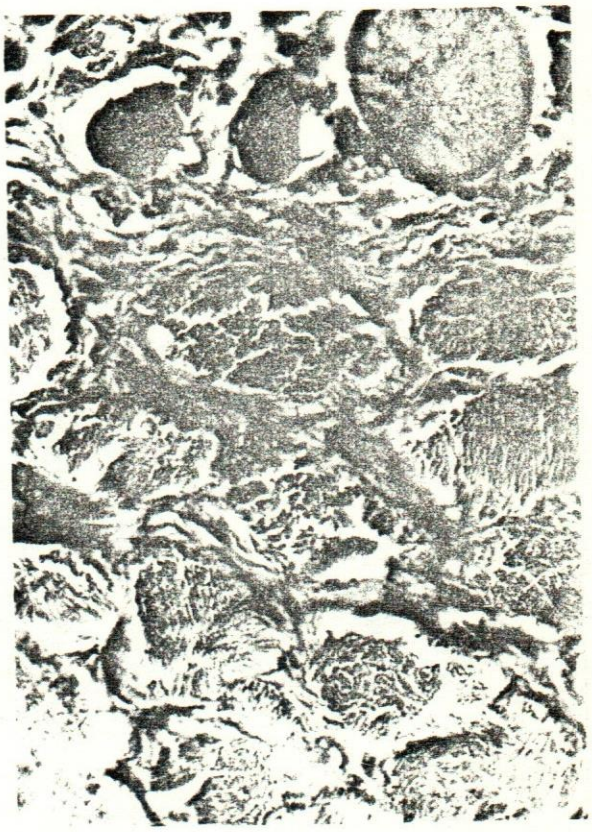

FIG. (5)

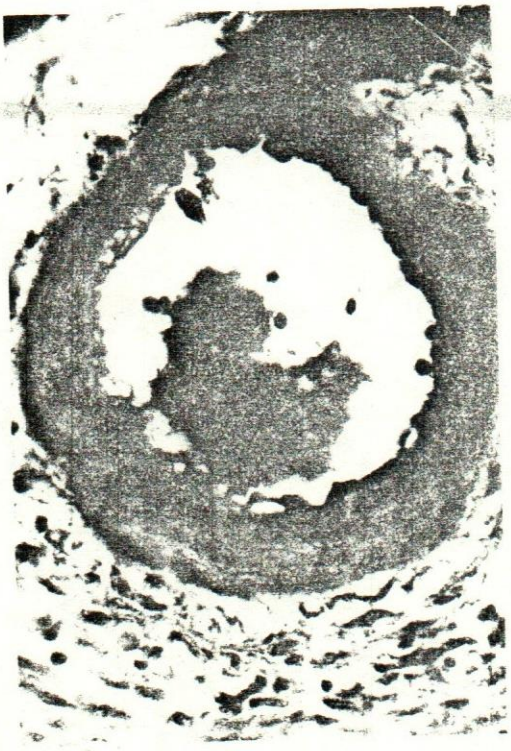

FIG. (6)

FIG. (7)