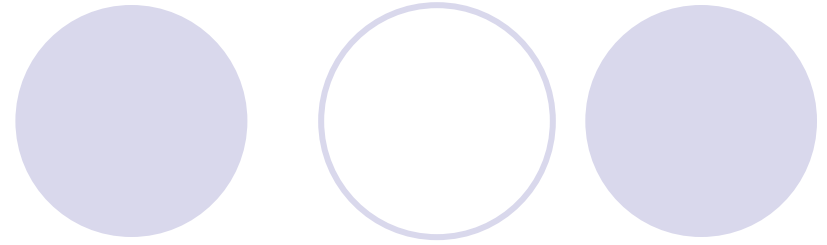


The slide features a decorative arrangement of six light purple circles. Three circles are positioned in a top row, and three are in a bottom row. The top-left circle is an outline, while the other five are solid. The text is centered horizontally and partially overlaid by these circles.

Blood & Tissue flagellates

Leishmania species

Classification :



- Phylum : Protozoa
- Subphylum: Sarcomastigophora
- Superclass: Mastigophora
- Class: Zoomastigophora
- Family : Trypanosomatidae

Classification of Leishmania species

- Visceral leishmaniasis
 - Leishmania donovani complex
- Cutaneous leishmaniasis
 - Leishmania tropica complex
 - L.tropica
 - L.major
 - L.aethiopica
- Muco-cutaneous leishmaniasis
 - Leishmania brasiliensis complex

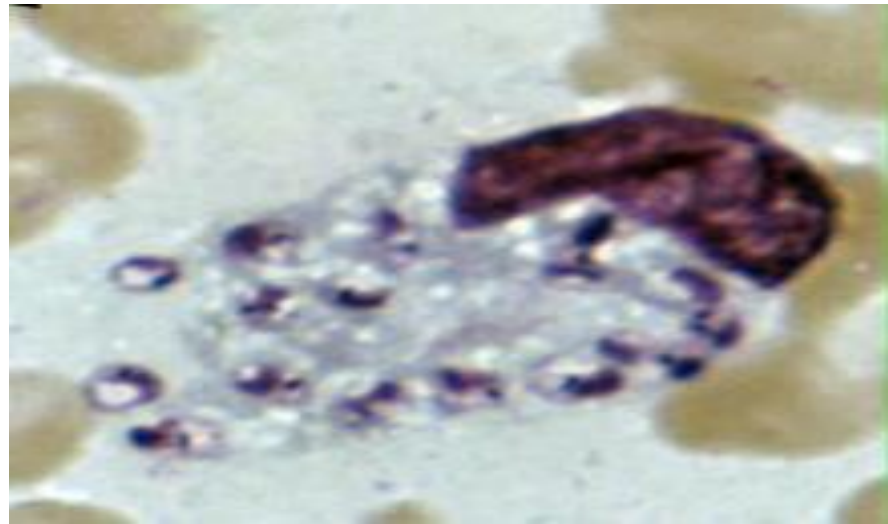
The image features six light purple circles arranged in two rows. The top row contains three circles, and the bottom row contains three circles. The text 'Leishmania donovani' is centered horizontally between the two rows of circles.

Leishmania donovani

History

- Leishman- London May 1903
- Donovan- Madras July 1903

○ Reported parasite in splenic smears of patients of Kala azar.



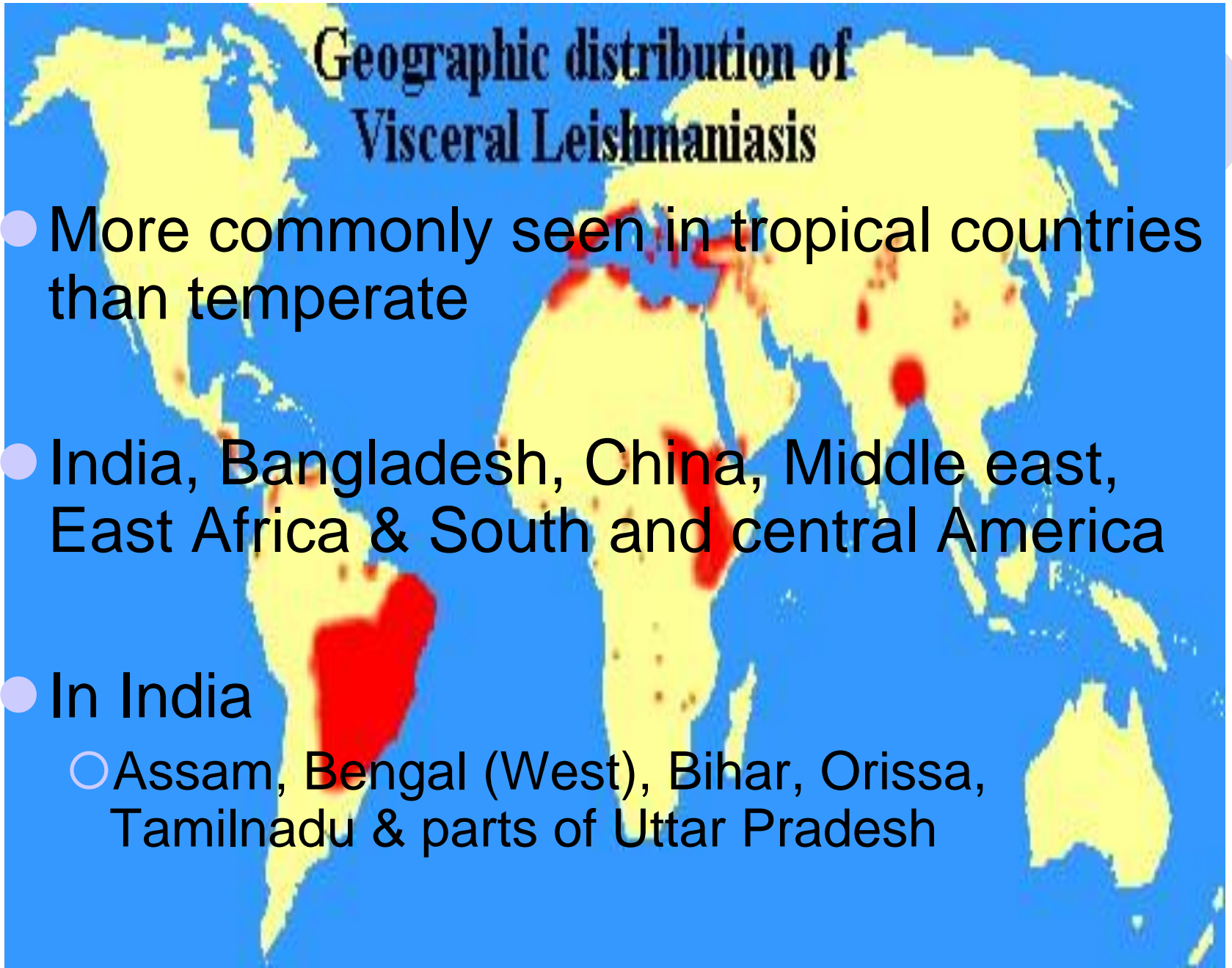


Habitat

- A parasite of R-E system
- Intracellular- in Macrophage, liver & splenic cells.

Geographic distribution of Visceral Leishmaniasis

- More commonly seen in tropical countries than temperate
- India, Bangladesh, China, Middle east, East Africa & South and central America
- In India
 - Assam, Bengal (West), Bihar, Orissa, Tamilnadu & parts of Uttar Pradesh



Morphology – 2 forms

- Amastigote - Aflagellar form
 - Inside R-E cells of definitive host-man
- Promastigote – Flagellar stage
 - Gut of sand-fly (insect)
 - Artificial culture

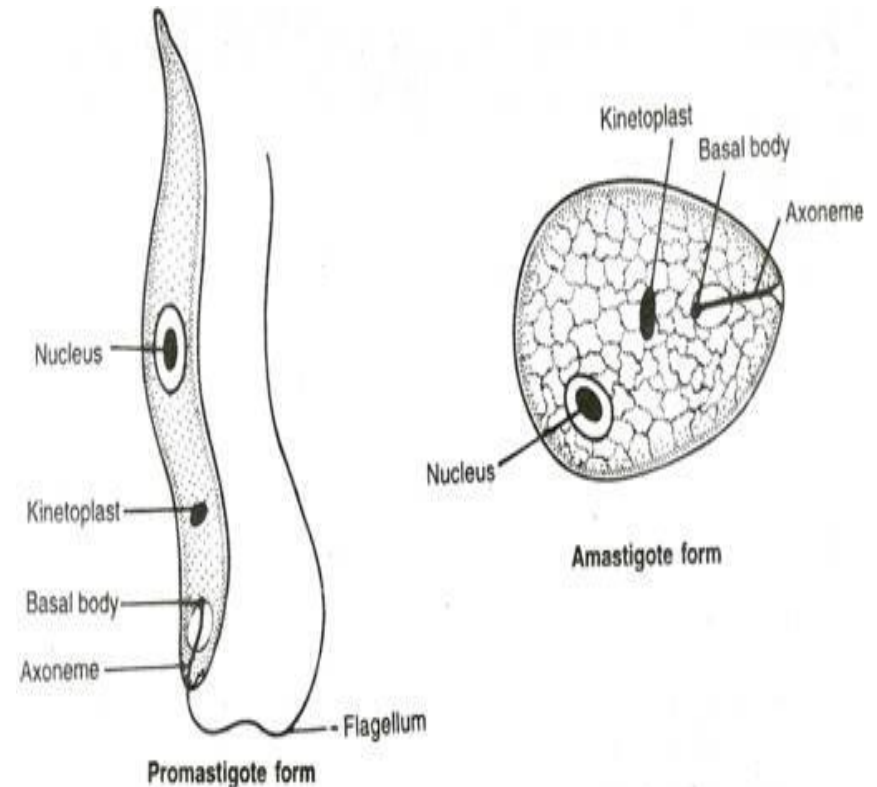
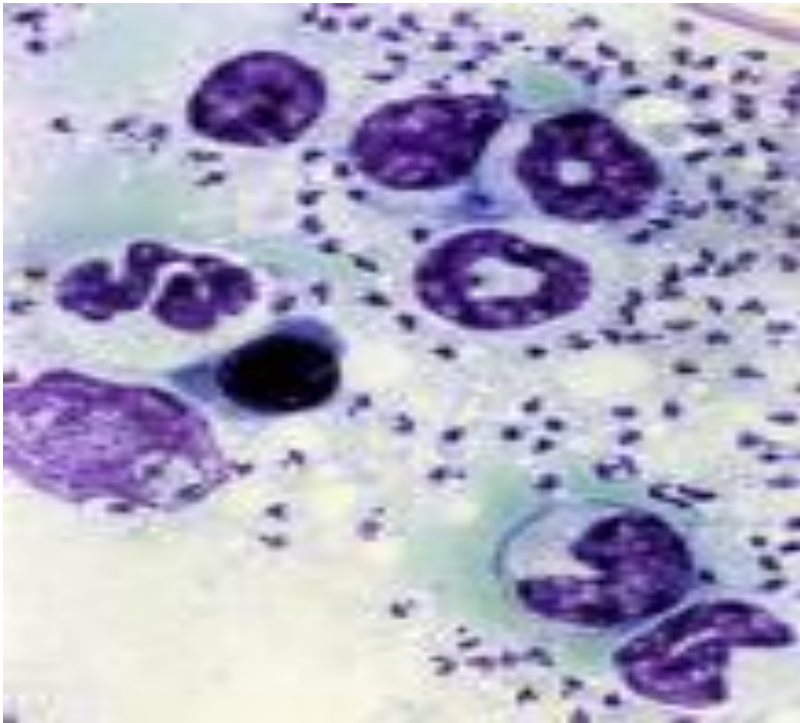


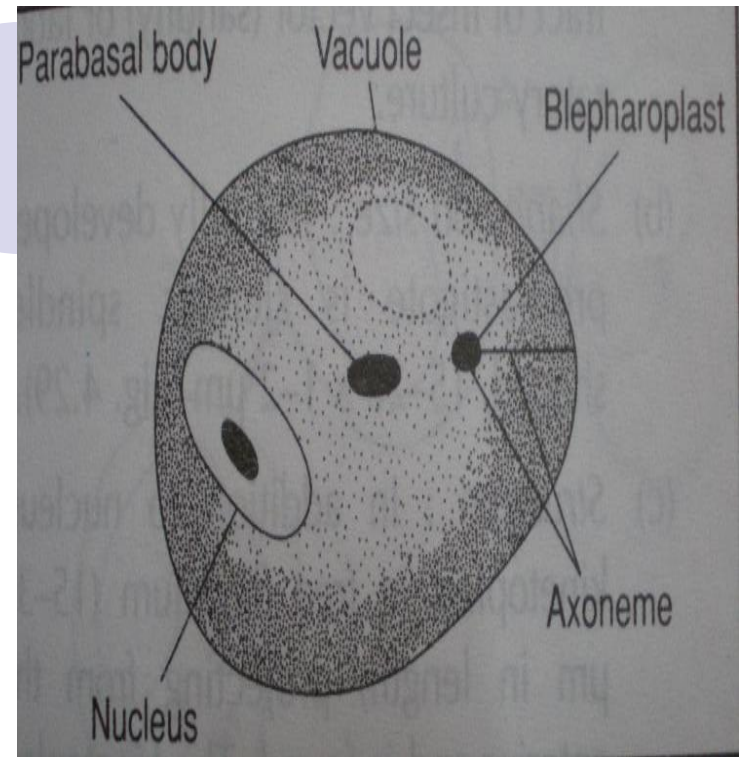
Fig. 178. Morphological forms of *Leishmania donovani*

Amastigote & Promastigote

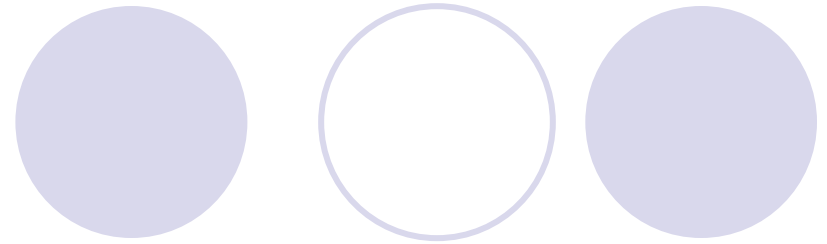


Amastigote form

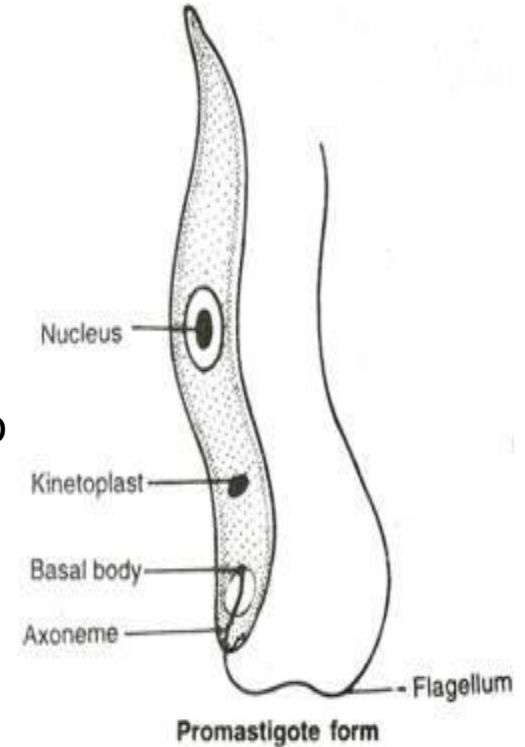
- Size – 2 to 4 micron
- Shape – round or oval body
- Cell membrane – delicate
- Nucleus-
 - 1 micron in diameter, round to oval
 - Middle of cell or along side of cell wall
- Kinetoplast
 - At right angle to nucleus
 - rod shaped body (Para basal body) or
 - tiny dot like structure (blepharoplast)
- Axoneme
 - Delicate filament extending from the kinetoplast to the margin of the body
- Vacuole
 - A clear unstained space lying along side the axoneme.



Promastigote stage



- Size
 - 15 to 20 micron in length * 2 to 3 micron in breadth
- Shape
 - long slender spindle shaped bodies
- Nucleus- centrally
- Kinetoplast
 - Lies transversely near anterior end.
- Axoneme
 - Delicate filament extending from the kinetoplast to the margin of the body
- Eosinophilic Vacuole
 - Light staining area lying in front of the kinetoplast.
- Flagellum
 - Same length as the body or even longer, projecting from front.
 - Does not curve around the body, so no undulating membrane.



Cultivation

A decorative graphic consisting of six circles arranged in two rows. The top row has three circles: a solid light purple circle on the left, a hollow light purple circle in the middle, and a solid light purple circle on the right. The bottom row has three circles: a solid light purple circle on the left, a hollow light purple circle in the middle, and a solid light purple circle on the right.

- NNN medium

- 2 part of salt agar & 1 part of defibrinated rabbit's blood
- Novy & Mac Neal prepared first, later modified by Nicolle
- Material is inoculated into water of condensation and incubated at 24 c.
- Presence of Ascorbic acid & hematin favors the growth of parasite
- Amastigote form change morphology to promastigote

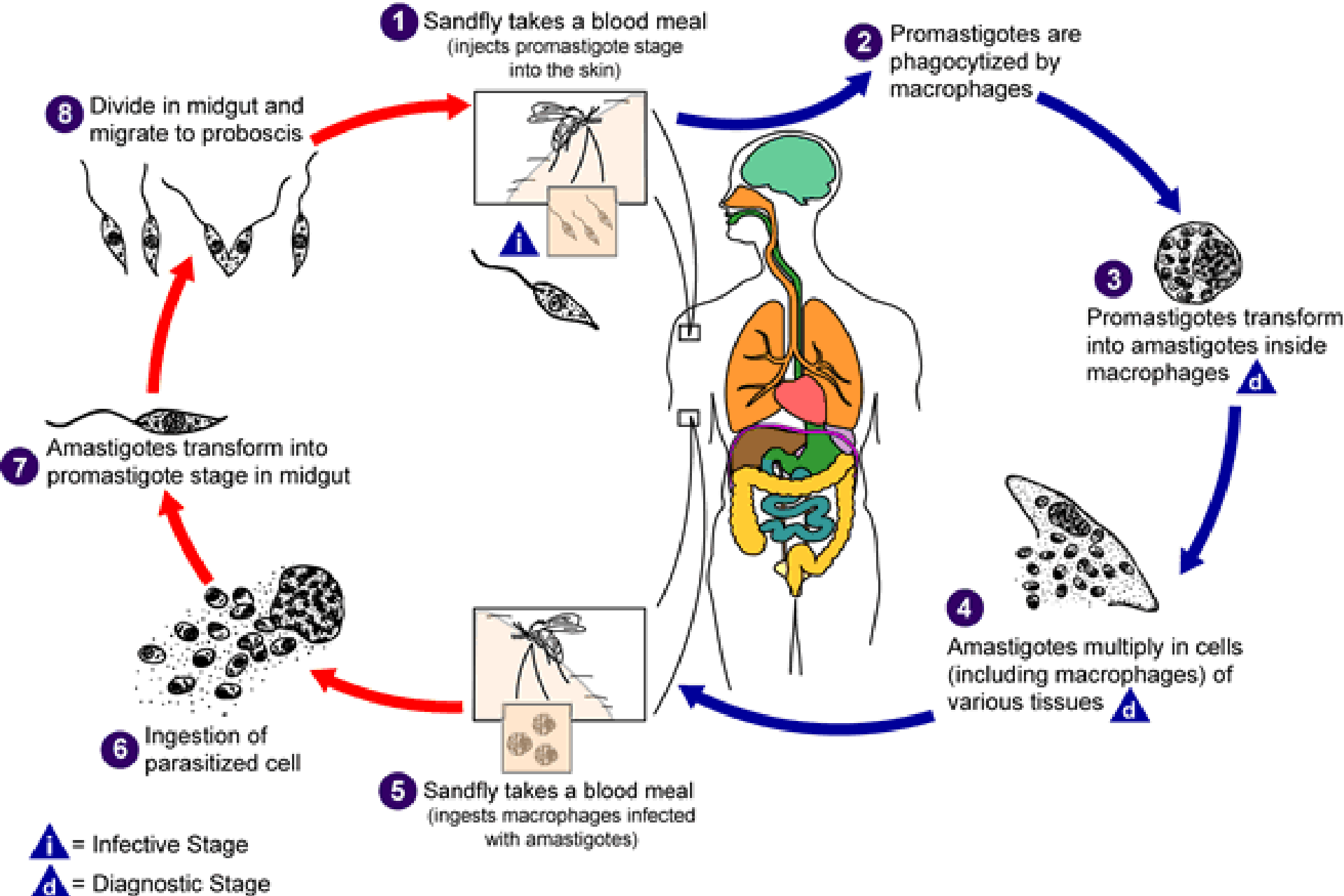
Life cycle

- Passes life cycle in two hosts.
 - Definitive – Man & other vertebrate host
 - Intermediate-Sand fly
- **Infective form- Promastigotes** present in buccal mucosa
 - Bite of infected sand fly- enter tissues
 - Taken up by macrophage, lose flagella and transform into amastigotes
 - Multiply by binary fission to reach enormous numbers
 - Macrophage distends & ultimately ruptures
 - Liberated amastigotes enter other macrophages
 - Few are present free in peripheral blood.



Sandfly Stages

Human Stages

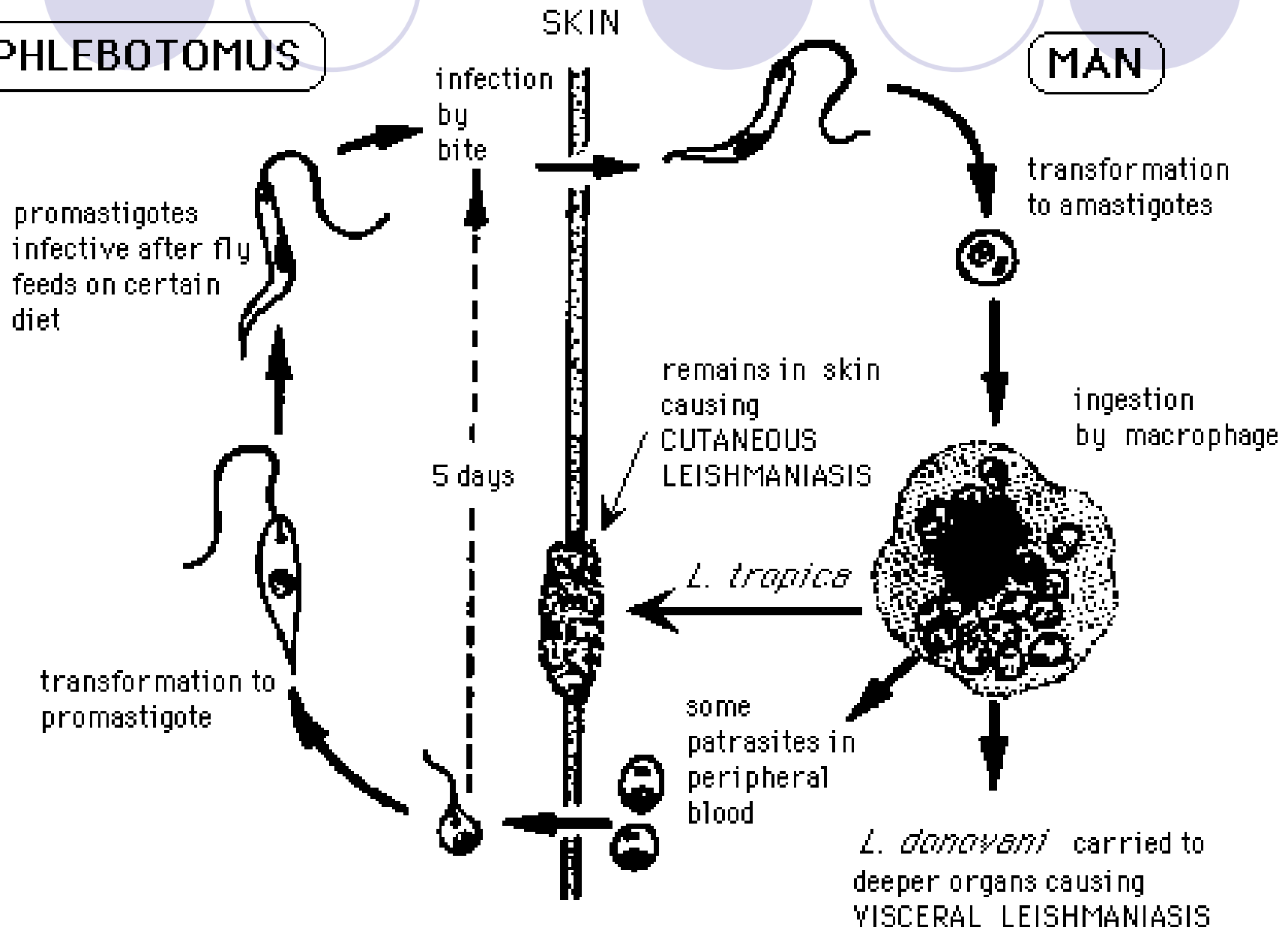


- When female sand fly bites infected person, **amastigotes present in peripheral blood** are taken up
- Reach midgut, change morphology
- **Converted into promastigotes**
- Multiply by binary fission
- Migrate to pharynx and buccal cavity in large numbers
- Mature promastigote block passages.
- Hence in order to take another blood meal sand fly has to liberate large number of parasites in person
- Process takes 6 to 10 days – **Extrinsic incubation period**

Life cycle of *Leishmania donovani* & *L. tropica*. (After Smyth, 1994)

PHLEBOTOMUS

MAN





Pathogenicity & clinical features

Incubation period : 3 to 6 months

Disease produced is called **kala-azar** or **visceral leishmaniasis**

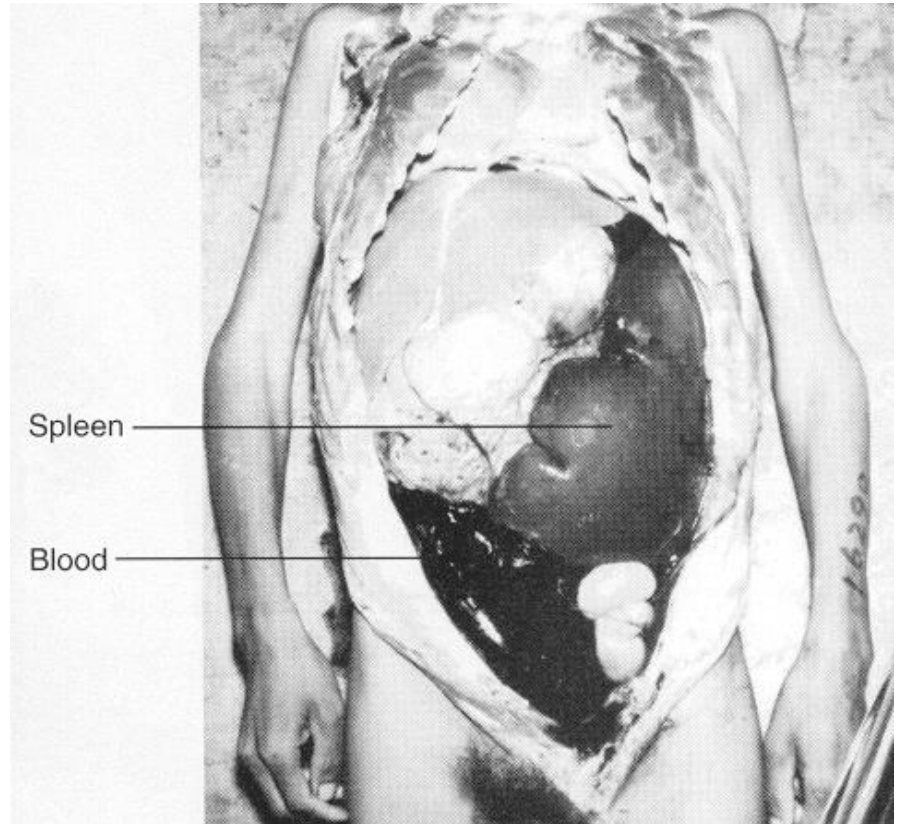
Pyrexia

early symptom, it may be continuous or remittent in type becoming intermittent at later stage

Splenic enlargement- one of the most striking feature of disease.

With progression it extends several inches below the costal margin filling up the entire abdomen.

Hepato-splenomegaly



Clinical features



- **Liver** is also enlarged but not as much as spleen
- **Skin changes**
 - Seen on face, hands, feet & abdomen
 - Dark pigmentation of skin-kala
 - Skin is dry, rough & harsh
- **Lymphadenopathy** is often seen in African & Chinese form of kala azar
- **Epistaxis** & bleeding from gum
- Infections in immuno-compromised person
 - One of the important opportunistic infection occurring In patients of AIDS
- **Anemia** in kala azar
 - Due to hemolysis occurring due to hypersplenism
 - Autoimmune basis-

Pathology or autopsy finding

● Spleen

- Capsule is thickened
- Soft & friable, cuts easily.
- Cut section – red or chocolate in color
- Trabeculae – thin & atrophic
- Reticular cells ↑

● Liver

- Kupffer cells & vascular endothelial cells ↑
- Sinusoidal capillaries are dilated and engorged
- Hepatocytes – not affected



Laboratory diagnosis

- Direct evidence

- Demonstration of organism in smear &/or culture from specimens like blood, splenic biopsy or bone marrow biopsy

- Indirect evidence

- Demonstration of circulating antibody non-specific or specific by various serological tests



Demonstration of organism in smear

- Blood

- By making a **thick blood film**

- By producing **straight leukocyte edge**

- By **centrifuging citrated blood**

- Culture

- 1 to 2 ml of blood is inoculated into water of condensation of NNN medium and incubated at room temp for 4 weeks with daily observation in first week and at weekly interval



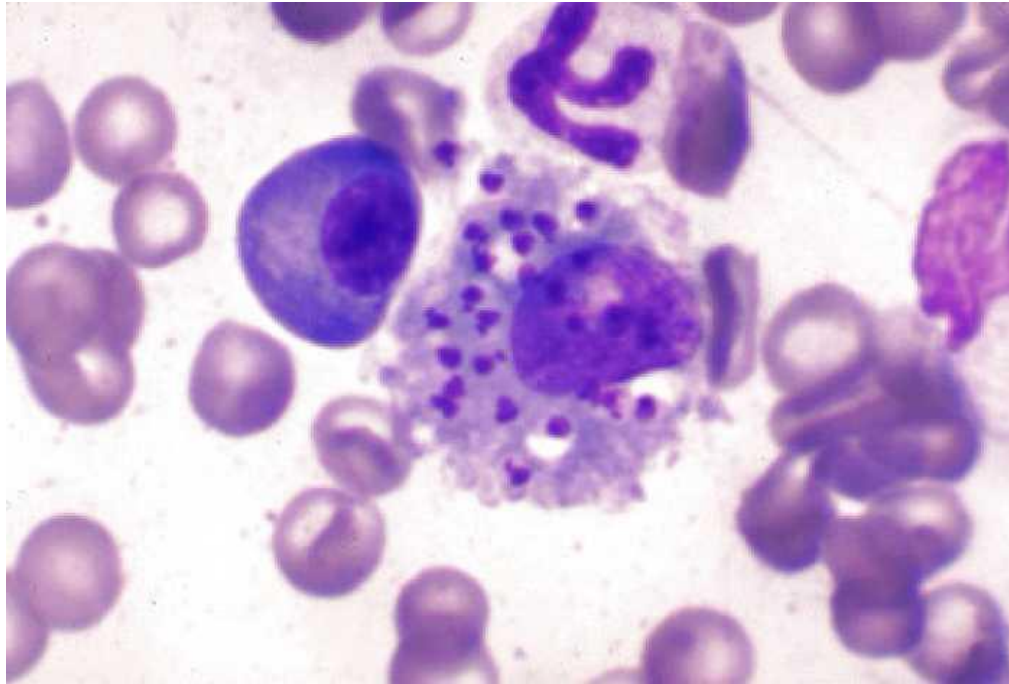
● Splenic puncture

- Important in those whose blood smear is negative
- Only drawback is **bleeding** which might continue from puncture site
- It is advisable to perform **bleeding time & pro-thrombin time** before doing it

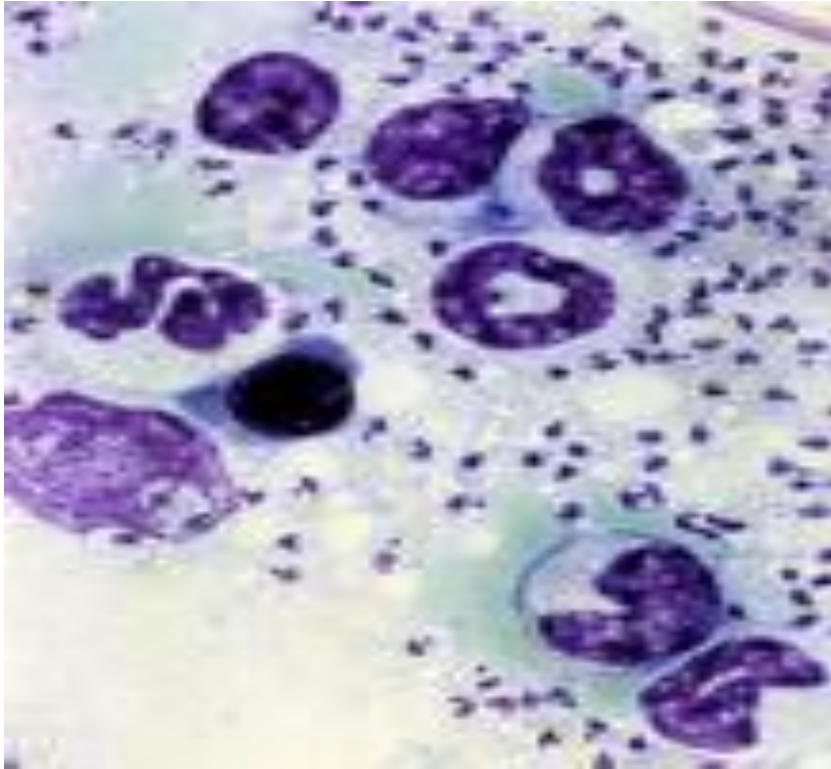
● Bone marrow biopsy

- A **certain** method of diagnosis particularly in early cases when spleen is not enough enlarged
- Compared to splenic puncture it is **safer** method as risk of hemorrhage is low
- But **more painful** and less sensitive than splenic puncture

Giemsa stained smear



L.donovani in smear & culture



Indirect evidence



- Blood count:
 - Leucopenia with marked diminution of neutrophils
 - Total count is often < 3000 , may be as low as 1000
 - Erythrocyte count is also low
- Serological tests
 - Nonspecific
 - Aldehyde test
 - Antimony test
 - Complement fixation test with W.K.K. antigen
 - Specific
 - Immunofluorescent test
 - Indirect haemagglutination test
 - Specific complement fixation test

Aldehyde test



- 1 to 2ml of serum + 1-2 drop of 40 % formalin
- If **jellification of milky white opacity** like white of a boiled egg occurring in **2 to 20 minute** – test is considered positive
- Due to an increase in serum gamma globulin
- Positive only after **3 months** duration of disease

Antimony test



- 1-2 ml of serum, add drop by drop 4 % **urea stibamine** solution in distilled water
- Formation of **profuse flocculent** precipitate indicates positive reaction
- Less reliable than aldehyde test

Complement fixation test with WKK antigen



- Antigen used is prepared from **human tubercle bacillus** as suggested by Witebsky, Klinghausen & Kuhn
- Helps in early diagnosis of disease when Aldehyde test is negative
- Positive by **3 weeks** of infection

Laboratory diagnosis



- Skin test (Leishmanin test)
 - Antigen -promastigotes of *L.tropica* - 10^6 per ml
 - 0.1 ml injected intradermally in inner surface of forearm
 - Area of induration of 5 mm or more after 48-72 hours of inoculation is considered positive
 - Negative leishmanin test is seen in patients of diffuse cutaneous leishmaniasis & active visceral type.

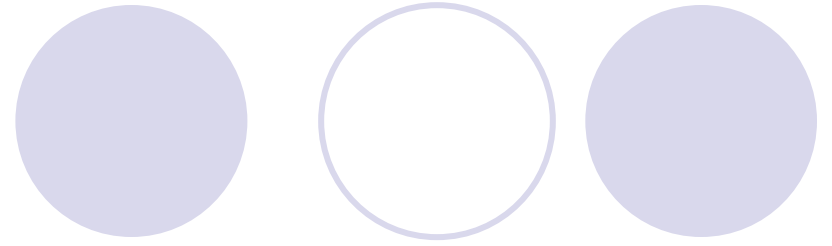
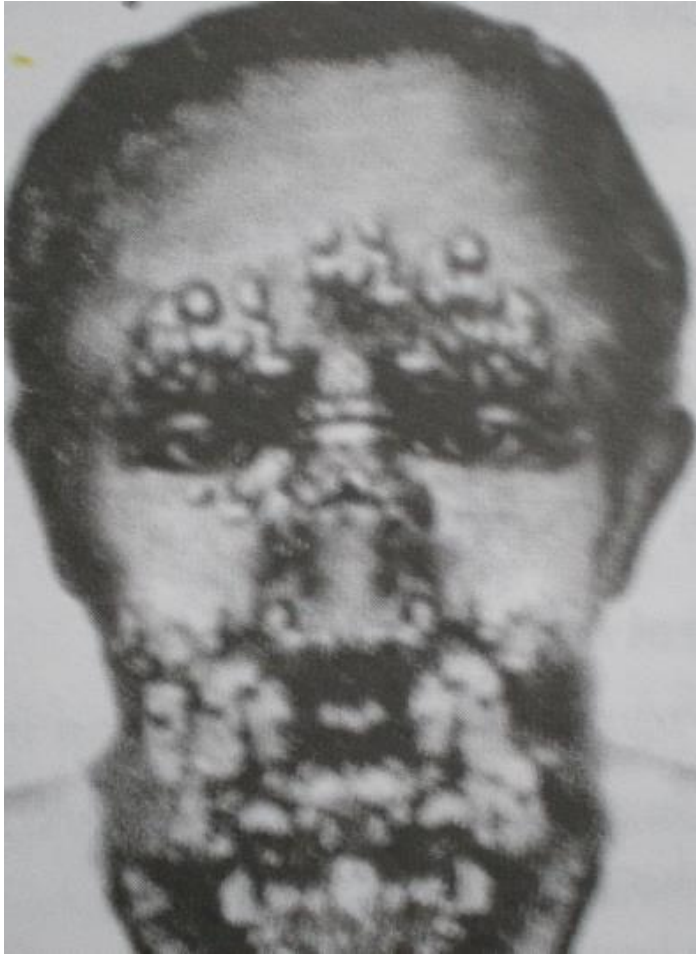
Post kala-azar dermal leishmaniasis (PKDL, Dermal leishmanoid)

- Cutaneous form of leishmaniasis occurring in about 2-10% of kala -azar patients generally one or two year after completion of successful antimonial treatment
- At this time visceral infection disappears but skin lesion persists
- Only found in India & African form of kala-azar
- Also found in spontaneously cured kala-azar

PKDL-Clinical manifestations

- **Hypo pigmented patches (macule)**
 - Earliest lesions on the trunk & extremities, face is less affected.
- **Erythematous patches**
 - Early lesion seen on nose, cheek and chin, having butterfly distribution
 - Photosensitive, becoming prominent during mid-day
- **Yellow-pink nodules**
 - Nodules replace earlier lesions, occasionally develop at the outset.
 - Mostly distributed on skin (mostly of face), rarely on mucus membrane of tongue & eyes
 - Granulomatous, soft and painless yellow to pink nodules of varying size , **do not ulcerate.**

PKDL



Diagnosis & treatment of PKDL

- Demonstration of Amastigote form of *L.donovani* in RE cells of skin in leishman stained smear from biopsy material obtained from nodular lesions.
- Smear from macules are often negative

Diagnosis & treatment of PKDL

- By pentavalent antimonial in double doses used for visceral lesions
- Standard treatment of kala-azar
 - **Sodium stibogluconate** – 20 mg/kg bodyweight up to 850 mg/day for 30 days
 - **Pentamidine** 3-4 mg/kg, 1 or 2 times/week for 2 weeks
 - **Amphotericin B** 0.25 to 1 mg/kg daily slow infusion for 8 days

Leishmania causing old world cutaneous leishmaniasis

- Causative agent

- *Leishmania tropica*

- *Leishmania major*

- *Leishmania aethiopica*

- Responsible for

- Cutaneous leishmaniasis- *Oriental sore*

- Also called as –

- *Delhi boil*

- *Baghdad boil*

Discovery of *Leishmania tropica*

Cunningham	1885	Observed parasite from patient of Delhi boil in Kolkata
Borovsky	1891	Make accurate description of morphology
Luhe	1906	Given name <i>Leishmania tropica</i>

Geographical distribution



Life cycle



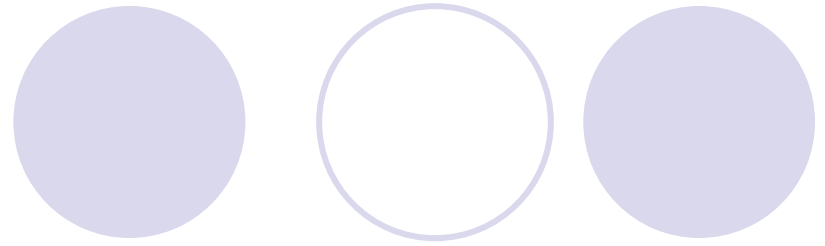
- Intermediate Host- Sand fly – Phlebotomus
- Definitive Host- Man
- Reservoir of infection
 - L.tropica- None
 - L.major- rodents
 - L.aethiopica- rock
- Mode of infection
 - Promastigote present in buccal mucosa enter tissues by bite of infected sand fly or by crushing of the infected sand flies into punctured wound.

Immunity



- CMI develops early and results in elimination of parasites.
- A single attack gives lifelong immunity
- Serum antibodies are not produced
- In non-immune person it produces diffuse cutaneous leishmaniasis (p.e. *L.aethiopica*)
- Attack lasts for 4-6 months (*L.major*) sometimes 1-2 year (*L.tropica*) followed by spontaneous cure in most cases

Clinical features



- Incubation period
 - 2-8 months (may up to 1 or 2 year)
- L.tropica
 - Cutaneous lesion- **Oriental sore or Delhi boil**
 - Small **papule** develops at the site of bite which soon becomes a raised **nodule** 2 to 5 cm in diameter
 - Majority of cases nodules **ulcerate**, having a clear cut margin, raised indurated edge surrounded by red areola
 - Limited in number- 2 to 3 or sometimes single & distributed over **exposed** part of body (face & extremities)
 - Heals spontaneously and in 1-2 years producing a depressed white scar leaving patient immune to reinfection

Oriental sore





- L.major

- Clinical picture is similar except lesions develop and heal more quickly than those of L.tropica.

- Ulcer is self healing in 3-6 months

- Infection gives cross immunity to L.tropica & L.aethiopica

- L.aethiopica

- Lesion similar to Oriental sore but may give rise to diffuse cutaneous leishmaniasis in patients with poor CMI

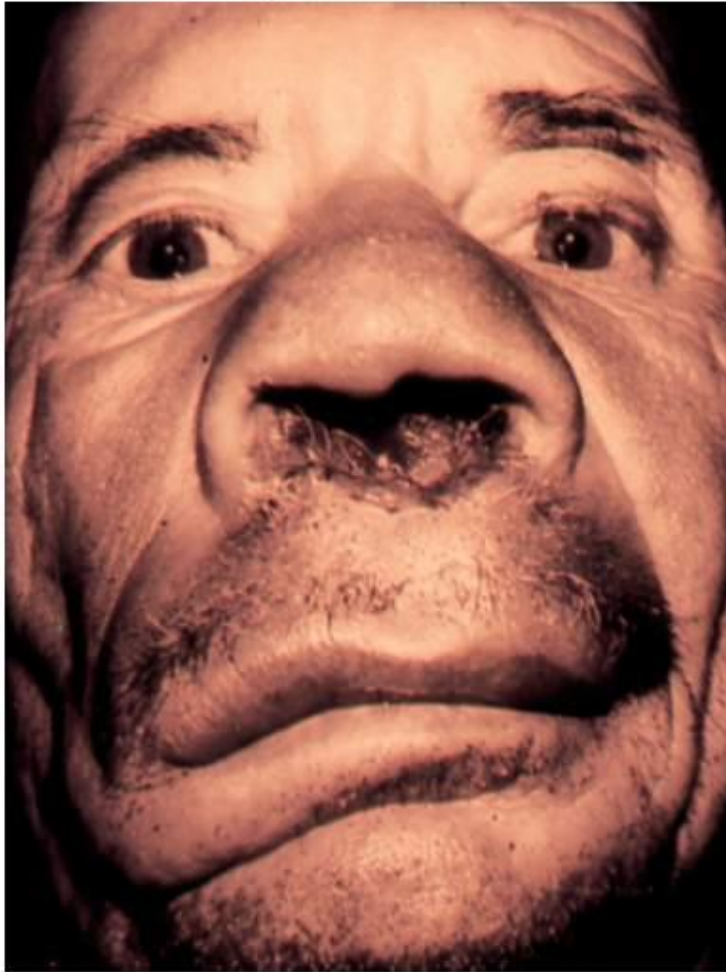
- Condition characterized by formation of disfiguring nodules over the surface of body which are difficult to cure

Diffuse cutaneous leishmaniasis

- Characterized by appearance of **diffuse nodular infiltrative** lesion which are neither destructive nor erosive but most disfiguring
- Starts as single lesion and spreads slowly over face, ears, extremities and buttock, until whole body is affected
- Histologically nodes consists only of **histiocytes** with relative absence of lymphocytes & plasma cells
- Leishmanin skin test is negative

Diffuse cutaneous leishmaniasis

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(a)



(b)

Laboratory diagnosis



- Microscopy

- Smear prepared from material obtained by a puncture of indurated edge of the sore or nodule & stained by Leishman stain
- Amastigotes are found in large numbers inside macrophage

- Isolation

- Culture into NNN medium will give promastigotes

Laboratory diagnosis



- Skin test (Leishmanin test)
 - Antigen -promastigotes of *L.tropica* - 10^6 per ml
 - 0.1 ml injected intradermally in inner surface of forearm
 - Area of induration of 5 mm or more after 48-72 hours of inoculation is considered positive
 - Negative leishmanin test is seen in patients of diffuse cutaneous leishmaniasis & active visceral type.

Leishmania causing new world cutaneous & muco-cutaneous leishmaniasis

- Causative agents

- Cutaneous leishmaniasis

- L.braziliensis subspecies

- L.b. guyaensis
- L.b. panamensis
- L.b.peruviana

- L.mexicana

- L.m. mexicana
- L.m.amazonensis
- L.m.pifani
- L.m.venezuelensis

- Muco-cutaneous leishmaniasis

- L. braziliensis subspecies braziliensis



Habitat

- Intracellular parasites inside macrophage cells of skin & mucus membrane of nose and buccal cavity.
- Promastigote forms occur in insect vector- Sand fly (*Lutzomyia*)

Geographical distribution

- Mainly seen in South & Central America



Epidemiology



- Mainly seen in central & south America
- Reservoir of infection – small forest rodents and dogs
- Transmission- Sand fly – Lutzomyia

Lutzomyia



Life cycle



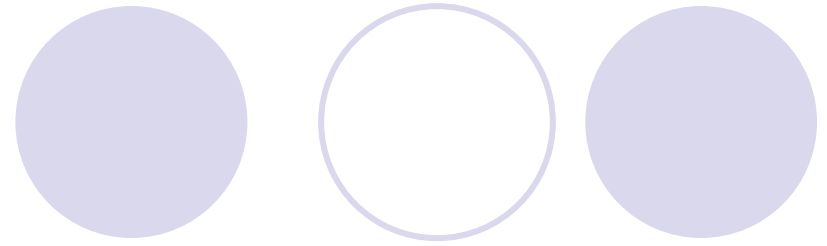
- Basic life cycle same except that the amastigotes- mononuclear cells of skin & mucus membrane
- Promastigotes- found in mid gut & buccal cavity of sand fly

Pathogenesis



- Similar to that of oriental sore
- Lesions are typically found in skin and mucosa is affected subsequently

Clinical disease



- Cutaneous leishmaniasis

- | | |
|--------------------|--|
| ○ L.b. guyanaensis | Forest Yaw |
| ○ L.b. panamensis | Skin ulcer-non healing |
| ○ L.b. peruviana | Uta- ulcer <u>c</u> heals
spontaneously after 4 month |
| ○ L.m. mexicana | Chiclero's ulcer |

L.b.guyanensis	Benign form (Forest yaw)	Oro-nasal mucosa
L.b.peruviana	Benign form-only dry papule on skin (Uta in peru)	No involvement of mucosa
L.b.pifanoi	Malignant form	Diffuse cutaneous leishmaniasis
L.b.mexicana	Chiclero's ulcer-a benign form without involvement of mucosa	Single cutaneous lesion on ear, face or hand which undergoes spontaneous healing

Muco-cutaneous leishmaniasis - (Espundia)

- A **severe and malignant** form of cutaneous leishmaniasis similar to oriental sore associated with **invasion of oro-nasal mucosa** in south America
- Lesion starts as a **papulo-pustular swelling** in skin localized around mouth, nostrils or eye or widespread on the face, elbows or knees
- Migrate on the mucosal surface of mouth, nose and naso-pharynx causing destructive and mutilating erosions leading to disfiguration often with **complete destruction of nasal septum, perforation of palate and damage to tissues of lips and naso-pharynx**
- Heals by scarring producing typical **tapir nose or camel nose**

Espundia-Mucocutaneous leishmaniasis

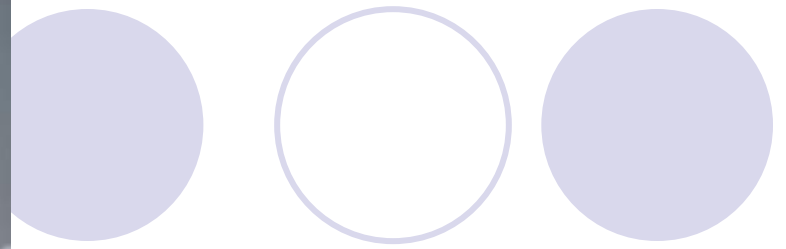


Camel nose = tapir nose





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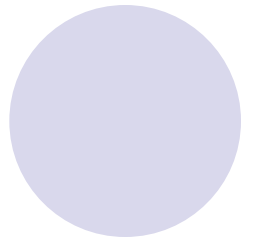
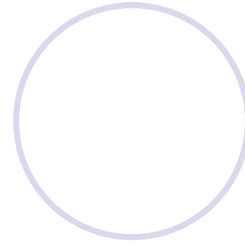
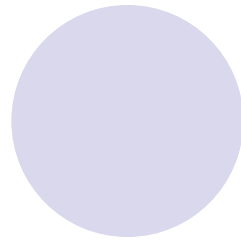
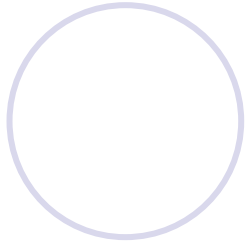
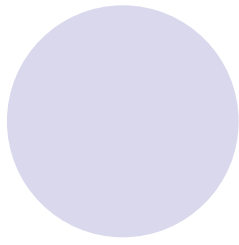


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Laboratory diagnosis

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- Microscopy
- Isolation
- Serology
- Skin test



Thank You