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MYCOBACTERIUM ULCERANS IN THE ARMADILLO

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MYCOBACTERIUM ULCERANS IN THE ARMADILLO*

I. BACKGROUND

Mycobacterium ulcerans is an acid-fast bacillus that grows optimally at 32°C and causes indolent, necrotizing skin ulcers in man. These ulcers are also called Buruli ulcers in Uganda, Searle's and Bairnsdale ulcers in Australia, and Kakerifu ulcers in Zaire.

A. Clinical features (For Figs. 1-38 and their legends, see Annexes 1 & 2)

The first sign is a slowly enlarging hard, circumscribed movable mass in the subcutaneous tissue (Fig. 1). The nodule is usually neither red, painful, tender, nor edematous but frequently itches. An X-ray may reveal mineralization (Fig. 2). As the mass enlarges, a vesicle forms, then breaks down into a gradually enlarging ulcer. Scratching, sometimes ruptures the vesicle. The perimeter of the ulcer is gently scalloped (Fig. 3) and, along with the base, is partially covered by white necrotic sloughs (Figs. 3, 4, 5, 6). The surrounding tissues are edematous. Patients feel well, remain active, and have no fever, regional lymphadenopathy, malaise, nor leukocytosis, even when the ulcers are very large (Fig. 7). As a rule the ulcer bed has no odor and secondary infections are not usually a problem. Sometimes scaling and altered pigmentation of the skin are prominent over the infected area and may precede ulceration. On rare instances, a whole extremity is involved before an open ulcer develops (Figs. 8, 9). Occasionally, the skin over a wide area may be loosened from the fascia of an entire arm or thigh, even though the ulcer is small. Ulcers have a natural tendency to heal even without medical care but complete healing may take months or years. Healing usually begins at the

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proximal margin (Figs. 5, 6) from which the epithelium grows over the base. Healing may proceed at the proximal margin while the ulcer extends at the distal margin. The final result may be a long, depressed scar (Fig. 10). In a few patients, contraction, deformity, and lymphedema may complicate healing (Fig. 11) and on rare occasions, relentless extension has led to amputation (Figs. 12, 14). Nine-tenths of the ulcers are on the limbs and about one-tenth are on the trunk (Fig. 13). Palms and soles are spared and face and scalp are only rarely involved.

B. Epidemiology

This disease has been identified in Australia, Bolivia, Cameroon, Congo (Brazzaville), Ghana, Malaysia, Mexico, New Guinea, Nigeria, Uganda, Republic of Zaire, and probably Peru and Northern Sumatra. Those afflicted tend to live in river valleys or in poorly drained swampy lowlands. In Uganda, patients live along the course of the Victoria Nile, in Zaire, along the Zaire River and its major tributaries, and in New Guinea, along the Kamusi River. Most of these areas are sparsely populated. Contagion is apparently not a factor and there is still no evidence to implicate an insect as vector or reservoir. Because of the high percentage of single lesions, most investigators believe that the organism's route of entry into man is through the skin. Some infections have developed at sites of hypodermic injections and other penetrating traumas and these examples support the view that infection follows percutaneous inoculation. All forms of minor penetrating trauma are therefore suspect. In Uganda, grasses used in thatching have been studied as possible vehicles for M. ulcerans, but without conclusive results. As with other mycobacteria, M. ulcerans survives exposure to ultraviolet light only briefly, so that an open reservoir, such as vegetation, seems unlikely. Because some endemic areas parallel slow moving rivers, some researchers believe the reservoir is in or near the open water. Many patients, however, never contact river water directly and fish and other aquatic life have not been shown to harbor M. ulcerans. Further, no aquatic or land animals are known to be naturally infected. Persons of all age groups may be infected but graphs depicting frequency show a peak in the second and third decades of life.

The incidence and prevalence of the disease are unknown but in one endemic area along the Victoria Nile almost 10 percent of recently settled refugees became infected in about 2 years. Generally, however, endemic areas tend to be sparsely populated, so that the total number of those exposed is probably small.

Nothing is now known of the epidemiology of the infection in the Americas, but as data develop, a comparison with other endemic areas will probably yield clues to the reservoir of M. ulcerans.

C. Pathogenesis

Sections through nonulcerated lesions reveal a central area of necrosis containing large numbers of acid-fast bacilli (Fig. 15). This area of necrosis is centered in the deep dermis or subcutaneous fat. It is symmetric and continuous, and involves all contiguous structures. As the infection progresses, bacteria and necrosis spread laterally--with necrosis preceding the bacteria--most rapidly in the subcutaneous adipose tissue. The viable margin is edematous and contains a few lymphocytes, histiocytes, and eosinophils. Cells in the involved tissues enlarge, lose their nuclei, but retain their outlines for several weeks or months. The capillary circulation is lost but larger vessels may maintain their contours and pass erythrocytes even though their walls are completely necrotic (Fig. 16). The necrosis may spread to deep fascia and muscle (Fig. 17) but only rarely is bone involved. Systemic spread has not been recognized. Layers of fibrin accumulate between the ghosts of fat cells (Fig. 18) and fat lobules, making the outlines of cells and lobules prominent. Stains for acid-fast bacilli reveal clumps and masses of bacilli in the necrotic sloughs of the ulcer bed and in adjacent necrotic adipose tissue (Fig. 19). The advancing edge of necrosis is distinctive because it lacks a significant vascular response or cellular exudate. Some of the larger vessels at the advancing margin are occluded by a proliferation of endothelium (Fig. 20). As the necrosis spreads superficially, dermal papillae become edematous and enlarged (Fig. 21), the epidermis is spongiotic and separates above the basal layer before sloughing (Fig. 22). The ulcer enlarges--following the advancing necrosis in the deeper tissues. Thus a

characteristic undermined margin develops. The overhanging flap is usually 1-3 cm but may reach 10-15 cm. Healing is recognized microscopically by the development of a granulomatous reaction at the margin (Fig. 23)--then by reepithelialization of the undermined edge, and finally by granulation tissue and reepithelialization of the ulcer bed. The extensive necrosis and other histologic features suggest that M. ulcerans elaborates a diffusing toxin that destroys the nuclei of somatic cells and perhaps also inhibits secondary bacterial growth. Krieg et al.^{4/} and Read et al.^{8/} have recently identified toxic products that may cause the spreading necrosis.

D. Treatment

Smaller lesions, especially those that are not yet ulcerated, are excised en bloc with a surrounding margin of noninfected tissue. This procedure, experience has shown, is curative. Larger lesions are carefully debrided, preserving the viable tissues. This is followed by skin grafting. Recurrent foci of infection are debrided and regrafted when recognized. Heat therapy has proved beneficial when the entire lesion can be warmed continuously under a cradle or by a heated jacket, to about 40°C. The higher temperature may combat the infection in one or more ways: (1) multiplication of M. ulcerans may be inhibited, (2) local cell-mediated immunity may be increased, and (3) granulation tissues may be stimulated. Rifampicin has also promoted healing in some patients, but has not yet been adequately evaluated.

E. Differential diagnosis

The following three conditions are confused with ulcers caused by M. ulcerans: (1) Tropical phagadenic ulcer, the most common cutaneous lesion in some parts of the tropics, may be distinguished by its putrid odor, its location below the knee, its hard, raised margin that is not undermined, the absence of acid-fast bacilli in the exudate and by the presence of fusospirochetal flora on its surface. (2) Cutaneous ulcers in patients with severe kwashiorkor may resemble closely ulcers caused by M. ulcerans but are associated with other signs of kwashiorkor especially the characteristic

"flaky paint" dermatitis that tends to be prominent around the ulcer. Ulcers in kwashiorkor may contain a mixed flora but not acid-fast bacilli. (3) The spreading necrosis and ulceration caused by venomous insects, scorpions and other animals, may also resemble M. ulcerans infection. A mixed bacterial growth throughout the necrotic tissues, lack of acid-fast bacilli, and more rapid healing on antibiotics, if the patient survives, are features that help distinguish these ulcers.

II. ANIMAL STUDIES

Mycobacterium ulcerans is slow growing, lightly pigmented and non-photochromogenic. It thus falls in Group III of Runyon's classification. M. ulcerans grows optimally at 32°C to 33°C and fresh isolates will not grow in vitro above 34°C. Stock cultures tend to lose their virulence but can be restored by animal passage.

A. Reaction in the mouse

A variety of animals have been inoculated including mice, rats, mystromys, guinea pigs, and calves. The calf and guinea pig are resistant but progressive infections develop in mice, rats, and mystromys and in these small rodents the infection follows essentially the same course. In the mouse for example, 7 to 12 weeks after inoculation of the footpad, it becomes swollen and red (Fig. 24). As the infection progresses the entire leg swells, the hair falls out and there is diffuse crusting and weeping. Eventually the entire leg may slough or autoamputate. In some experiments dissemination throughout the mice's skin has caused swelling of the entire mouse, giving it a ballooned somatic contour. Histologically the infection in the mouse is characterized by severe edema, an acute inflammatory reaction, and necrosis.

B. Reaction in the guinea pig

The reaction in the guinea pig is quite different. When inoculated with viable bacteria or with toxic products from cultures of M. ulcerans,

the sites become swollen and red, 1 to 4 days later (Fig. 25). A scab forms between 10 and 15 days and this sloughs off between 18 and 25 days. Microscopic study (Figs. 26-28) reveals an abscess in the dermis with surrounding acute and chronic inflammatory cells and vascular congestion. Foci of necrosis are present in the underlying muscle and adipose tissue. Reepithelialization of the abscess is a prominent feature. With viable organisms clumps of intra- and extra-cellular acid-fast bacilli are present in the dermis, but the reaction is the same as when the toxic fractions are inoculated (Fig. 29).

The characteristics of the infections in the mouse and the guinea pig are distinctly different from the human infection and thus they are not ideal models for comparison with human infections.

C. The armadillo

The armadillo (Figs. 30, 31) was suggested as an animal model for M. ulcerans by Dr. Chapman H. Binford because of the success with M. leprae in this animal. Mycobacterium leprae grows best in the cooler parts of the human body and the nine-banded armadillo, Dasypus novemcinctus, develops disseminated lepromatous leprosy. The armadillo has a normal body temperature of 33°C to 35°C and this low body temperature may allow the M. leprae to proliferate.

In an initial study, four armadillos were inoculated intradermally on the medial thighs with a suspension of M. ulcerans. Two months later two of the animals developed preulcerative lesions (Fig. 32) and by 12 weeks, indolent undermined ulcers characteristic of the lesions seen in humans were present (Figs. 33, 34). Histologically the lesions contained broad areas of coagulation necrosis and clumps of acid-fast bacilli (Figs. 35-38).

Since this pilot study 18 additional animals have been inoculated on the abdomen where the temperature is two degrees warmer than the thigh. Small erythematous nodules developed within 3 weeks but these receded and did not recur. Erythematous areas appeared on the thighs and some of the animals developed a generalized edema and died within a few days, but microscopic sections revealed no evidence of a disseminated M. ulcerans

infection. Organisms were present at the sites of inoculation and at the erythematous foci on the thighs. More recently additional armadillos have been inoculated on the thighs and have developed nodules. They are being observed for early signs of ulceration and other evidence of progressive infection.

The chronic undermined ulcer of the armadillo resembles closely the ulcers seen in humans. Furthermore, the histologic changes in both the human and armadillo are essentially identical. Because infection in the armadillo parallels that seen in humans the armadillo is the best animal model so far studied for investigation of M. ulcerans.

As noted previously, M. ulcerans produces a toxin that diffuses and causes necrosis and inflammation of guinea pig skin. This toxic factor is present in the culture filtrate and the cytoplasmic fluid and in the particulate fraction. Studies to isolate, purify, and elucidate the antigenicity and physical-chemical properties are in progress.

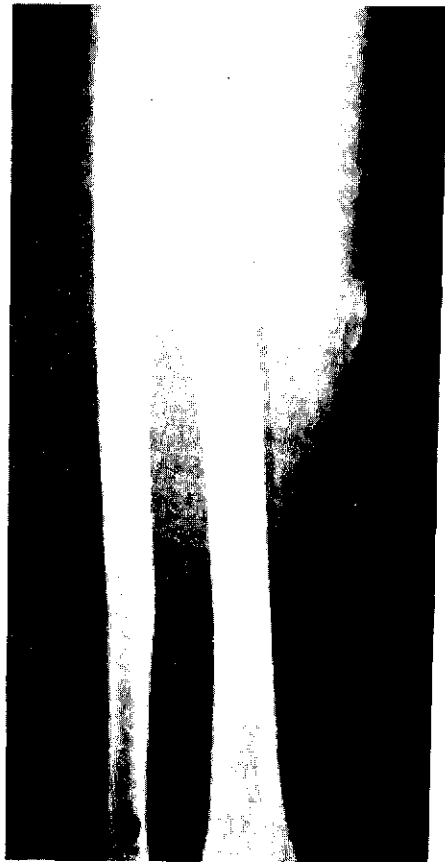
The effects of the purified toxin on the armadillo will be studied and should be revealing. The effectiveness of antitoxin for therapeutic treatment and prophylaxis will be determined. Research will also be conducted on the armadillo's immunologic response to infection. This research is significant in that it will create an awareness of this disease in the Americas and this should lead to a diagnosis of more cases and a more thorough understanding and subsequent control of the disease by better preventive measures. Earlier and more effective treatment will prevent the late sequelae of contraction deformity, lymphedema, and amputation.

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ANNEX 1

FIGURES



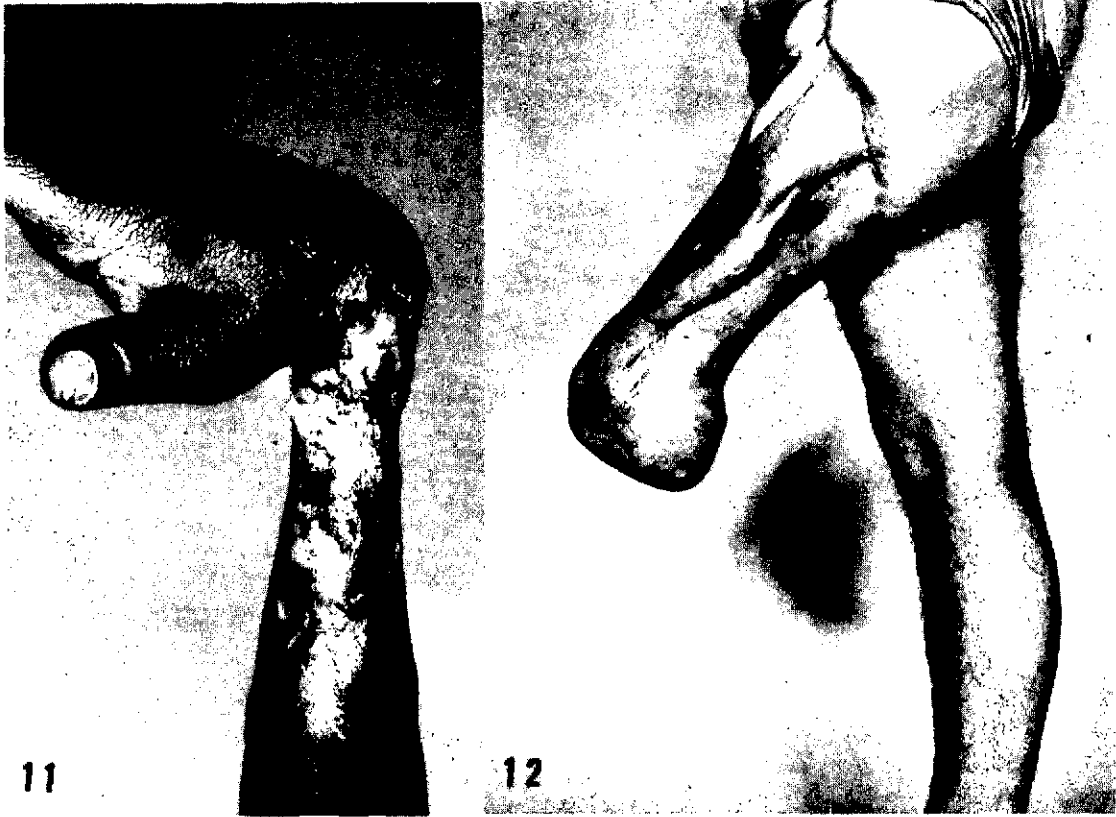


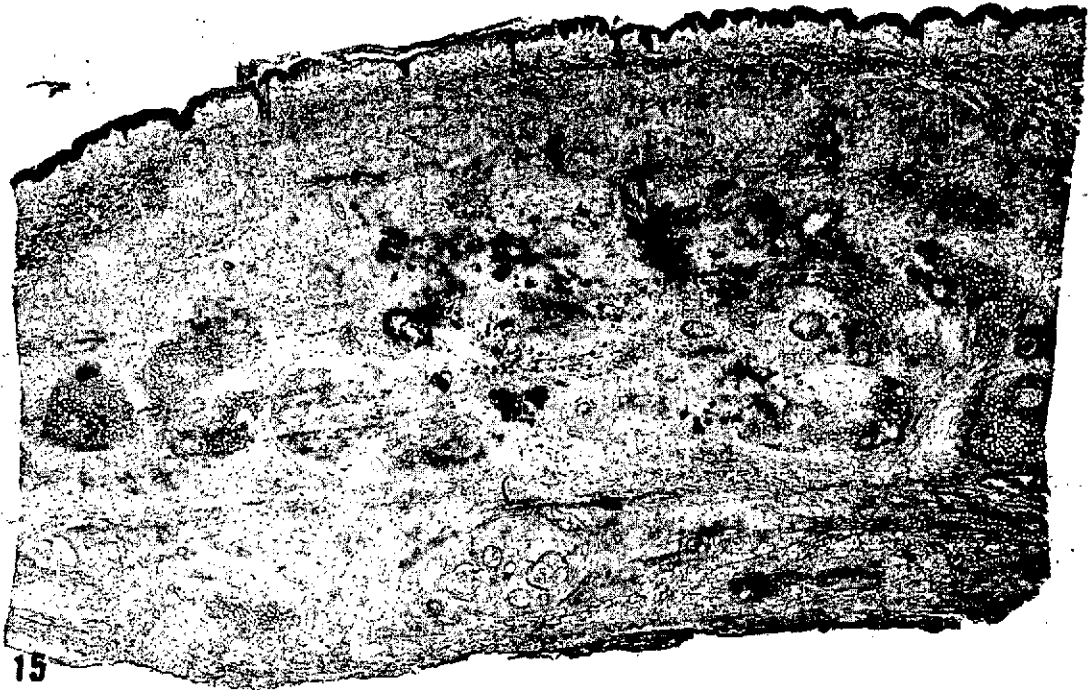
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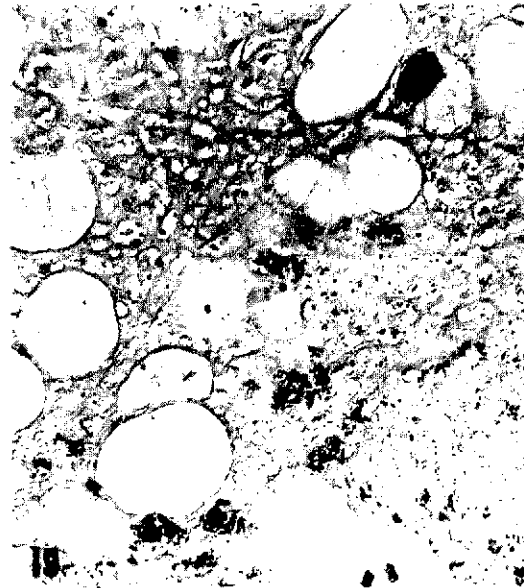
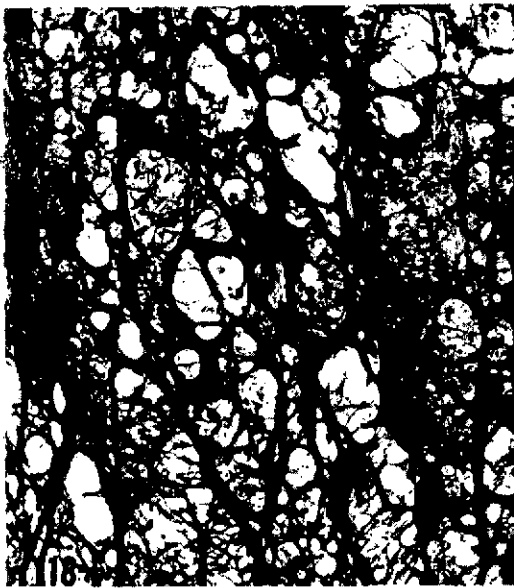
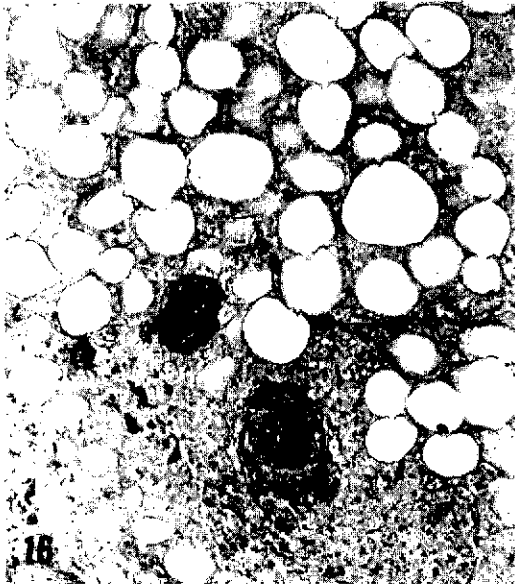


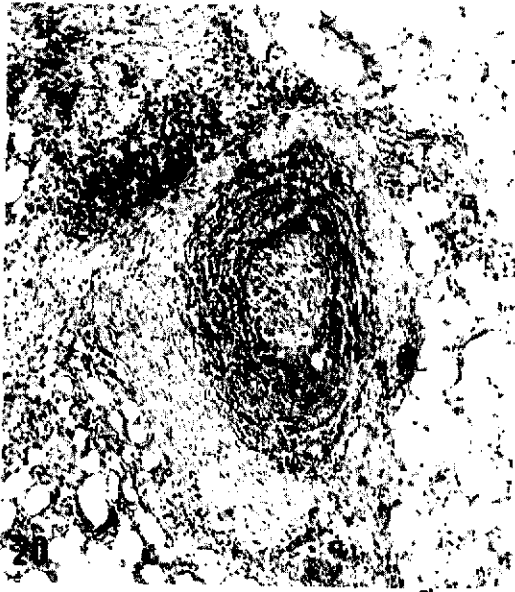
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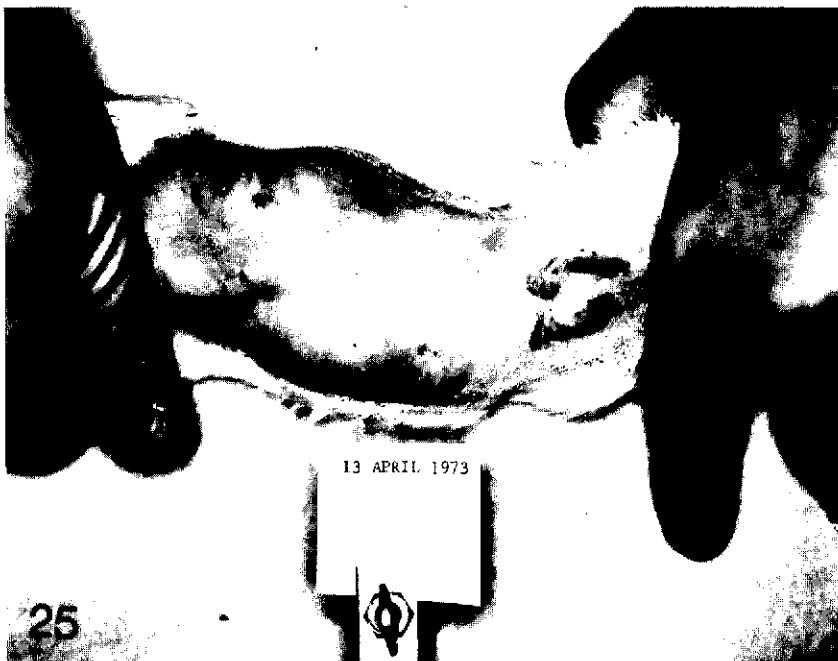


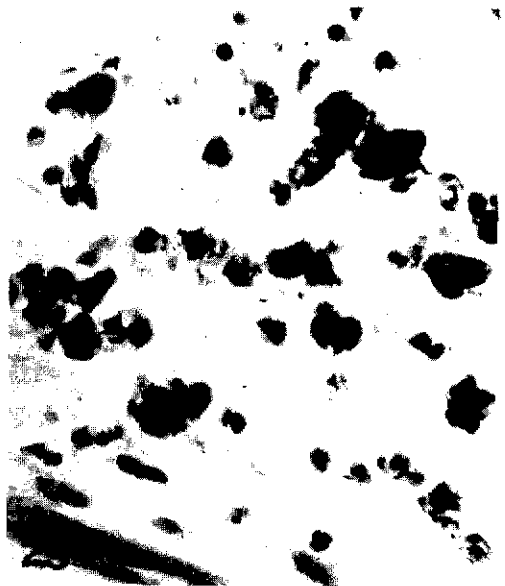
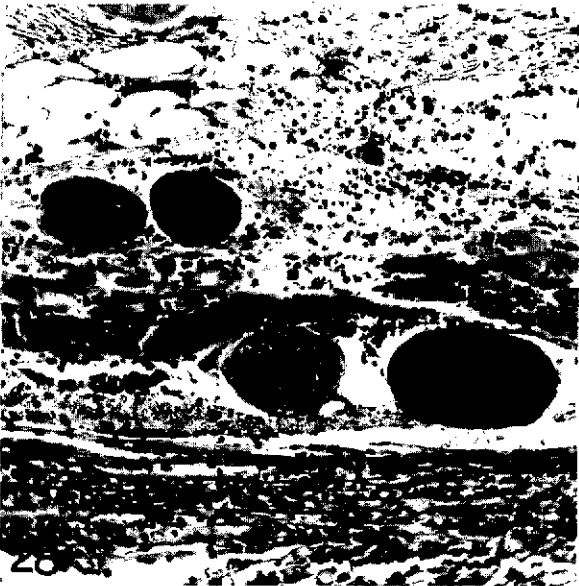


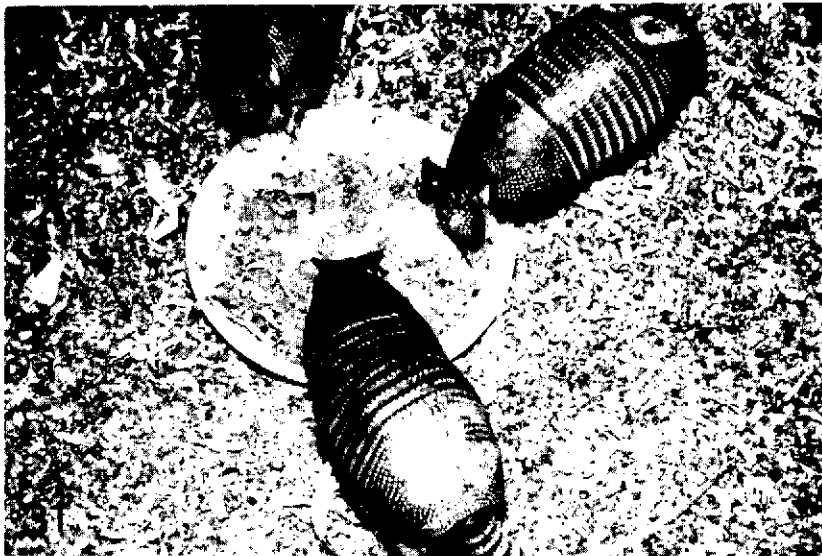
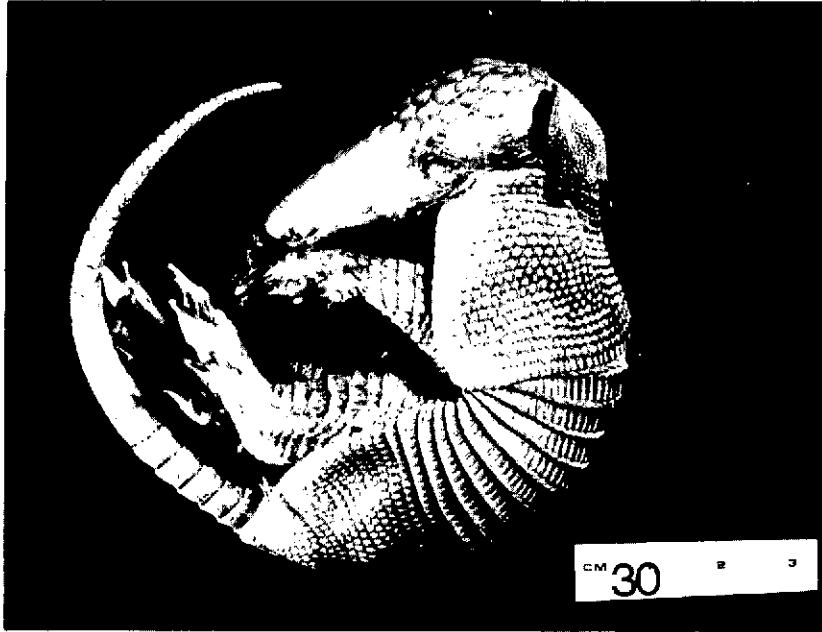








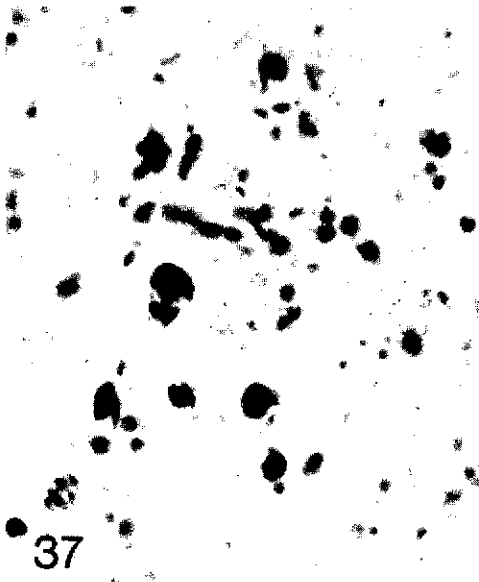




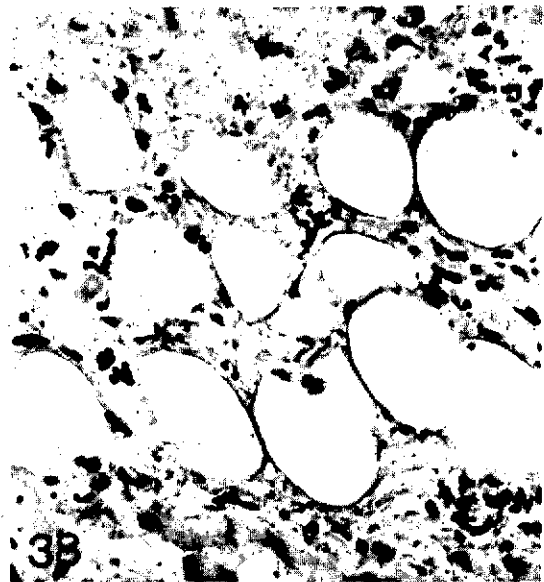




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ANNEX 2

LEGENDS

LEGENDS

- Fig. 1. There is a nontender hard movable mass approximately 5 x 3 cm. in the lateral aspect of the midforearm. A small vesicle has formed.
(Armed Forces Institute of Pathology Photograph No. 65-3139)
- Fig. 2. A roentgenogram reveals mineralization of soft tissue in the indurated area. (AFIP Photograph No. 65-2988)
- Fig. 3. Midforearm and wrist of a 16-year-old Uganda boy whose ulcer developed over a four-week period. The edges are undermined.
(AFIP Photograph No. 65-2981)
- Fig. 4. A 34-year-old Nigerian physician developed M. ulcerans infection of right elbow while doing postgraduate work in New York City. He had been in the United States about 7 weeks. Debridement and grafting led to complete healing in about two months.
(AFIP Photograph No. 70-11607)
- Fig. 5. A young woman from Uganda with an ulcer of her right thigh which is healing along the proximal margin. The distal margin remains undermined and is extending distally.
(AFIP Photograph No. 69-3236)
- Fig. 6. A young boy from Kimpese, Republic of Zaire. His ulcer is beginning to heal along the proximal margin but there is persistent infection at the lateral and distal margins.
(AFIP Photograph No. 70-2181)
- Fig. 7. A 7-year-old boy from Uganda had involvement of the entire leg. In spite of the size of this ulcer, he walked into the hospital and had no systemic signs or symptoms, nor regional lymphadenopathy. Repeated debridement and grafting led to complete healing without complication and with normal locomotion.
(AFIP Photograph No. 65-2976)

- Fig. 8. A ten-year-old Uganda boy presented with a one-month history of increased swelling and dermatitis of the left leg. Over a three-month period, sinuses formed -- draining first watery fluid, then necrotic sloughs -- gradually enlarging to form ulcers.
(AFIP Photograph No. 65-2985)
- Fig. 9. Ulcers have formed and there is extensive necrosis of dermis and subcutaneous adipose tissue. Repeated debridement and grafting led to complete healing.
(AFIP Photograph No. 72-17287)
- Fig. 10. The spiraled scar on the leg of this woman from Mayo, Uganda, is characteristic of a healed M. ulcerans infection. Its length indicates that it progressed down the leg with healing at the proximal margin and extension at the distal margin. The depression results from loss of subcutaneous adipose tissue but the band of normal tissue has preserved lymphatic drainage.
(AFIP Photograph No. 65-6408-2)
- Fig. 11. A healed ulcer of a ten-year-old boy who lived in Uganda, along the Nile, near the Sudan border. The ulcer is now healed but involved the tendons leading to contraction deformity, and because the entire circumference of the wrist was necrotic, there is a persistent lymphedema of the hand.
(AFIP Photograph No. 65-2982)
- Fig. 12. An American Peace Corpsman spent two years in Nigeria and developed an M. ulcerans infection which subsequently led to a below knee amputation. The infection recurred above the knee causing loss of tissue in the thigh. The infection is almost completely healed.
(AFIP Photograph No. 70-11768)

Fig. 13. About 9/10 of the ulcers are on the limbs and 1/10 involve trunk, head, face, neck, and scalp. Palms and soles are spared. This large ulcer has been present for several months.

(AFIP Photograph No. 74-4472)

Fig. 14. This girl had a persistent and extensive ulcer which led to amputation of her left arm at the shoulder.

(AFIP Photograph No. 74-5288)

Fig. 15. This is a section of the lesion shown in Figs. 1 and 2. There is a central area of necrosis centered in the deep dermis. In this plane of section, the necrotic tissue almost touched the epidermis. Clumps and masses of acid-fast bacilli are located in the central portion of the necrotic area. Excision led to healing without recurrence.

(Ziehl-Neelsen X 7.25, AFIP Photograph No. 65-1413)

Fig. 16. Characteristic necrosis reveals swollen fat cells without nuclei. Cells and lobules are separated by fibrin. The capillary circulation is gone but some of the larger vessels have open lumens even though their walls are necrotic.

(H&E X 110, AFIP Photograph No. 65-1433)

Fig. 17. Specimens taken to include deep tissue usually reveal necrotic changes in muscle similar to those in the fat. Some muscle fibers are vacuolated, have lost their nuclei, are swollen and separated by fibrin.

(H&E X 110, AFIP Photograph No. 65-1439)

Fig. 18. A fibrin stain (phosphotungstic acid-hematoxylin) reveals coarse strands between dead fat cells.

(PTAH X 145, AFIP Photograph No. 65-5856)

Fig. 19. Clumps of acid-fast bacilli lie in and between ghosts of fat cells.
(Ziehl-Neelsen X 165, AFIP Photograph No. 65-1444)

Fig. 20. This vessel at the margin of the necrotic zone is almost completely occluded by proliferating endothelial cells.
(Movat stain X 70, AFIP Photograph No. 65-1447)

Fig. 21. As the process extends toward the surface the dermal papillae become edematous and sometimes greatly enlarged -- as seen here. Epithelial proliferations appear as an interlacing network.
(H&E X 50, AFIP Photograph No. 65-1442)

Fig. 22. At the point of incipient ulceration (seen in Fig. 1) the epidermis is spongiotic with segments of suprabasilar separation.
(Movat X 70, AFIP Photograph No. 65-1410)

Fig. 23. Healing is associated with a granulomatous reaction at the margin of necrotic tissue in the ulcer bed.
(H&E X 80, AFIP Photograph No. 64-5096)

Fig. 24. *M. ulcerans* infection in mouse foot pad (right). The left is not infected.

(AFIP Photograph No. 70-7384)

Fig. 25. Four swollen red lesions at the site of intradermal injections of 0.1 ml of cytoplasmic fluid into a guinea pig. Tuberculin and phosphate buffer were also inoculated as controls, but no lesions developed.

(AFIP Photograph No. 73-3898-1)

Fig. 26. Section of guinea pig skin from site of inoculation 13 days after intradermal injection of 0.1 ml of cytoplasmic fluid. The abscess developed at the site of inoculation and occupied the entire dermis. Dermal vessels are congested, and the underlying muscle and adipose tissues are focally necrotic and infiltrated with inflammatory cells, including histiocytes, eosinophils, neutrophils, lymphocytes, and plasma cells. The abscess contains fibrin and degenerating neutrophils, and the sides of the cavity are lined by a layer of squamous epithelium.

(Movat X 14, AFIP Photograph No. 73-8098)

Fig. 27. Cutaneous muscle fibers (the panniculus carnosus) of guinea pig three days after inoculation of 0.1 ml of culture filtrate. Normal and necrotic muscle fibers are shown. The necrotic fibers (arrows) are swollen, have lost their striations, are tinctorially altered (orange rather than red), and have a granular cytoplasm.

(Movat X 305, AFIP Photograph No. 73-7496)

Fig. 28. In the deep dermis, edema and inflammatory cells, degenerated muscle fibers, and four cross-sections of thrombosed vessels are present.

(Movat X 130, AFIP Photograph No. 73-8096)

Fig. 29. Guinea pig skin, upper dermis, four days after inoculation of a washed, phosphate-buffered suspension of M. ulcerans. Clumps of acid-fast bacilli are present within macrophages just beneath the scab. The middle and lower dermis contain smaller numbers of organisms, mostly within macrophages. Some of the macrophages containing organisms have degenerated.

(Ziehl-Neelsen X 700, AFIP Photograph No. 73-7463)

Fig. 30. Baby armadillo (2 days old).

Fig. 31. Adult armadillos (2-3 years old).

Fig. 32. Pre-ulcerative lesion on abdomen of armadillo. The infection developed 8 weeks after inoculation of M. ulcerans isolated from mouse foot pads.

Fig. 33. Thighs of armadillo showing necrotic, undermined ulcers which developed 12 weeks after inoculation of M. ulcerans.

Fig. 34. Close-up of lesion shown in Figure 33.

Fig. 35. Section through the margin of the ulcer shown in Figures 33 and 34. There is necrosis of the dermis and re-epithelialization of the ulcer margin as seen in infections of man.

(AFIP Photograph No. 73-9651)

Fig. 36. Coagulation necrosis of the dermis of the armadillo. Chronic inflammatory cells surround the necrotic zone.

(H&E X 130, AFIP Photograph No. 73-9653)

Fig. 37. Intracellular and extracellular acid-fast bacilli from the area of necrosis.

(Ziehl-Neelsen X 395, AFIP Photograph No. 73-9650)

Fig. 38. The necrotic fat cells are swollen, have lost their nuclei, and are separated by accumulations of fibrin -- as seen in human infections.

(H&E X 395, AFIP Photograph No. 73-4826)