*The Gulf Journal of Dermatology & Venereology*

# HISTOPATHOLOGY OF CUTANEOUS LEISHMANIASIS

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## Introduction

The histopathology of cutaneous leishmaniasis shows some parallels with those of other cutaneous infectious granulomas. Infection of the skin by leishmania organisms results from the introdution of promastigotes into the skin from the bite of a sandfly of the subfamily phlebotominae[1]. Invasion of the skin by Leishamania organisms leads to a tissue reaction that may range from a mixed inflammatory cell infiltrate with a large number of macrophages containing leishmania amastigotes, to a highly organized and predominantly epithelioid cell granuloma with very few or no demonstrable organisms. Variation in the clinical and histological manifestations depend upon the strain of the organism, the size of the initial inoculum and the immunologic status of the individuals in the endemic or nonendemic areas. The strain of leishmania organism ceorrelates well with the tropism of the organism, either limited to the skin as with leishamania tropica, aethiopica, infantum, and mexicana or the skin and the mucous membrane such as leismania braziliensis or to the viscera with leishmania donovan[2]. Strains of leishmania organisms also have variations in their genetically determined virulence. For instance leishmania tropica minor, in the Middle East usually results in the development of a single dry ulcer where as leishmania tropica major often results in the formation of multiple weeping ulcerated lesion[3,4].

Once inside the human host, the parasites are ingested by dermal macrophages and exist as intracellular nonflagellated organisms (amastigotes)[5]. If the patient's cellular's mediated immunity is unimpaired, a single self-healing lesion most commonly develops. If the patient's cell mediated immunity is deficient, disease will take the form of diffuse cutaneous leishmaniasis with multiple organisms present in widespread disease. This is analogues to the relation with leprosy in which an intact cellular immune response results in the lepromatous tissue reaction[6]. The delayed hypersensitivity response is the primary mechanism of killing the organims.

Leishmania amastigotes are found in tissue sections as round or oval bodies measuring between two to four micrometers. The amastigote cytoplasm stains pale blue with Hiemsa, Wright, feulgen, or Romanovski stain[5,6,7]. The cytoplasm stains a pale blue-gray with hematoxylin and eosin. The intracellular rod-shaped kintoplast and nucleolus appear less than one micrometer in diameter and stain reddish purple.

With electron microscopy, the organism are located within the cytoplasm of tissue macrophages. The leishmania amastigotes show a pellicle composed of two membranes. There is intracellular flagellum which appears as a small protuberance surrounded by an invagination of cellular membrane[8,9]. In cross section the flagellum is composed of nine strands, a thik central surrounded by eight thinner strands[8,9]. Adjacent and at a a right angle to the flagellum is the rod-shaped kintoplast[8,9]. The organism contains a well-developed golgi apparatus, liposomes, and mitochondria. There is a large nucleolus containing several nucleoli. The nucleus is surrounded by a double membrane.

The histologic findings follow those of the clinical setting. For that reason we have divided the histologic findings into those of acute lesions which in the "New World" (Central and South America) would include chiclero's ulcer, uta and espundia, and the "Old World" (North Africa, Mediterranean Basin, the Middle East, Southern Asia, and China) would include the oriental sore Salek, Baghdad boil, etc[10,11,12,13]. The chronic lesions would include lupoid leismaniasis/Leishmania recidivans in those patients with intact cellular mediated immunity and diffuse cutaneous leishmaniasis in those individuals with poor cellular mediated immunity[14]. The chronic lesions also include the rarer port kala-azar dermal leishmaniasis and the leishmanids.

## ACUTE CUTANEOUS LEISHMANIASIS

Acute lesions of cutaneous leishmaniasis may

present as papules, nodules, or plaques which are crusted or ulcerated. There is histological similarity between the acute lesions of Old World leishmaniasis (oriental sore, baghdad boil) and the New World lesions of acute leishmaniasis (uta, chiclero's ulcer). Early in the course of disease, there is mixed inflammation[15]. The inflammation is dense and diffuse throughout the dermis with a rare Grenz zone between the inflammation and the epidermis.. The infiltrate is composed predominantly of histiocytes with a mixture of lymphocytes. Plasma cells may be found primarily within the macrophages, but when organisms are rare[16]. Organisms are found primarily within the macrophages, but when organisms are numerous, they may also be seen extracellularly. Granuloma formation is rare early in the course of an acute lesion[17]. When the infiltrate fills the dermis, there may be loss of the adnexal structures. As the lesion progresses, epithelioid granulomas are surrounded by mild to moderate infiltrate of histiocytes and lymphocytes. A large number of plasma cells may be seen in late lesions of acute cutaneous leishmaniasis. Leishman bodies (amastigotes within macrophages) are identifiable in approximately half of the cases of acute cutaneous leismaniasis in this late stage[17]. Necrosis of the dermal collagen is rare[18]. Loss of elastic fibers and adnexal strutures manifest clinically scarring.

The epidermal changes are variable. There may be hyperkeratosis with or without parakeratosis. Both atrophy and epidermal acanthosis may be seen. At times, the acanthosis may approach pseudoepitheliomatous epidermal hyperplasia[17]. Follicular plugging and hydropic degeneration of the basal cell layer are occasionally seen[17]. Intraepidermal microabscesses are rare. It is rare to find Leishmania organisms present within the epidermis[17].

Mucocutaneous leishmaniasis is most commonly due to Leishmania braziliensis and is found almost exclusively in the new world. As in other lesions of acute cutaneous leishmaniasis, the epidermal findings are variable. Epidermal atrophy, necrosis, and pseudoepitheliomatous hyperplasia may all be seen[18]. Some authors have divided the life atrophy with an underlying superficial and deep mixed infiltrate of lymphocytes[16]. This is followed by the granulomatous proliferative phase[16]. This phase may be marked by pseudoepithelioid granulomas within the dermis. Organisms are still visible within the histiocytes. In the late granulomatous necrotizing phase, there is necrosis of dermal collagen. Erosion into cartilage and underlying bone may be seen[19].

## CHRONIC CUTANEOUS LEISHMANIA

Early authors differentiated Leishmania recidivans from chronic lupoid leishmaniasis. Berlin differentiated lupoid leishmaniasis from Leishmania recidivans in that the latter develops only after apparent healing of the primary lesion and is limited to its scar[20]. Most authors, ouselves included, consider these entities to be identical. Single or sometimes multiple papular lesions develop at the edge of scars from previously healed acute lesions of leismaniasis and progress gradually into an infiltrated scaly plaque which may show yellowish discoloration on diascopy. The lesion may progress peripherally into a circular or semicircular pattern with central healing. The epidermis in Leishmania recidivans/lupoid leishmaniasis shows variable changes. In areas in which the underlying dermal infiltrate approaches the epidermis, the epidermis may show pseudoepitheliomatous hyperplasia. However, in lesions in which there is a Grenz zone intact between the dermal infiltrate and the epidermis, the epidermis may appear normal[21]. A lichenoid tissue reaction including epidermal atrophy, follicular plugging, and focal hydropic degeneration of the basal cell layer with loss of pigment into melanophages in the papillary dermis may also be seen[18,21]. In the dermis is a dense and diffuse dermal infiltrate[22]. The infiltrate involves the superficial to deep dermis in a dense and diffuse dermal infiltrate[22]. The infiltrate of lymphocytes[21,23]. There are occasional plasma cells, neutrophils, and eosinophils[21,23]. Organisms present within histiocytes are rare[18,22]. Atrophy and loss of the pilosebaceous structures is a common feature[21]. The scarring which is evident clinically, is perhaps best appreciated in sections stained for elastic tissue which is evident clinically, is perhaps best appreciated in sections stained for elastic tissue which reveal extensive loss of elastic fibers.

## DISSEMINATED CUTANEOUS LEISHMANIASIS

Disseminated cutaneous leishmaniasis occurs in individuals with an anergic response to the leishmania organism. This is usually caused by Leishmania aethiopica in the Old World and Leishmania

mexicana amazonensis in the New World. Clinically and histologically this form of leishmaniasis is similar to lepromatous leprosy. There is an infiltrate of histiocytes in a diffuse pattern throughout the dermis. The histiocytes contain multiple amastigotes, and appear vacuolated[24,25]. Staining for fat Sudan IV stain reveals that the vacuoles do not contain lipid[26]. Necrosis is rare. Lymphocytes, plasma cells, and multinucleated giant cells are few[19]. Towards the periphery of the histiocytic infiltrate, a few epithelioid granulomas may be found. The epidermal changes are variable. The epidermis may show focal necrosis and ulceration, atrophy, or epidermal hyperplasia.

## POST FORMS OF CUTANEOUS LEISHMANIASIS

Several unusual and rare clinical presentations of cutaneous leishmaniasis have been described. Erysipeloid leishmaniasis has been described in Iranian women presenting clinically as erythematous indurative patches and plaques over the face and nose resembling erysipelas[27]. Histologically, there are macrophages containing large numbers of anastigotes filling the upper dermis and extending into the deeper dermis along adnexal structures[27]. A variable number of lymphocytes and plasma cells are present.

Visceral leishmaniasis has been described in patients immunosuppressed by HIV infection. Cutaneous lesions may also occur in these patients. These are marked histologically by an infiltrate of macrophages containing leishman bodies filling the upper dermis[28]. Extracellular organisms may be noted as well as organisms in eccrine gland secretory cells and acrosyringium[28]. Few lymphocytes may be present. Macrophages containing leishman bodies have also been described in lesions of kaposi's sarcoma in patients with AIDS[29].

## DIFFERENTIAL DIAGNOSIS

The differential diagnosis of acute cutaneous leishmaniasis include other infectious granulomas. The histologic differential diagnosis would include sporotrichosis, blastomycosis histoplasmosis, yaws, a syphilitic gumma, tuberculosis cutis, anthrax, granuloma inguinale, rhinoscleroma, and furunculosis. Identification of the organisms in the acute lesion should help to differentiate cutaneous leishmaniasis from these other infections. Rhinoscleroma, histoplasmosis, granuloma inguinale, and blastomycosis may also show parasitized macrophages requiring special stains for differentiation. The intracellular organisms of rhinoscleroma is also approximately 2-3um in size, however, rhinoscleroma is marked by a large number of plasma cells with formation of Russel bodies[30]. The organisms of blastomycosis are larger, 8-15um. The organisms of blastomycosis, histoplasmosis, and sporotrichosis all stain with PAS and metheranine silver[30]. Granuloma inguinale is marked by a formation of a small abscess containing neutrophils. Older lesions of acute cutaneous leishmaniasis may show large number of plasma cells in the perivascular infiltrate resembling lesions of secondary or tertiary syphilis.

Chronic lesions of leishmaniasis (lupoid leishmaniasis/Leishmania recidivans) present a greater diagnostic challenge[21]. The histologic findings closely resemble those of lupus vulgaris, lepromatous leprosy, and sarcoidosis. Leishmania organisms may occasionally be found on staining with Giemsa. Like wise, acid-fast stains may reveal mycobacterium tuberculosis or mycobacterium leprae. Tuberculoid leprosy has a tendency to form granulomas along the course of cutaneous nerves, which is a feature not found in Leishmania recidivans. In cutaneous sarcoidosis the granuloma is more purely epithelioid in type. Asteroid or Schaumann's bodies may be present in cutaneous sarcoid, but are not specific.

## IMMUNOPATHOLOGY OF LEISMANIASIS

The immunopathology of many granulomatous processes has come under greater scruting on the cellular level. Much of this recent work is helpful in correlating the clinical and immunologic status of the patient to the histologic findings.

The immunopathology of leismaniasis is predominatly that s T-cell mediated immune response[18]. Lesions of cutaneous leishmaniasis show an abundance of T-cells with an activated phenotypoe expressing interleukin- (CD25+) receptors, transferrin receptors (CD71+) or major histocompatibility complex class II molecules on their surface. There is an approximately equal number of helper/inducer T-cells (CD4+) and suppressor/cytotoxic T-lymphocytes (CD8+0 in the localized lesions[31,32]. The majority of T-cells bear the alpha/beta T-cell receptor, however, approximately 20 to

30% bear the gamma/delta T-cell receptor. The number of gamma/delta cells are seen in other granulomatous infiltrates such as tuberculoid leprosy[33]. The T-cells which bear the gamma/delta T-cell receptor release factors which induce macrophage aggregation and proliferation which are important for granuloma formation[16]. The gamma/delta T-cell receptors are absent in mucocutaneous leishmaniasis[16]. In localized cutaneous leishmaniasis, the predominant cytokines involved are interleukin-2 and gamma interferon[31,32]. Gamma interferon activatesmacrophages. Mucocutaneous leishmaniasis in contrast is associated with decreased gamma interferon and increased interleukin-4 and interleukin-10 production[34]. This correlates well with both murine models and the clinical course of leishmaniasis, in which interleukin-2 and gamma interferon are associated with healing where as interleukin-4 and interleukin-10 are associated with disease progression[35].

The T-helper cell-type 1 (TH1) are the T-lymphocytes which produce interleukin-2 and gamma interferon which activate macrophages. In contrast the T-helpers cell type-2 (TH2) secrete interleukin-4,5, and 10 which inhibit some cellular mediated immune responses. In diffuse cutaneous leishmaniasis there is a predominance of TH2 over TH1 lymphocytes[31,32]. Phagocytic ingestion of leishmania organisms by the macrophages is initiated by binding of the organisms which involves the CD11/CD18 integren subfamily[28]. Further study into the inter-relation of these cellular elements is required to complete our understanding of the immunopathology of leismaniasis.

## POLYMERASE CHAIN REACTION

The polymerase chain reaction is a method to copy specific DNA sequences to a more easily detectable and analyzable level[36]. This allows for a small amount of DNA to be detected from both fresh samples of tissue as well as formalin fixed and paraffin embedded tissue. Three elements are required in order for the polymerase chain reaction to occur 1) the template DNA in the tissue sample to be tested, 2) an oligonucleotide primer for the target DNA, and 3) a thermal stable DNA polymerase and excess of deoxyribonucleotide triphosphides[37]. The target double stranded DNA in the tissue is denatured by heating, forming single stranded DNA[36,37]. Synthetic oligonucleotide primers are added which anneal to complimentary single stranded DNA in the target template DNA[36,37]. The reaction cooled in the presence of an excess of nucloetides and DNA polymerase. The primers are then extended yielding two complimentary strands of DNA[38]. The process is repeated with an exponential increase in target DNA until enough target DNA is present for identification[38]. The target DNA is visualized most commonly by electrophoresis. The sensitivity of the polymerase chain reaction is increased by the use of primer bases to sequences that have more than one copy per cell. For Leishmania organisms those include the kinetoplast DNA[39]. Guillaume et al were able to use the polymerase chain reaction to detect a highly conserved portion of the kintoplast DNA in both New World and Old World genomic Leishmania[39]. Piarroux developed a polymerase chain reaction for Old Word genomic Leishmania using a primer from a conserved lesion region of Leishmania infantum DNA[40]. This primer was used for detecting Leishmania infantum, Leishmania donovani, Leishmania chagasi, Leishmania major, and Leishmania tropica[40]. Lopez et al used a primer directed to kinetoplast DNA of Leishmania braziliensis. Using skin biopsy specimens of localized cutaneous and mucocutaneous disease, the authors compared the polymerase chain reaction to a combination of conventional methods (80% vs 77%), where as in the rural setting, the polymerase chain reaction was able to demonstrate increased sensitivity than conventional methods (69% vs 27%)[41]. Rodriguez et al was able to demonstrate increased sensitivity of the polymerase chain reaction when compared to tissue culture or tissue biopsy in localized cutaneous leishmaniasis[42]. By hybrizing the polymerase chain reaction product to new small kDNA probes specific to L. mexican or L. braziliensis the authors were able to differentiate between the two taxa[42].

In lupoid leishmaniasis/Leishmania recidivans, organisms are much more scarce.

Momeni et al were able to identify Leishmania DNA in 30 of 63 lesions of lupoid leishmaniasis. The sensitivity compares favorably to conventional methods, in which 19 of 65 lesions were culture positive, and 10 of 65 lesions were positive on examination with Giemsa stain of skin biopsy material[21].

Immunoperoxidase stains have also been used to detect anastigote organisms in biopsy tissue. Likewise, fluorsceinated antibodies can be used in

deparafinized tissue previously fixed in Ridley's solution[43].

## SUMMARY

The histopathology of cutaneous leishmaniasis is marked by a T-cell mediated immune response ranging from well-defined epithelioid granuloma formation with few orgnaisms in patients with an intact immune response to a diffuse infiltrate of histiocytes with numerous organisms in patients with a poor immune response.In this respect, there is a similarly to infection by mycobacterium leprae. The tropism of the infectious Leishmania organism, the of the innoculum, and the underlying immune stains of the patient will determine the clinical and histologic findings. Further study into the immunopathology should lead to greater uinderstanding of the pathologic findings and improved treatments.

Furthermore, the use of polymerase chain reaction and other methods of verification utilizing immunoperoxidase staining should lead to greater diagnostic confidence.

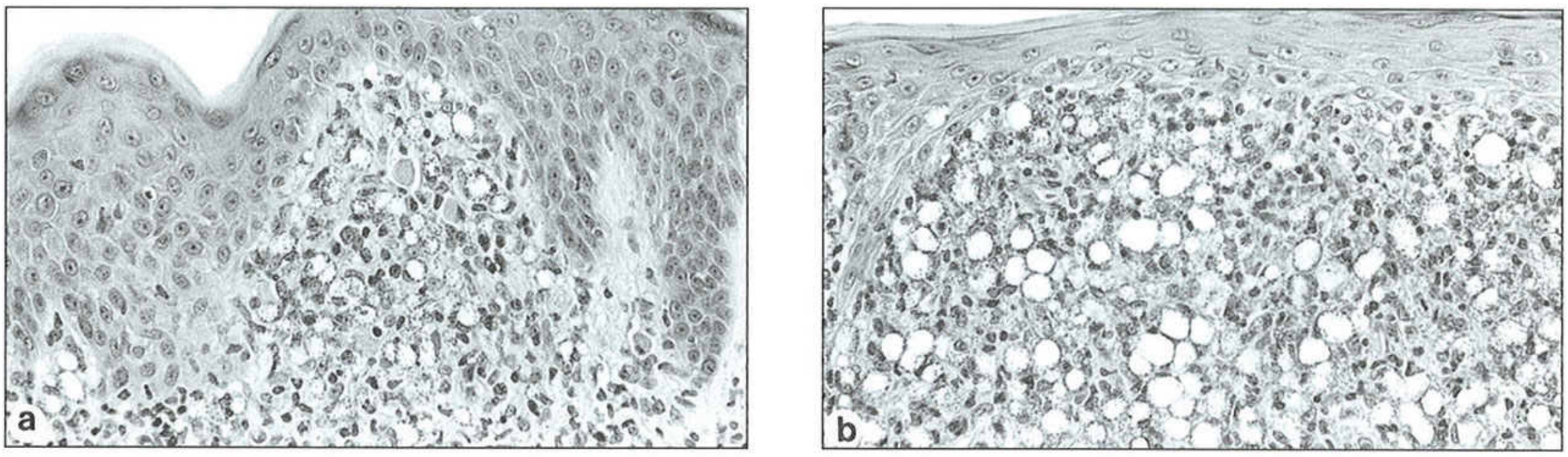


*Figure 1 :Acute cutaneous leishamaniasis. A&B : Diffuse dermal infiltrate of macrophages containing numerous leishmania organisms. H&E x 225*

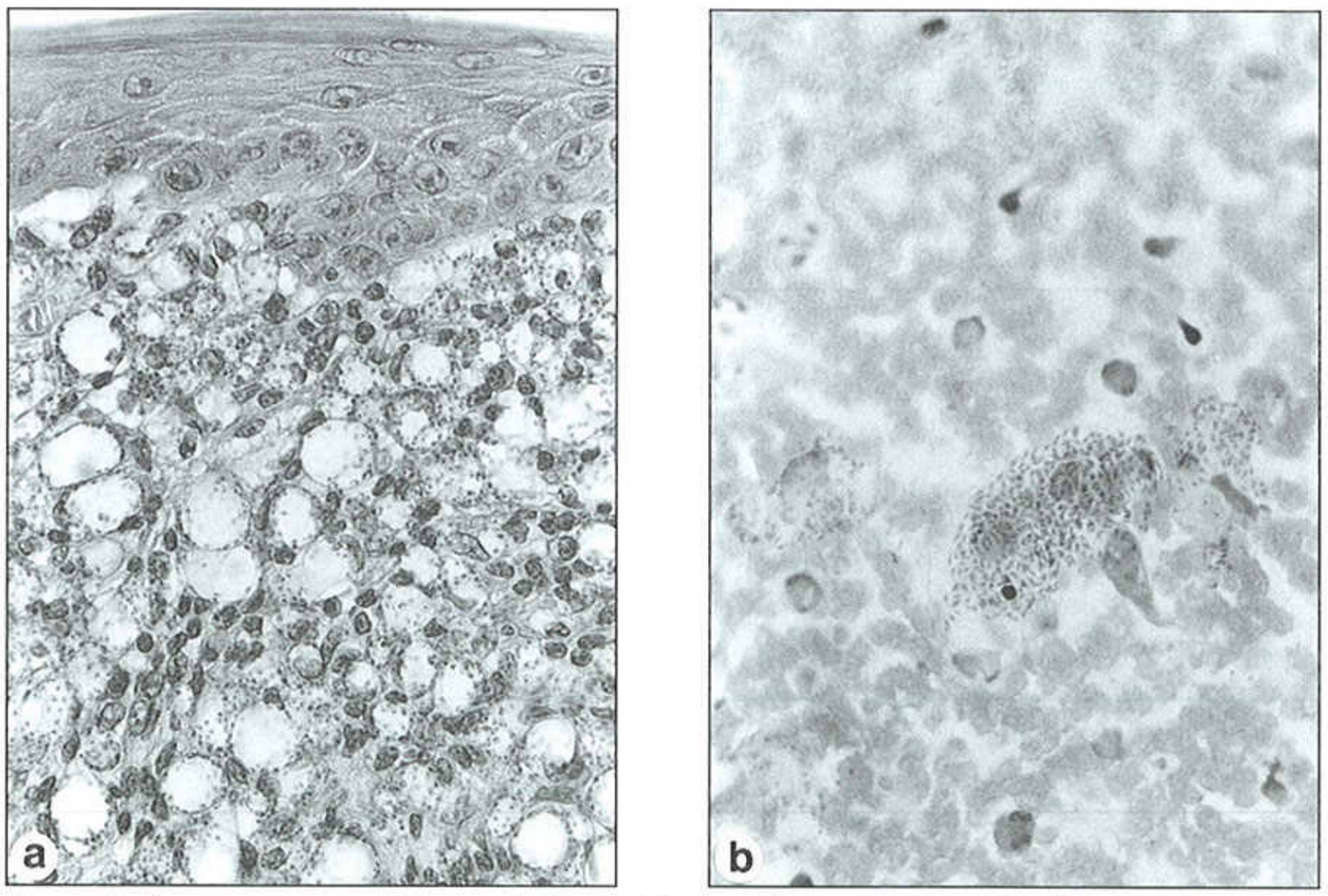


*Figure 2 :Acute cutaneous leismaniasis. A : Show a large number of leishmania organisms within dermal macrophages. H&E x 400. B : Macrophages containing organisms in a tissue smear. Giemsa x 1000*

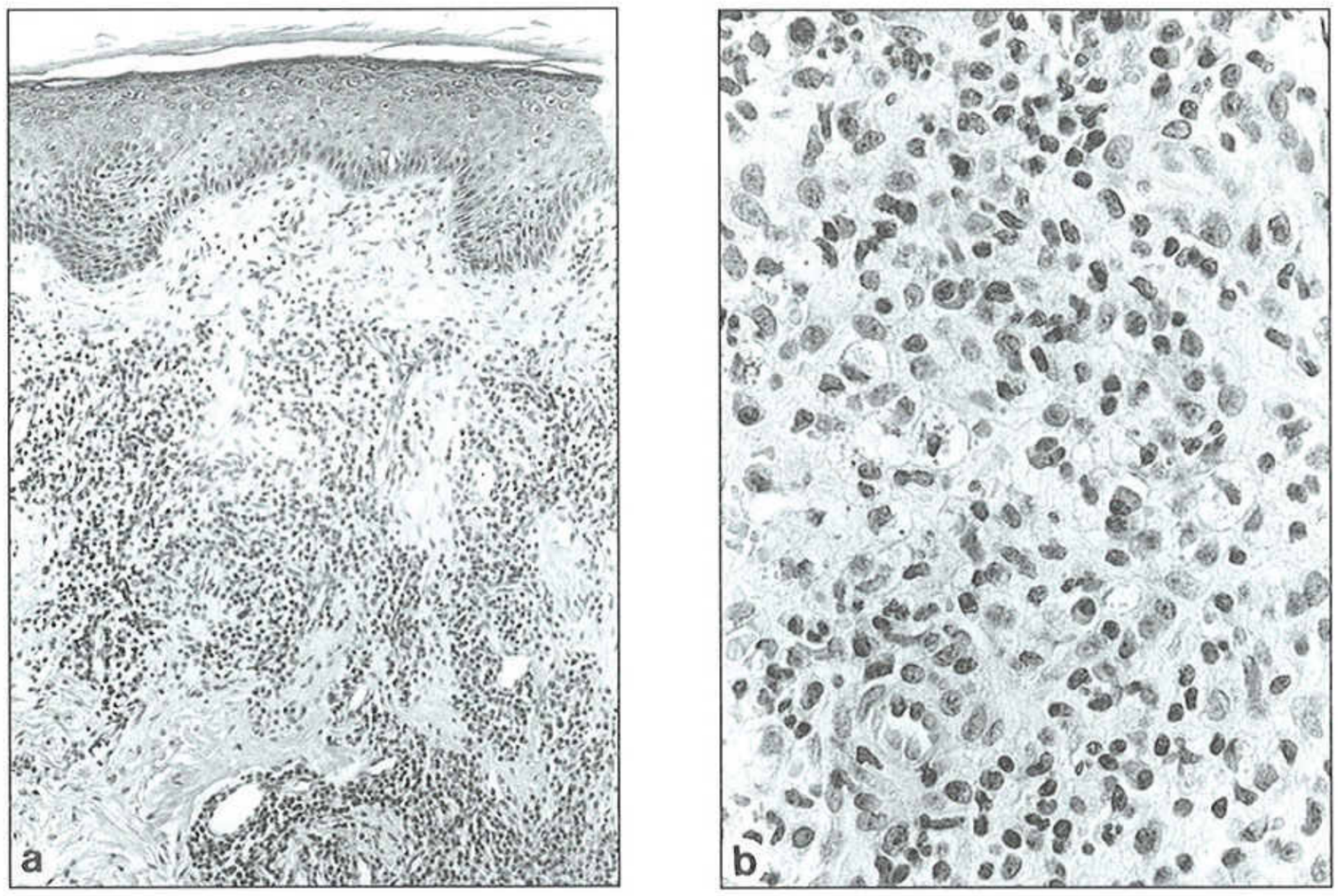


*Figure 3 :Subacute lesion of cutaneous leishmaniasis. A : Shows a patchy perivascular dermal infiltrate of lymphocytes, histiocytes, and plasma cells. H&E x 225. B : Higher magnification reveals several macrophages containing leishmania organisms. H&E x 400*

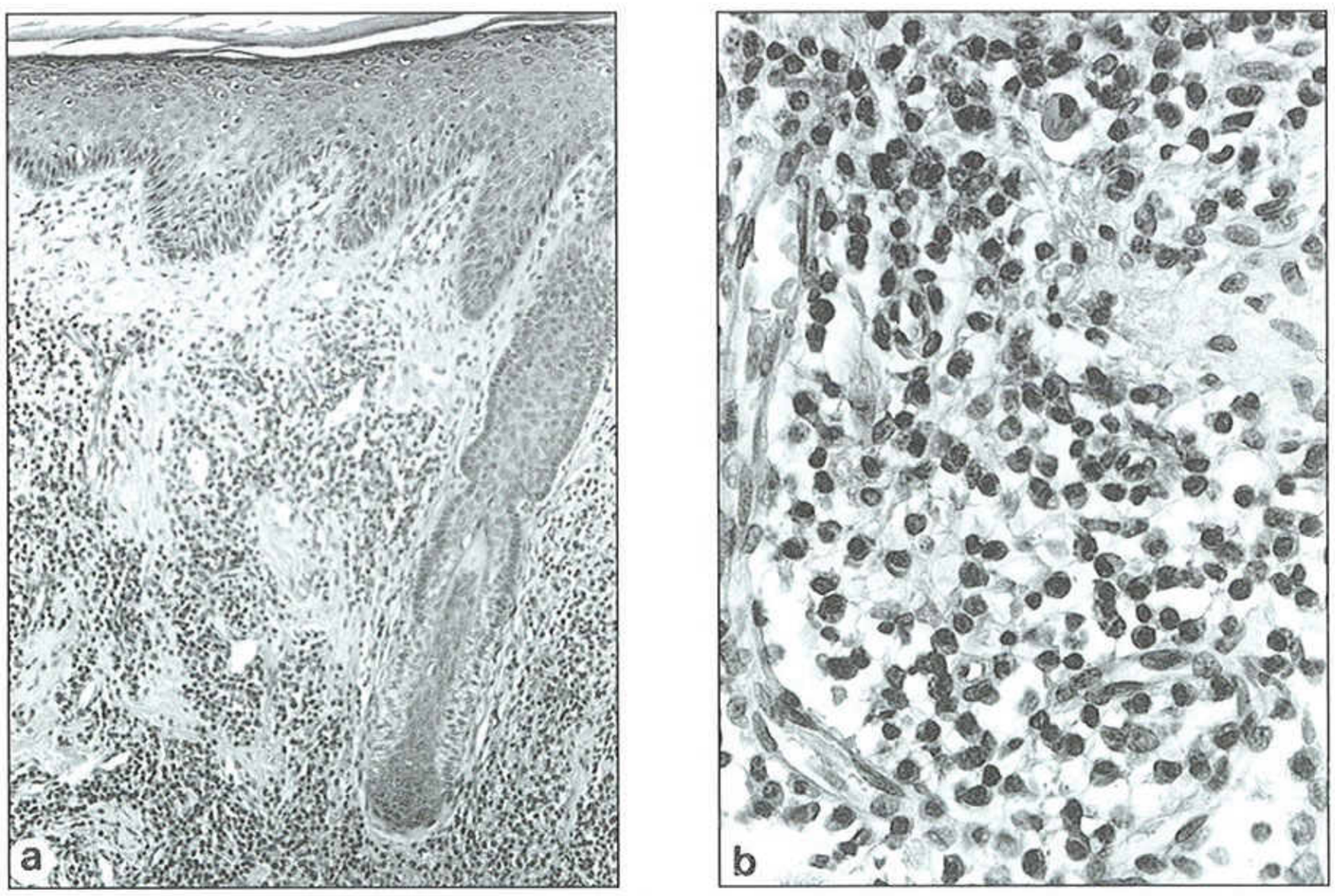


*Figure 4 :Subacute lesion of cutaneous leishmaniasis. A : Shows a patchy perivascular infiltrate of lymphocytes and plasma cells. H&E x 225. B : Higher magnification reveals a number of plasma cells giving the impression of a syphilitic lesion. H&E x 400*

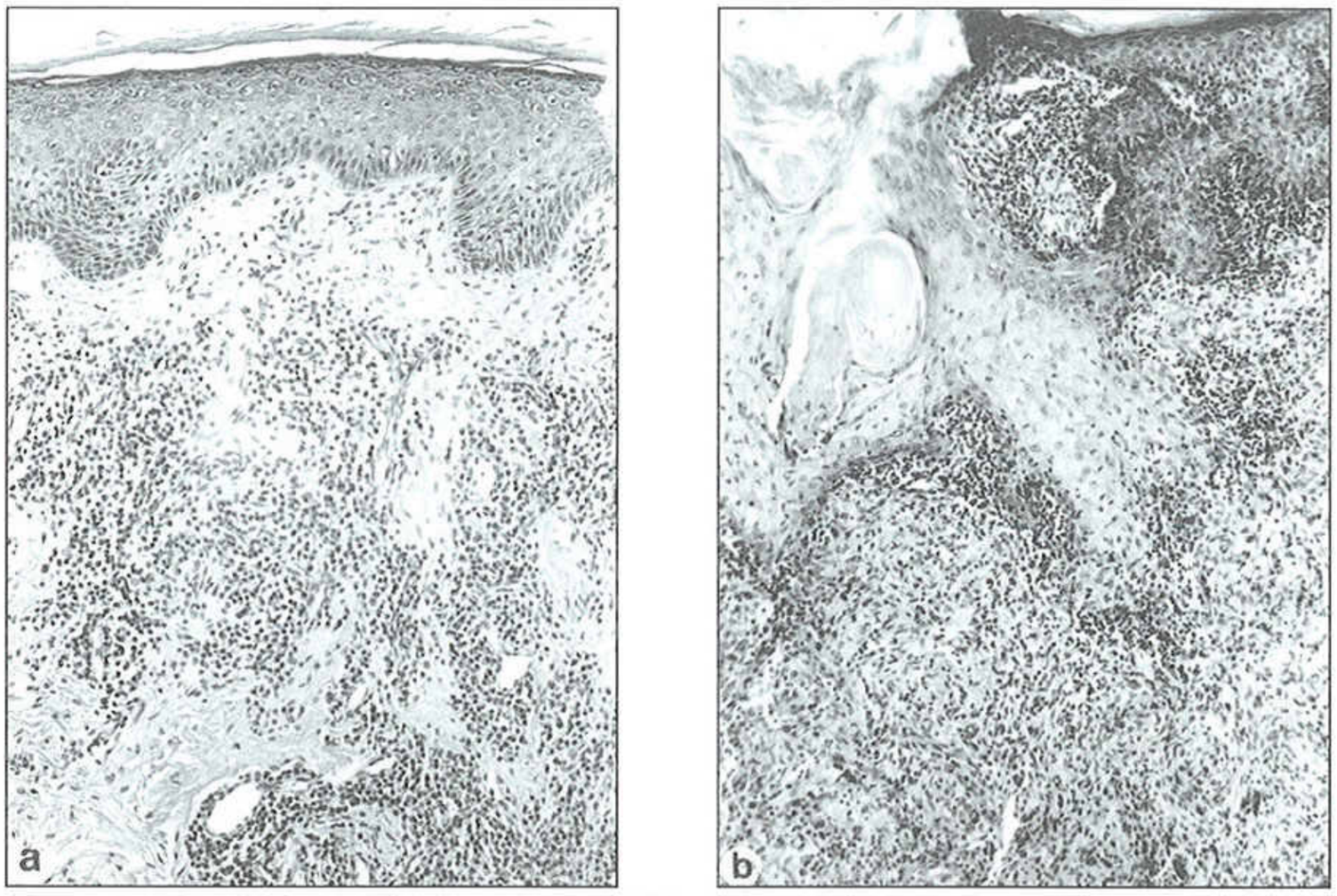


*Figure 5 :Chronic lupoid leishmaniasis (Leishmania recidivans). A : Shows an atrophic epidermic overlying heavy dermal involvement by lobulated masses of epithelioid cells surrounded by lymphocytes and plasma cells. H&E x 125. B : Pseudoepitheiomatous hyperplasia of the epidermis overlying dermal epithelioid cell granuloma. H&E x 125*

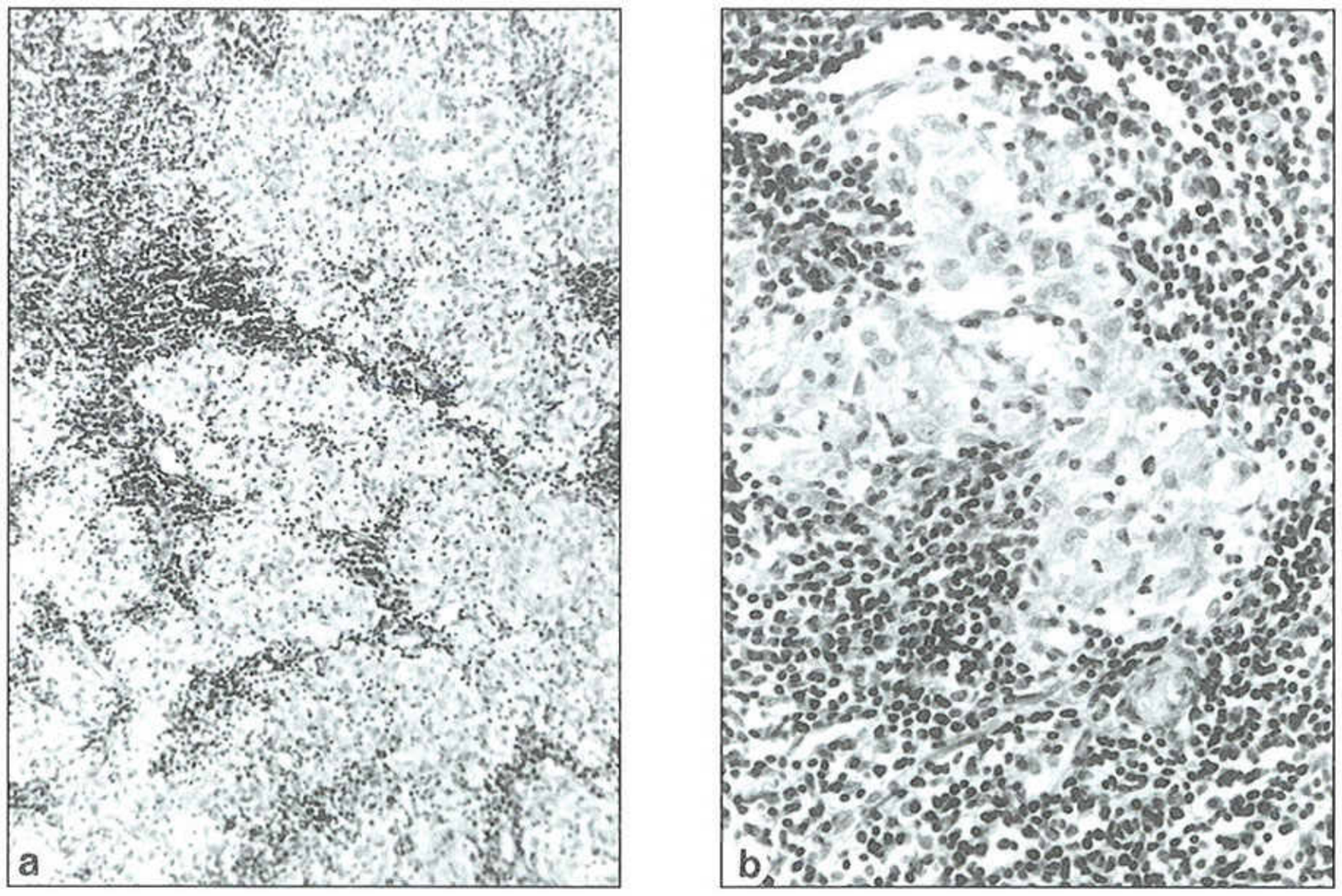


*Figure 6 :Chronic lupoid leishmaniasis. A : Shows a diffuse dermal epithelioid cell granuloma resembling those seen in lupus vulgaris or sarcoidosis. H&E x 125. B : Shows the epithelioid cell granulomas surrounded by lymphocytes and plasma cells. H&E x 225*

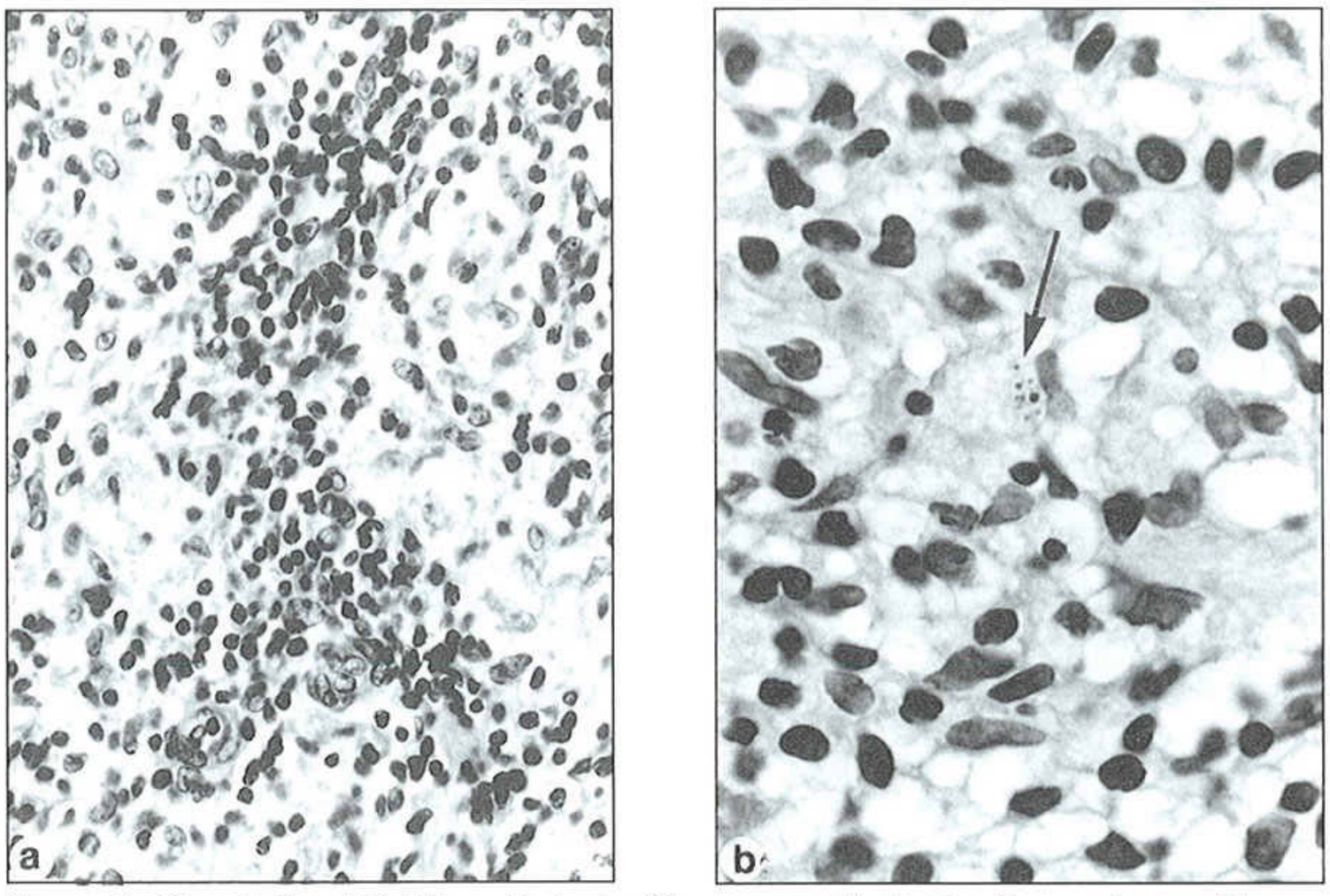


*Figure 7 :Chronic lupoid leishmaniasis. A : Shows an epithelioid cell granuloma with some lymphocytes and plasma cells. H&E x 225. B : Rarely, macrophages containing a few organisms may be present. H&E x 400*

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