

## History of Leishmaniasis (from Wikipedia)

Descriptions of conspicuous lesions similar to cutaneous leishmaniasis (CL) has been discovered on [tablets](#) from King [Ashurbanipal](#) from the 7th century BC, some of which may have been derived from even earlier texts from 1500 to 2500 BC.

[Muslim](#) physicians including [Avicenna](#) in the 10th century AD gave detailed descriptions of what was called [Balkh](#) sore.

In 1756, Alexander Russell, after examining a [Turkish](#) patient, gave one of the most detailed clinical descriptions of the disease.

Physicians in the [Indian subcontinent](#) would describe it as Kala-azar (pronounced *kālā āzār*, the [Urdu](#), [Hindi](#) and [Hindustani](#) phrase for *black fever*, *kālā* meaning black and *āzār* meaning fever or disease).

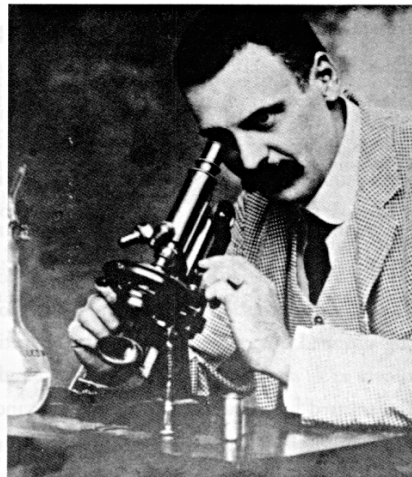
As for the [new world](#), evidence of the cutaneous form of the disease was found in [Ecuador](#) and [Peru](#) in pre-Inca potteries depicting skin lesions and deformed faces dating back to the first century AD. 15th and 16th century texts from the [Inca](#) period and from [Spanish colonials](#) mention "valley sickness", "Andean sickness", or "white leprosy", which are likely to be CL.

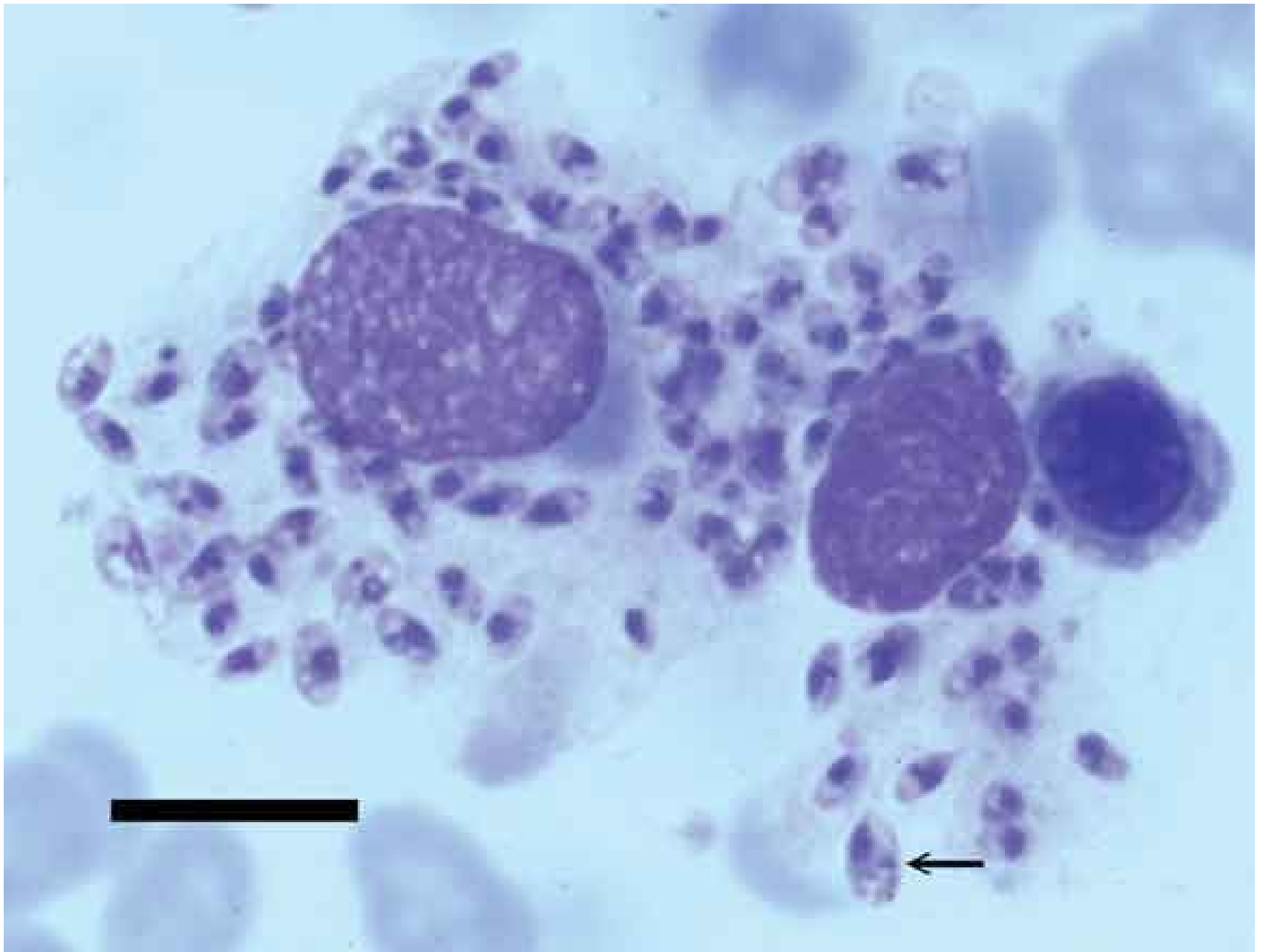
## Discovery of *Leishmania donovani* as cause of kala-azar

In 1900 when William Leishman, a Scottish army doctor, found *Leishmania donovani* in stained smears from the spleen of a soldier suffering from a fever contracted at Dum-Dum in India. His observations were published in 1903.

At the same time Charles Donovan, a Professor of Physiology at Madras University, described a similar parasite in smears made from a splenic biopsy.

Prior to Leishman and Donovan's discovery kala azar was considered to be a communicable malaria-like disease that showed relapses, emaciation, as well as enlargement of the liver and spleen, and spread slowly across the continents along the trade routes.





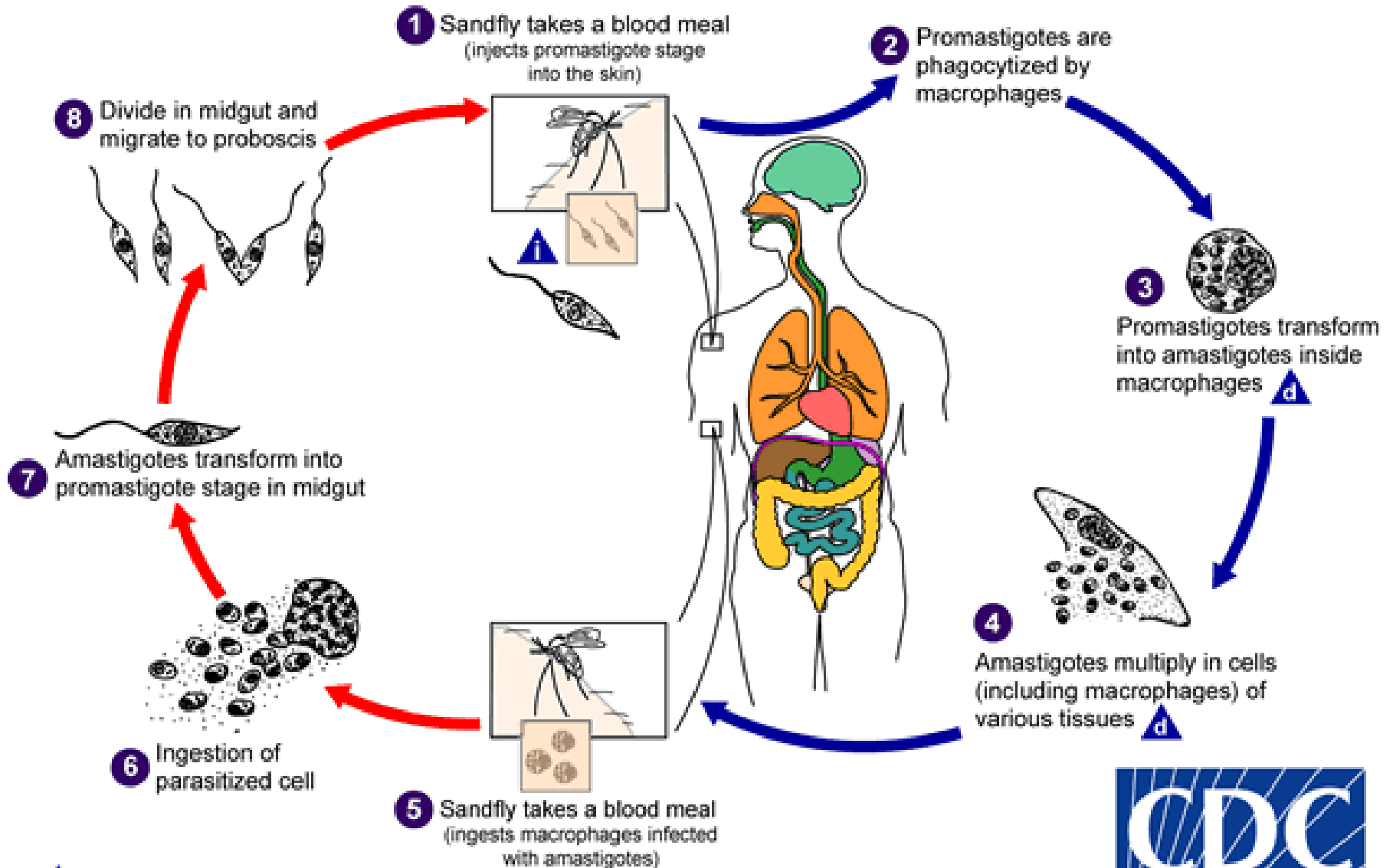
# Discovery of Sandfly as Vector

In 1924 the Kala-Azar Commission noted **that the distribution of a sandfly (*Phlebotomus argentipes*) in India closely overlapped the distribution of the disease.** But directly demonstrating transmission by sandflies was more difficult until 1939 when Smith, Haldar and Ahmed made the discovery that if sandflies after taking a bloodmeal were fed on raisins instead of being given additional blood meals, the flagellates grew so numerous that they blocked the pharynx as happens with plague bacilli in fleas. These workers then **subjected hamsters to the bite of blocked sandflies and each became infected.** Human transmission of leishmaniasis was demonstrated in 1941 when Adler and Ber successfully infected “volunteers” with *L. major* by the bite of *P. papatasi*, and in 1942 Swaminath, Shortt and Andersen allowed 6 human “volunteers” to be bitten by infected *P. argentipes* and all developed kala-azar.



## Sandfly Stages

## Human Stages



**i** = Infective Stage  
**d** = Diagnostic Stage



## The insect vector

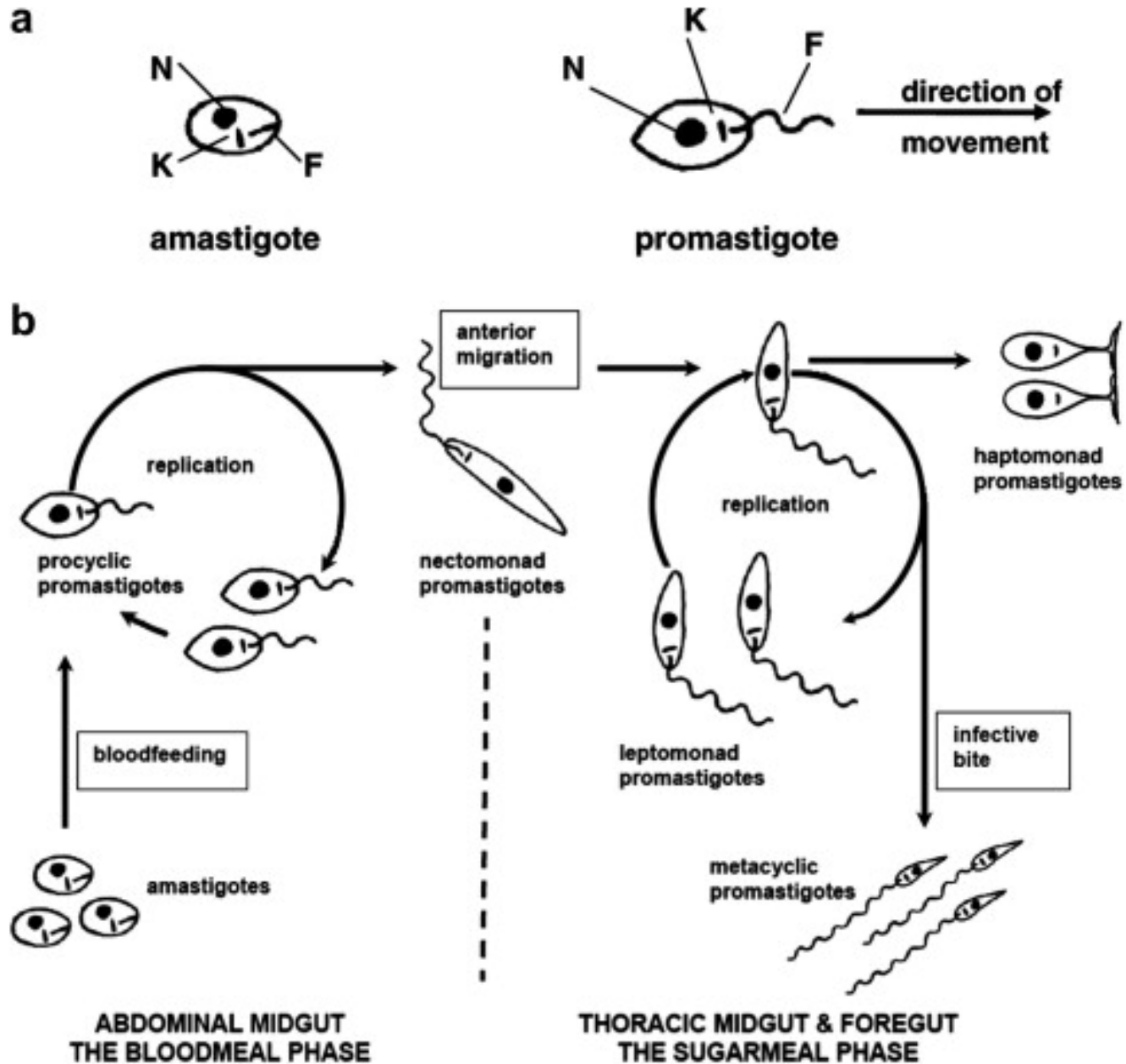
**Sandflies (*Phlebotomus* in Old World and *Luzotomia* species in New World) are “pool feeders”. The female sand flies insert their saw-like mouthparts into the skin and agitate them to produce a small wound into which blood flows from capillaries. This tissue damage releases skin macrophages and freed *Leishmania* amastigotes into the pool of blood, which are taken up into the fly posterior midgut. The *Leishmania* then develop into several distinct developmental stages as they migrate anteriorly to the stomodeal valve, which forms a junction with the foregut.**

**Takes 6-9 days to complete their development.**

## Life cycle of *Leishmania* in Sandfly – Coevolution of vector and host

1. The amastigotes transform into weakly motile “**procyclic promastigotes**”. These replicate in the “bloodmeal”.
2. Within 4 hours, the bloodmeal is enclosed by the peritrophic matrix (PM) – a chitin and protein mesh secreted by the midgut. The PM protects the promastigotes from digestive enzymes.
3. *L. major* secretes a neuropeptide that arrests hindgut peristalsis and prevents the cells from being excreted.
4. Within 48-72 hrs the PM is degraded by chitinases (some from the parasite), releasing the elongated **very motile non-replicating nectomonad promastigotes**. These migrate towards the anterior midgut, some attaching to the microvilli, until they reach the stomodeal valve.
5. The nectomonads transform into **leptomonad promastigotes** that resume replication. These secrete the promastigote secretory gel (PSG). The PSG contains a high MW filamentous **proteophosphoglycan** which fills the anterior midgut into the foregut.
6. Some transform into **non-replicating metacyclic promastigotes**.
7. The fly regurgitates and expels the PSG and the metacyclic promastigotes.

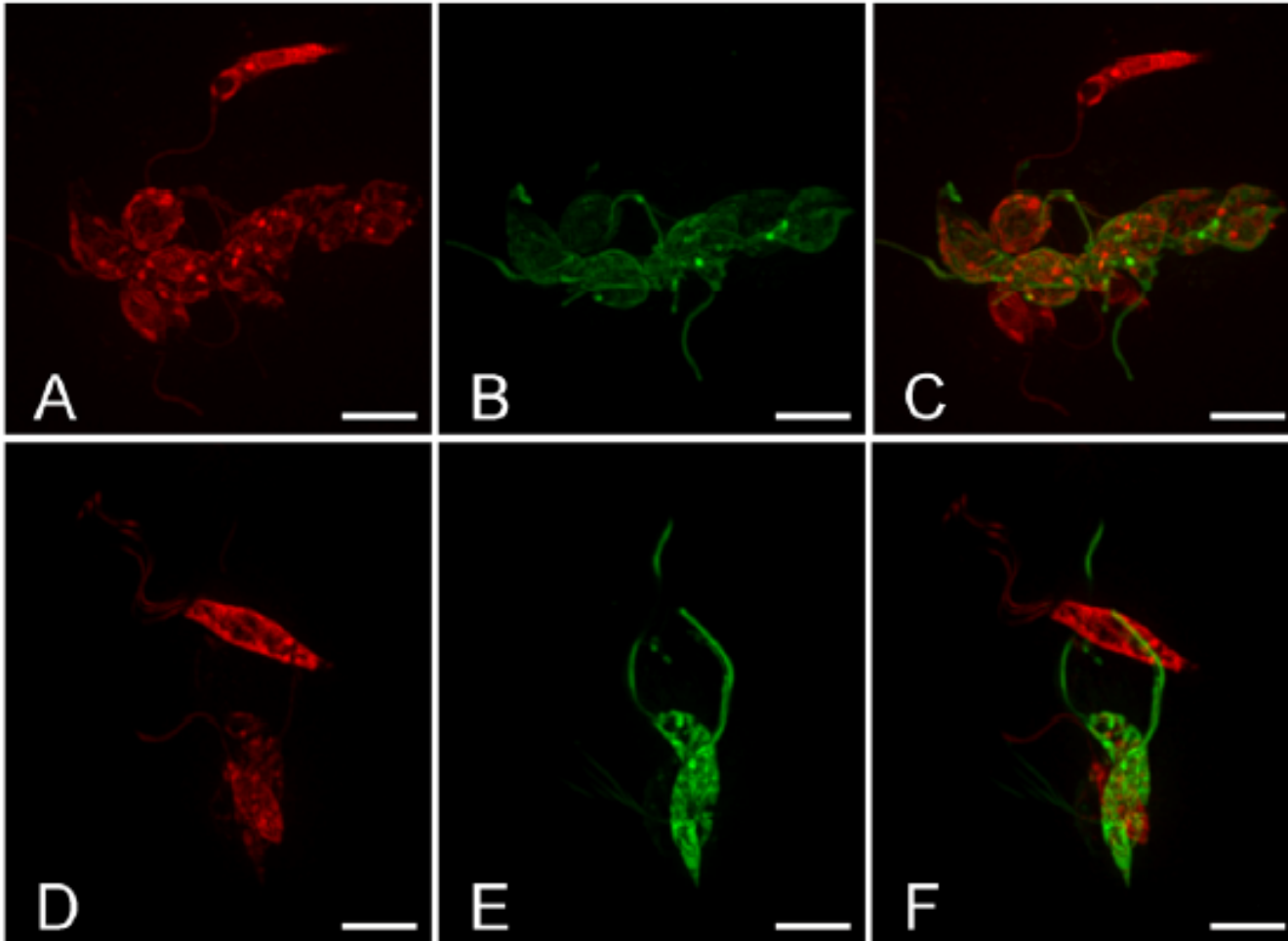
# Developmental Stages of *Leishmania* in Sand Fly



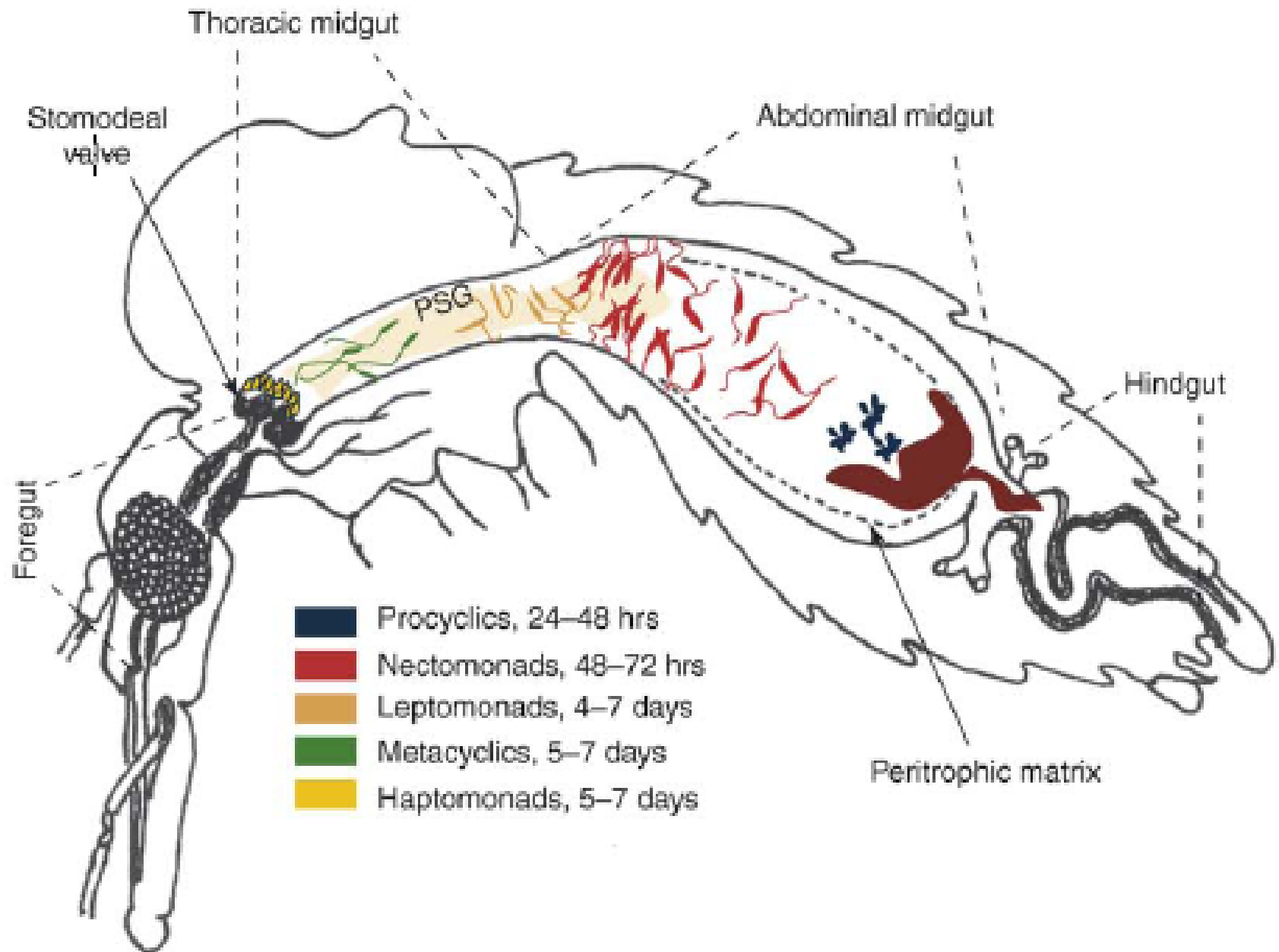


# A sexual phase may occur in the early stages in the sandfly

Transfected promastigotes with plasmids expressing GFP or RFP. Sandflies were coinfecting. Saw green-red hybrids in early phase.



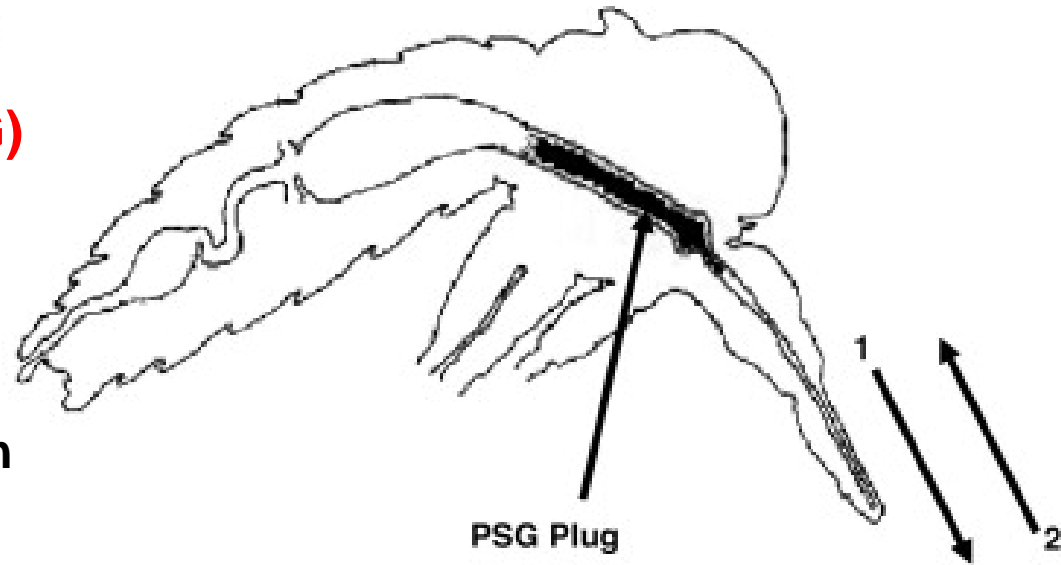
Sadlova et al  
(2011). PLOS  
one 6: 1-9.



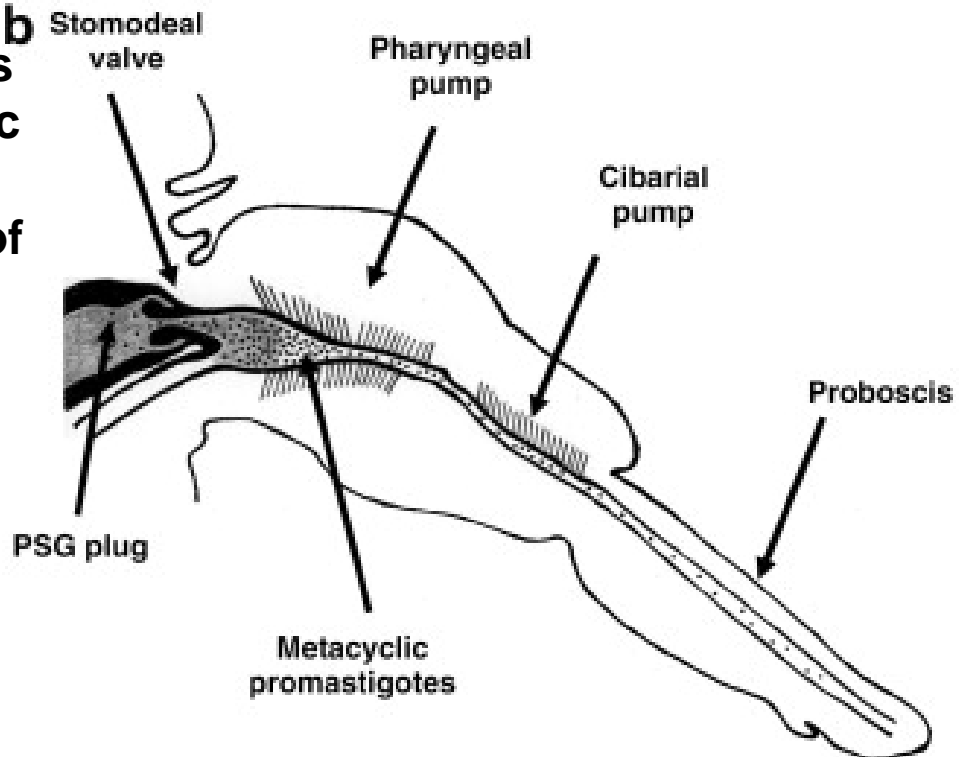
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## Promastigote secretory gel (PSG)

The plug must be partially egested before blood feeding can occur, thereby injecting both **metacyclic promastigotes** and **PSG** into the skin of the mammalian host. (b) Detail of the anterior midgut and foregut. The PSG plug (shaded) forces the stomodeal valve open and extends into the pharynx region. Metacyclic promastigotes (stippling) are concentrated at the anterior pole of the plug but are found along the foregut in both the cibarium and proboscis.



b



# Role of sandfly saliva and parasite PSG in exacerbation of disease

## Saliva:

Required for blood feeding activity since it has potent **vasodilatory and antihaemostatic** properties.

Modulates the immune response to favor parasite survival and replication

## PSG:

Leads to increase in pathology and parasite numbers when co-inoculated with metacyclic promastigotes.

# Leishmaniasis

Zoonotic infection affecting 12 million people worldwide in 88 countries. Annual incidence is 1.5 million with the cutaneous form and 0.5 million with the visceral form.

- **Old World cutaneous leishmaniasis** - *L. tropica* - in urban areas of the Mediterranean basin, the Middle East, Pakistan and parts of India.
- **Cutaneous leishmaniasis** - *L. major* - in rural areas, usually dry desert regions of Central Asia, Southern Russia, Middle East and Africa.
- **New World cutaneous leishmaniasis** - *L. braziliensis* and *L. mexicana* - in Mexico and central and South America.
- **Visceral leishmaniasis** >80 countries in Africa, Asia, southern Europe (*L. infantum*) and S. America (*L. chagasi*). About 90% of the 500,000 new cases each year occur in 5 countries: Bangladesh, India, Sudan, Nepal, Brazil. India alone contributes to ~50% of all cases in the world.
- **Leishmania/HIV** co-infection is considered to be a real “emerging disease”, especially in southern Europe, where 25-70% of adult VL cases are related to HIV infection, and 1.5-9.5% of AIDS cases suffer from newly acquired or reactivated VL

# CUTANEOUS LEISHMANIASIS:

## Old World Cutaneous Leishmaniasis (CL)

### *L. major*

Causes a moist, cutaneous, ulcerlike lesion at the site of the bite; it starts as a papule that runs an acute course (1-3 weeks) with early ulceration and a surrounding zone of inflammation, that usually heals in two months to a year leaving a depressed unpigmented scar, and lasting immunity.

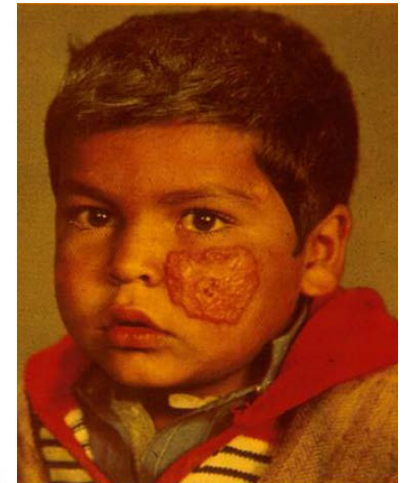


FIGURE 45. Oriental sores on face and forehead. (Courtesy of Ash and Spitz. Pathology of Tropical Diseases, Armed Forces Institute of Pathology, #80,717.)

It is transmitted by *Phlebotomus spp.* from gerbils, dogs or rodents to human or human-to-human. It is generally found in sparsely populated rural areas.

## *L. tropica*

**Causes a dry cutaneous lesion that persists for months before ulcerating; it is also called Oriental sore, Delhi boil, Baghdad boil, Aleppo button, Jericho boil. The disease is characteristically found in more densely populated urban areas. Reservoir = dogs.**



# New World Cutaneous Leishmaniasis

## *L. mexicana*

The ulcer usually heals spontaneously in a few months. However, when the bite occurs on the ear it results in chronic lesions known as chiclero's ulcer. Because the cartilage of the ear pinna is poorly vascularized the immune response is weak, and in 40% of cases the result is mutilation of the pinna. Found principally in Central America and Mexico where it occurs in the forest dwelling people who harvest latex from the chicle trees to be used in the manufacture of chewing gum. In Belize it is also known as *bay sore*.



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Fig. 13.9 "Chiclero's ulcer", due to *L. (L.) mexicana*. Almost total destruction of the the external ear in a chiclero from Belize with an infection of many years duration. (From Lainson and Strangways-Dixon 1963.)



# Muco-cutaneous leishmaniasis

*L. (Vianna) braziliensis, guayanensis, panamensis*

Inoculation of promastigotes by the bite of a sandfly results in a small, red, skin papule that is itchy and ulcerates in 1-4 weeks as in the case of cutaneous leishmaniasis; this ulcer usually heals spontaneously within 6-15 months.

However, there is metastatic spread of the promastigotes from the site of the bite via the lymphatics. Disease is called **espundia**; the metastatic lesion involves the **nasal and buccal mucosa** causing destruction and malformations of the **cartilage and soft tissues**. The ulcerations can involve the **nose, pharynx, palate and lips** resulting in camel nose or parrot beak. Invasion of the larynx may result in a loss of speech. In Brazil 1/3 of the espundia cases are in children.

May take many years for it to spread from the initial site of bite. Death may occur from secondary infections or respiratory complications.

# Mucocutaneous leishmaniasis

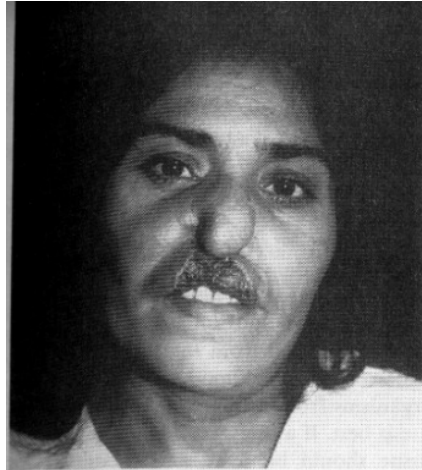
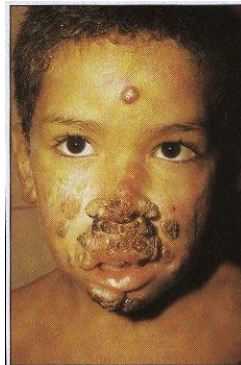


Figure 4-13. Mucocutaneous leishmaniasis in a Honduran female, probably due to *L. b. braziliensis* or *L. b. panamensis*.



## *L. guyanensis*:

In Uruguay and Venezuela, the disease is called *pian bois*, and the lesions are flattened ulcerated plaques that remain open and oozing all over body. Similar to wet lesions of *L. major*.

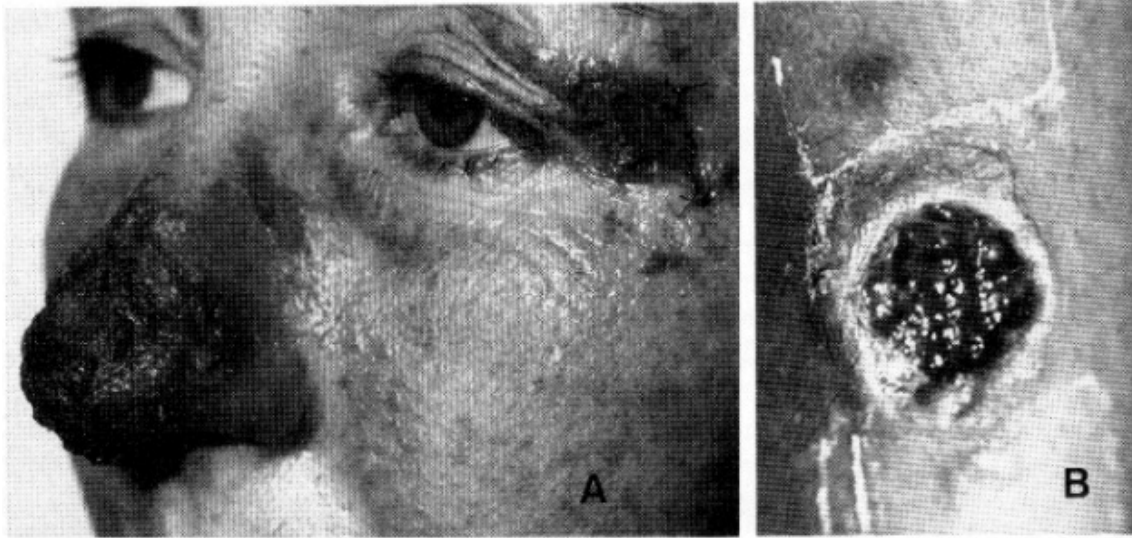
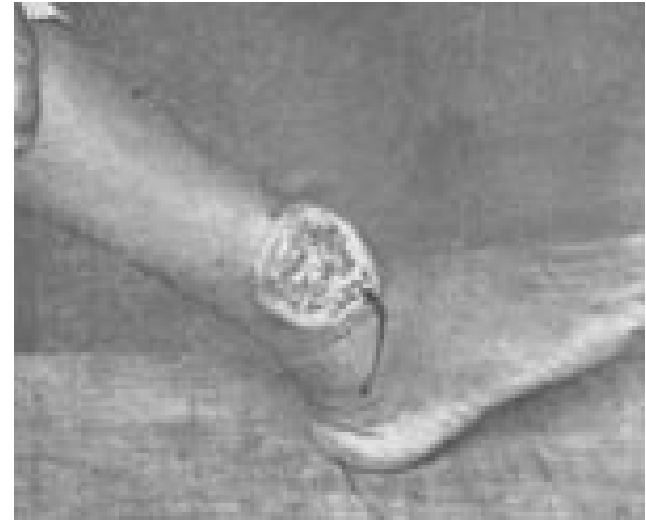


Figure 4-12. Cutaneous leishmaniasis. A. Lesions of outer nose and corner of eye due to *L. braziliensis guyanensis*. B. "Wet" lesion of arm caused by *L. major* acquired in Senegal.



## Diffuse Cutaneous Leishmaniasis

In some patients, especially in Venezuela, a diffuse cutaneous form develops which is characterized by a macrophage granuloma and thickening of the skin. This condition looks like lepromatous leprosy and sometimes like fungal disease, and is often misdiagnosed. Probably due to a failure of immune response. May be due to *L. mexicana* or *L. amazonensis*.



Fig. 4-18. Diffuse anergic cutaneous leishmaniasis. (AFIP 72-2312-13.)



Fig. 13.5-6 Anergic diffuse cutaneous leishmaniasis (ADCL) due to *L. (L.) amazonensis*. Note the amputation of some fingers (Fig. 13.5) and toes (Fig. 13.6), simulating lepromatous leprosy, with which disease ADCL is often confused. Pará, Brazil. (Fig. 13.5 from Lainson 1982b).

# Animal Reservoirs

**202 Rodent reservoirs of Brazilian rain forest** Most cutaneous leishmaniasis in the New World is a zoonosis associated with rodents of the rain forest. Rodents such as *Proechimys guyanensis* are typical animal reservoirs for forest leishmaniasis in both Brazil and Central America. *L. mexicana amazonensis* has been isolated from this species. The Brazilian rain forest is the typical habitat of *L. m. amazonensis*.

**203 Animal reservoirs in Panama** In Panama a wide variety of animals serve as reservoirs for *L. b. panamensis*. The hosts include species of monkeys, marmosets, and even such bizarre animals as the three-toed sloth shown here.



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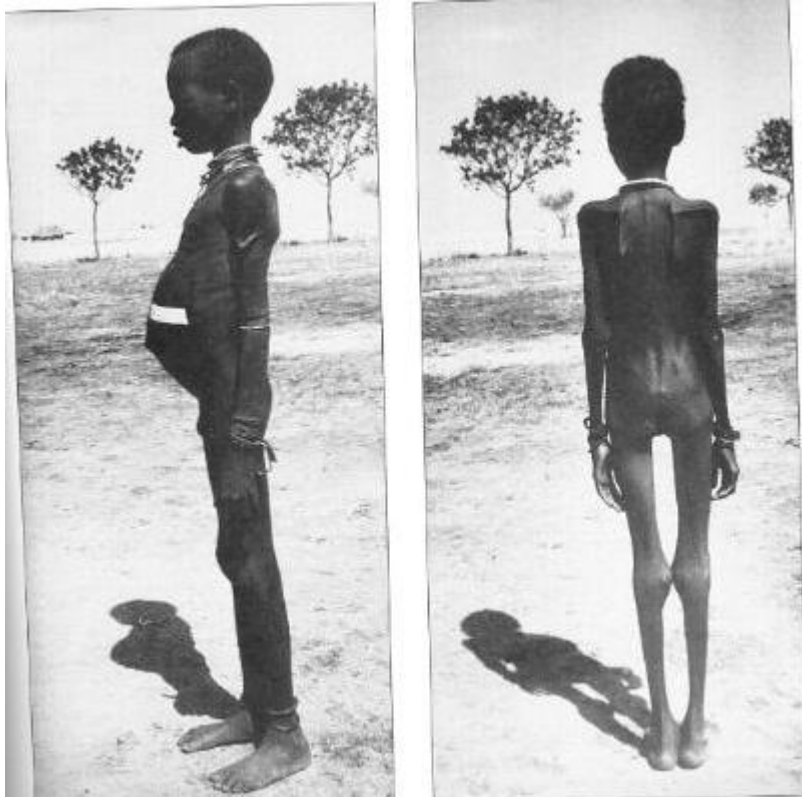


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## VISCERAL LEISHMANIASIS

*L. donovani* causes the classic type found in India. This is a metastatic disease. Parasites are only occasionally seen in blood, but are present in the spleen and lymph nodes. The incubation period is 1-4 months. Disease is characterized by fever, anemia, splenomegaly, wasting, imbalance of serum proteins (A/G ratio is reversed) and hyperpigmentation of the skin. The death rate is very high if left untreated. *L. infantum* causes the Mediterranean form of kala azar and has dogs, jackals and foxes as reservoirs. Humans are accidental hosts.



Kala-azar, Shandong, China ca 1950'

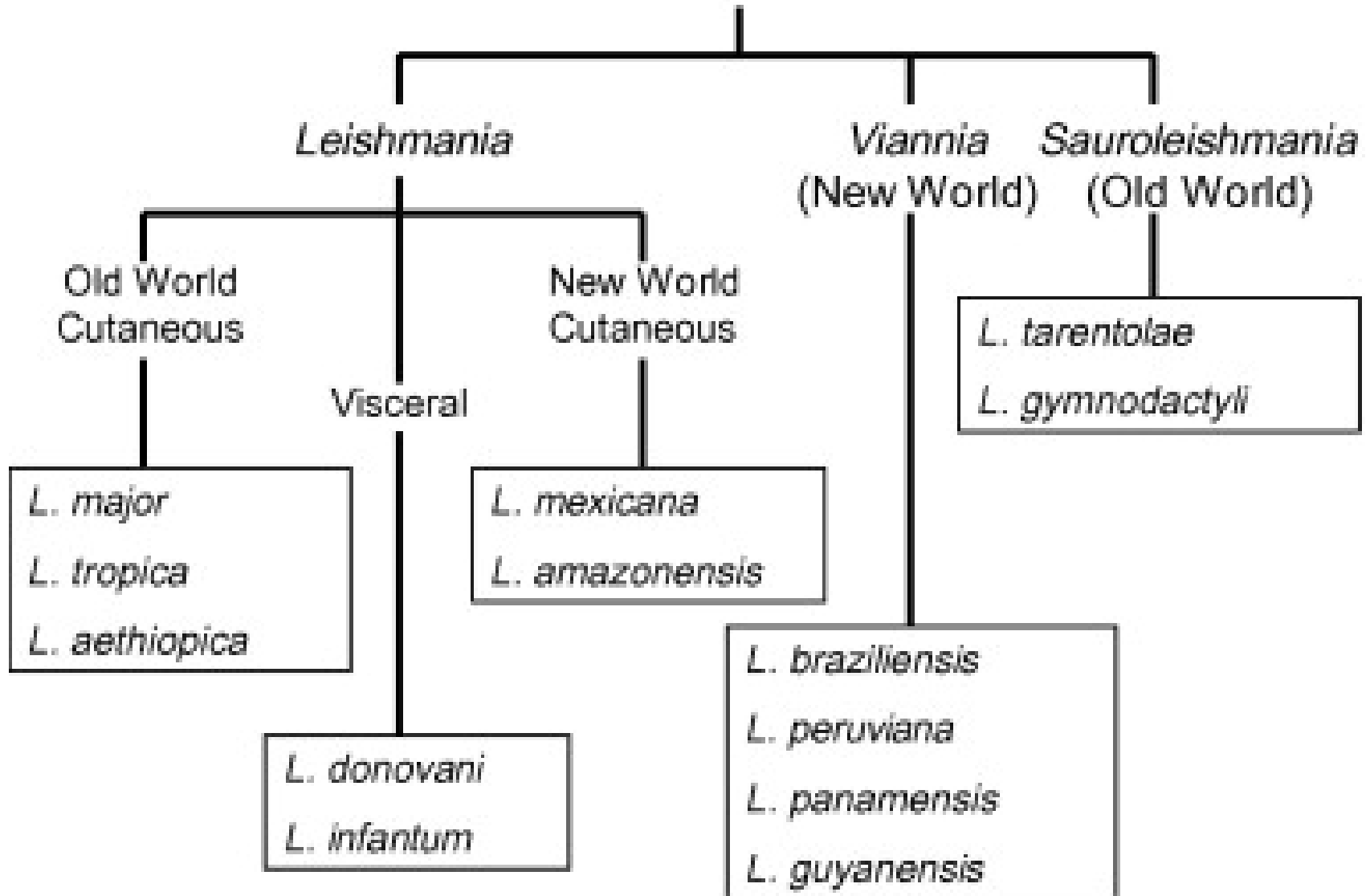


KP Chang

## Summary of the clinical aspects of Leishmaniasis

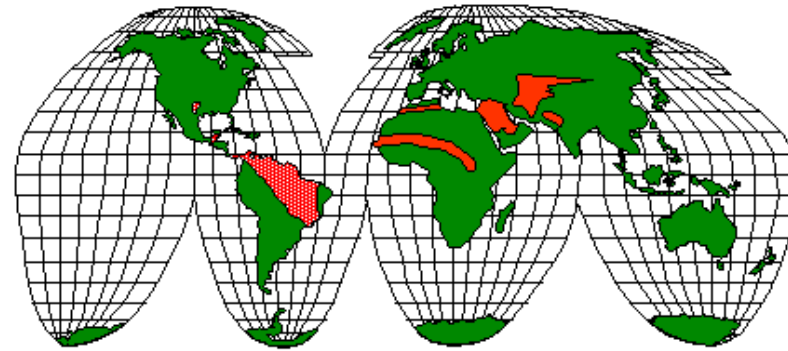
- **Localized cutaneous leishmaniasis (LCL):** Crusted papules or ulcers occur several weeks to months (and in rare cases) after sandfly bite inoculation on exposed skin. Lesions usually heal spontaneously.
- **Diffuse cutaneous leishmaniasis (DCL):** Analogous to lepromatous leprosy, individuals with DCL cannot mount a cell-mediated immune response to the *Leishmania* parasite. Consequently, patients develop multiple, widespread cutaneous papules and nodules, and they are anergic to leishmanin skin testing (LST).
- **Recidivans cutaneous leishmaniasis (RCL):** A relatively uncommon clinical variant of leishmaniasis, RCL appears as a recurrence of lesions at the site of apparently healed disease years after the original infection. RCL lesions typically occur on the face, and RCL presents as an enlarging papule, plaque, or coalescence of papules that heals with central scarring. Relentless expansion at the periphery may cause significant facial destruction similar to the lupus vulgaris variant of cutaneous tuberculosis.
- **post-kala azar dermal leishmaniasis (PKADL):** Endemic to India and the Sudan, this form of leishmaniasis develops months to years after the patient's recovery from VL. Cutaneous lesions demonstrate great variability, ranging from hypopigmented macules to erythematous papules and from nodules to plaques. As in leprosy, the wide clinical spectrum of PKADL reflects the immune response of the individual to the *Leishmania* organism. Lesions may be numerous and persist for decades. Isolated parasites from the lesions are identical to those causing the original visceral disease.
- **Mucocutaneous leishmaniasis (MCL):** Predominantly a New World disease, this form of leishmaniasis may not manifest clinically until years after localized cutaneous disease apparently has healed. In a poorly understood manner, certain species of *Leishmania* migrate to the upper respiratory tract where relentless destruction of the oropharynx and nose ensues. Gradually, the migration results in extensive mid facial destruction and, occasionally, in death.
- **Visceral leishmaniasis (VL) (kala azar):** *Leishmania* parasites localize to the reticuloendothelial system, rather than to the skin, and produce a potentially lethal widespread systemic disease.

# Genus Leishmania

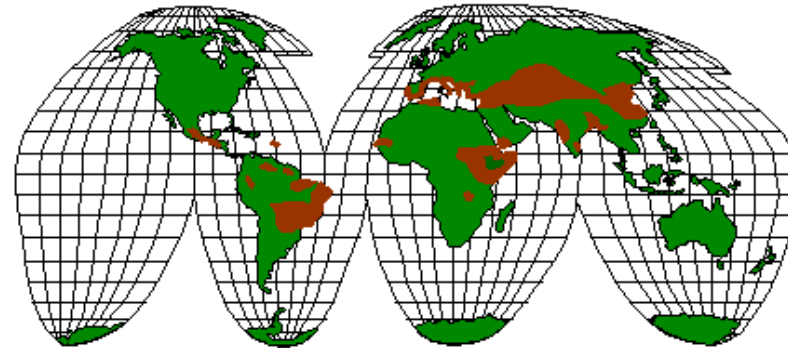





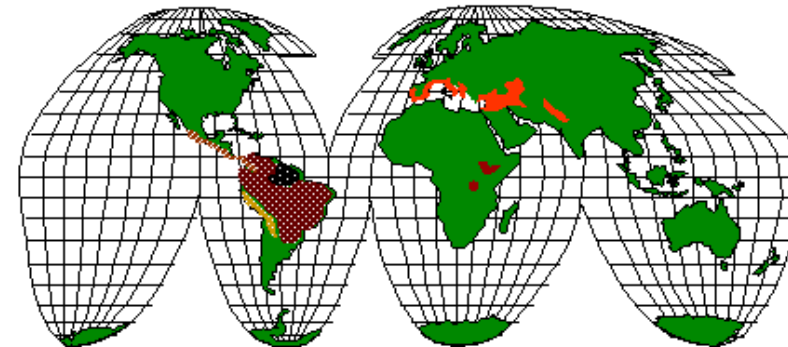
# Distribution of Leishmania species



 L.m.mexicana  L.m.major



 L. donovani and canine leishmaniasis

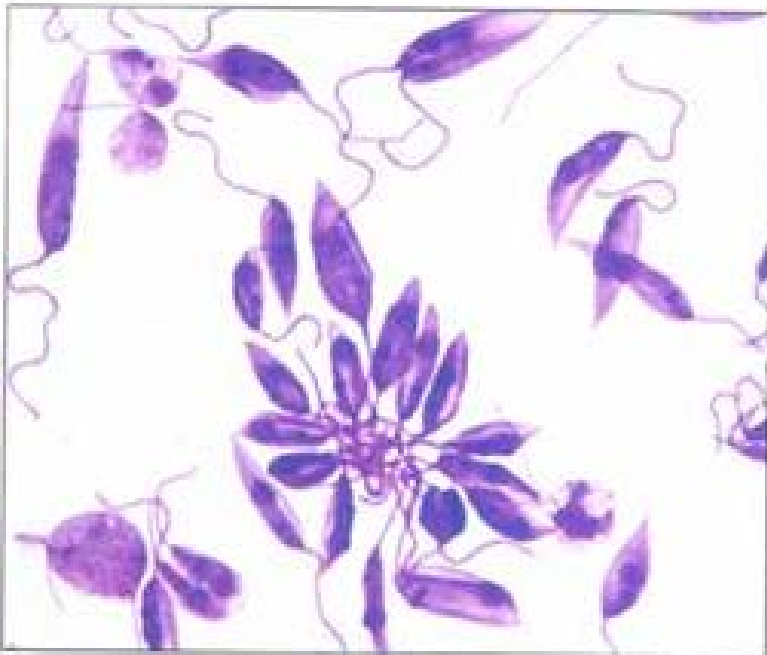


 L.b.braziliensis  L.aethiopica  
 L.b.panamensis  L.tropica  
 L.b.peruviana  L.b.guayanensis

# A “Cocktail Moment”

## Has Leishmaniasis Become Endemic in the U.S.?

Believed to be all but absent from the U.S., the *Leishmania* parasite has infected more than 1000 hunting dogs



Unwanted visitor. *Leishmania* parasites can kill.



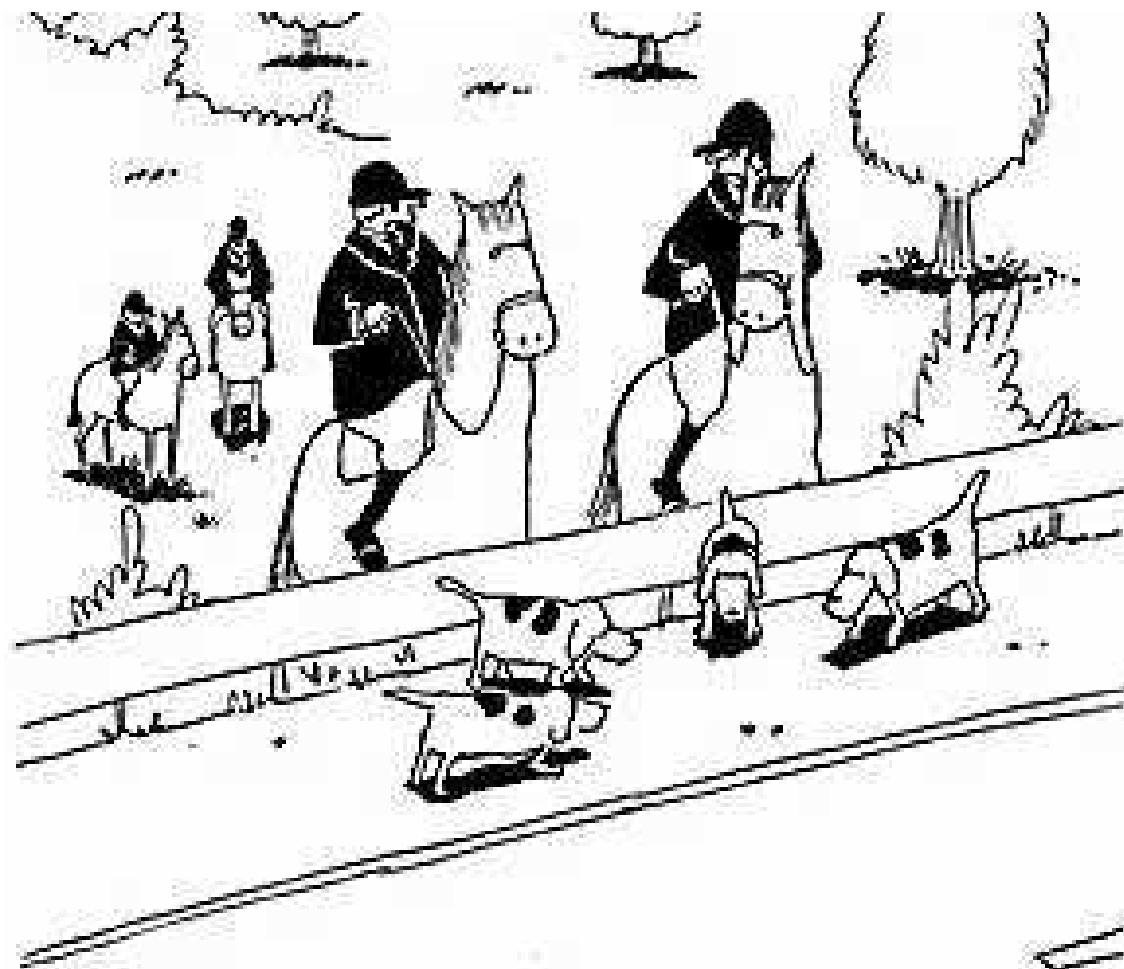
Fly bites dog? Normally, *Leishmania* is transmitted by sandflies, but it's not clear how U.S. foxhounds become infected.

**Foxhounds infected with**  
*L. infantum*



**Foxhounds infected with**  
*T. cruzi*





. NAF.

"So, let me get this straight, you're saying  
the fox had a car waiting for him?"

# Treatment

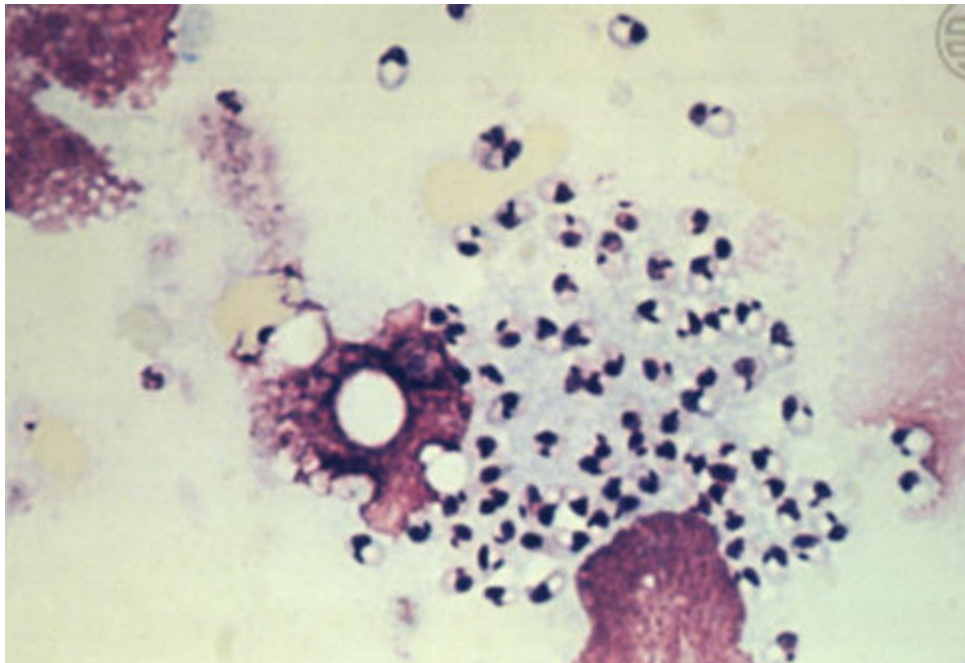
- Kala azar is treated today essentially as it was in 1940. The major drug is Sodium stibogluconate or **Pentastam**, a derivative of antimony, which was developed in 1930! Severe reactions including death occur in 10% of those treated. It is very expensive, and the recommended one month treatment costs around \$150. Drug resistance has also developed. Up to 70% of infected patients in India are resistant to this drug.
- **Pentamidine isothionate** has been used in antimony-resistant visceral leishmaniasis, but although the initial response is often good, the relapse rate is high and it is associated with serious side-effects.
- Pentamidine is also used for New World cutaneous leishmaniasis, but it usually heals spontaneously.
- **Amphotericin** is used with or after an antimony compound for visceral leishmaniasis unresponsive to the antimonial alone.

## New Drugs

- **Liposomal Amphotericin B** - single dose cures visceral leishmaniasis!
- **Paramomycin**. A 21-day course of paramomycin produces a definitive cure in >90% of patients with visceral leishmaniasis.
- **Miltefosine**. This is a membrane signaling pathway inhibitor. This can be taken orally and is very effective against visceral and dermal leishmaniasis. In clinical trials has a 95% cure rate!
- There is no treatment for muco-cutaneous leishmaniasis.

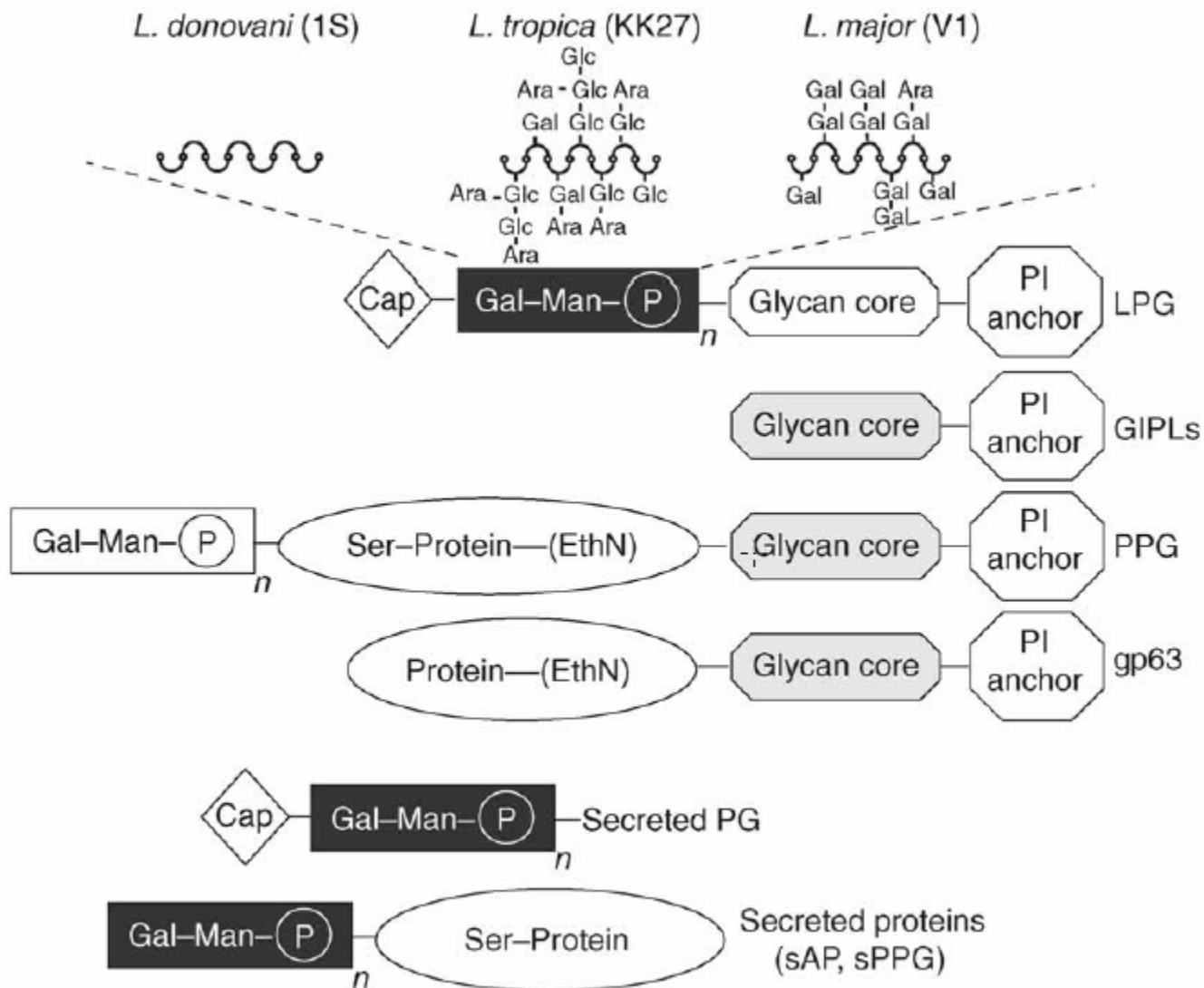
## Diagnosis of Cutaneous Leishmaniasis

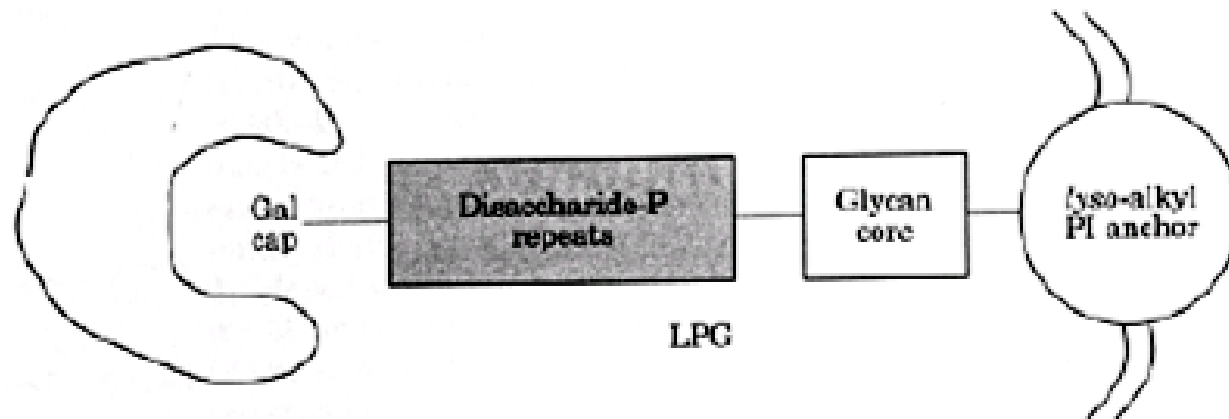
Microscopic detection of amastigotes (LD bodies) in stained smears made from the edges of the ulcer. Culturing parasites from the lesion can also be used in diagnosis.



# The Parasite Surface

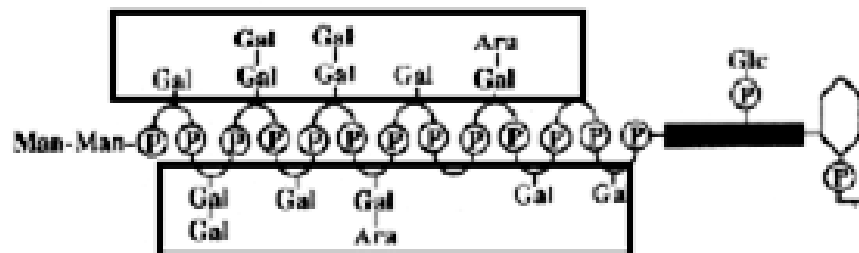
The surface of *Leishmania* promastigotes is covered by glycoproteins.



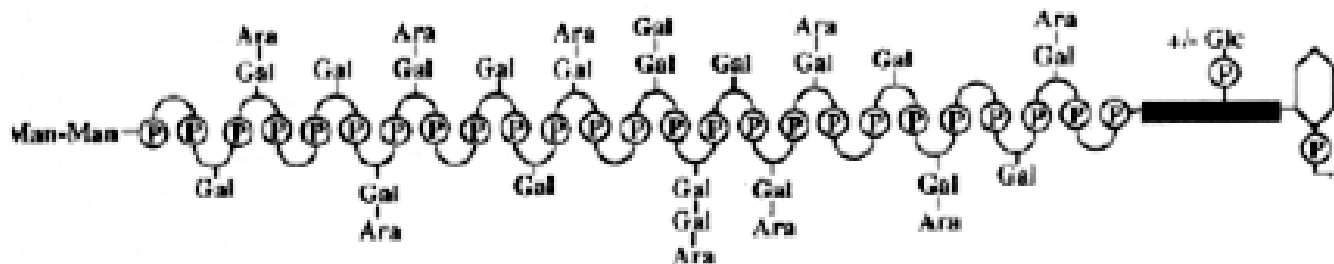


**Metacyclic promastigotes do not bind to the gut receptor due to shielding of galactose residues with arabinose residues.**

Procyclic Promastigote  
LPG (fly midgut)



Metacyclic  
Promastigote  
LPG (fly  
midgut)



= phosphorylated disaccharide backbone,  $PO_4-6Gal\beta1-4Man$



= Hexosaccharide core,  $Gal\alpha1-6Gal\alpha1-3Gal\beta1-3Man\alpha1-3Man\alpha1-4GlcN$



# Nectomonads bind to sand fly galactin receptor - PpGalec

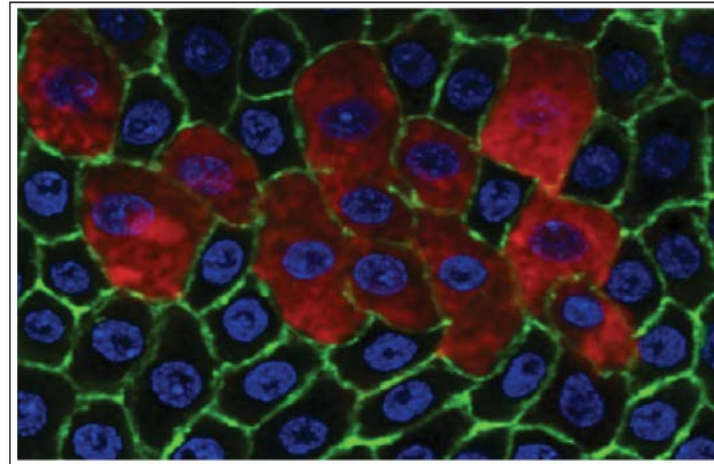
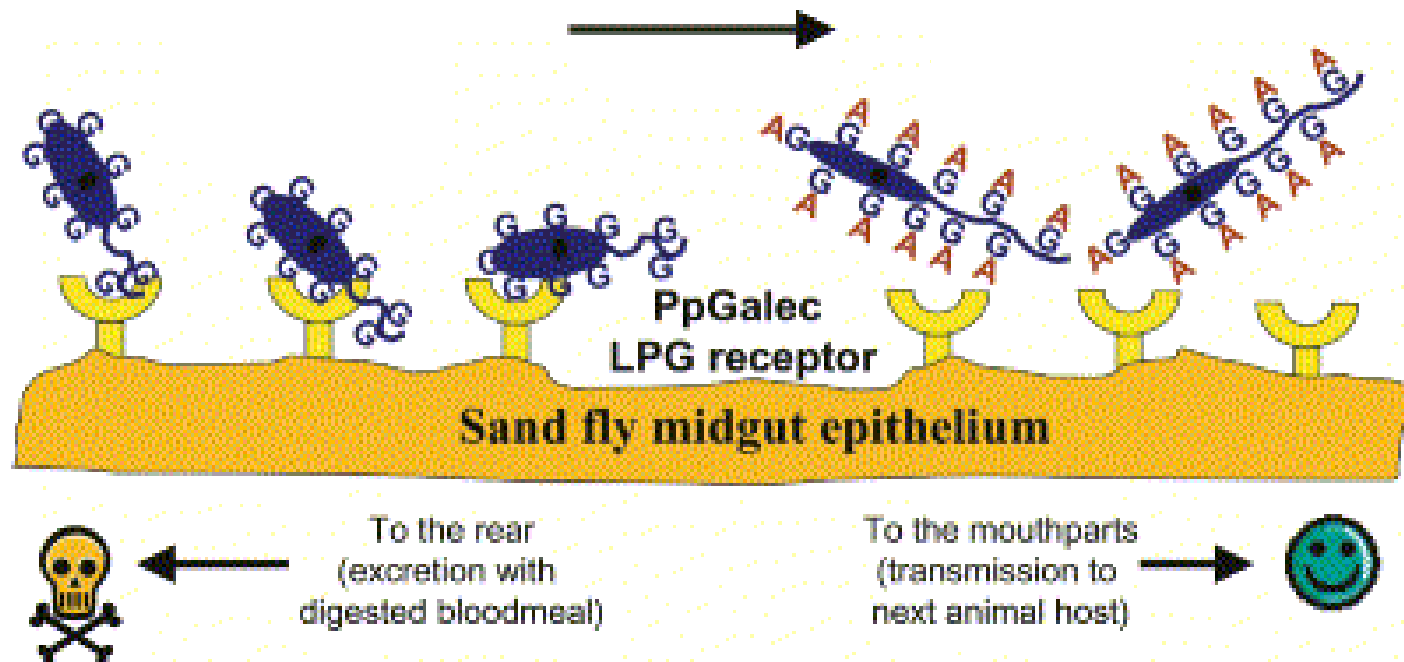
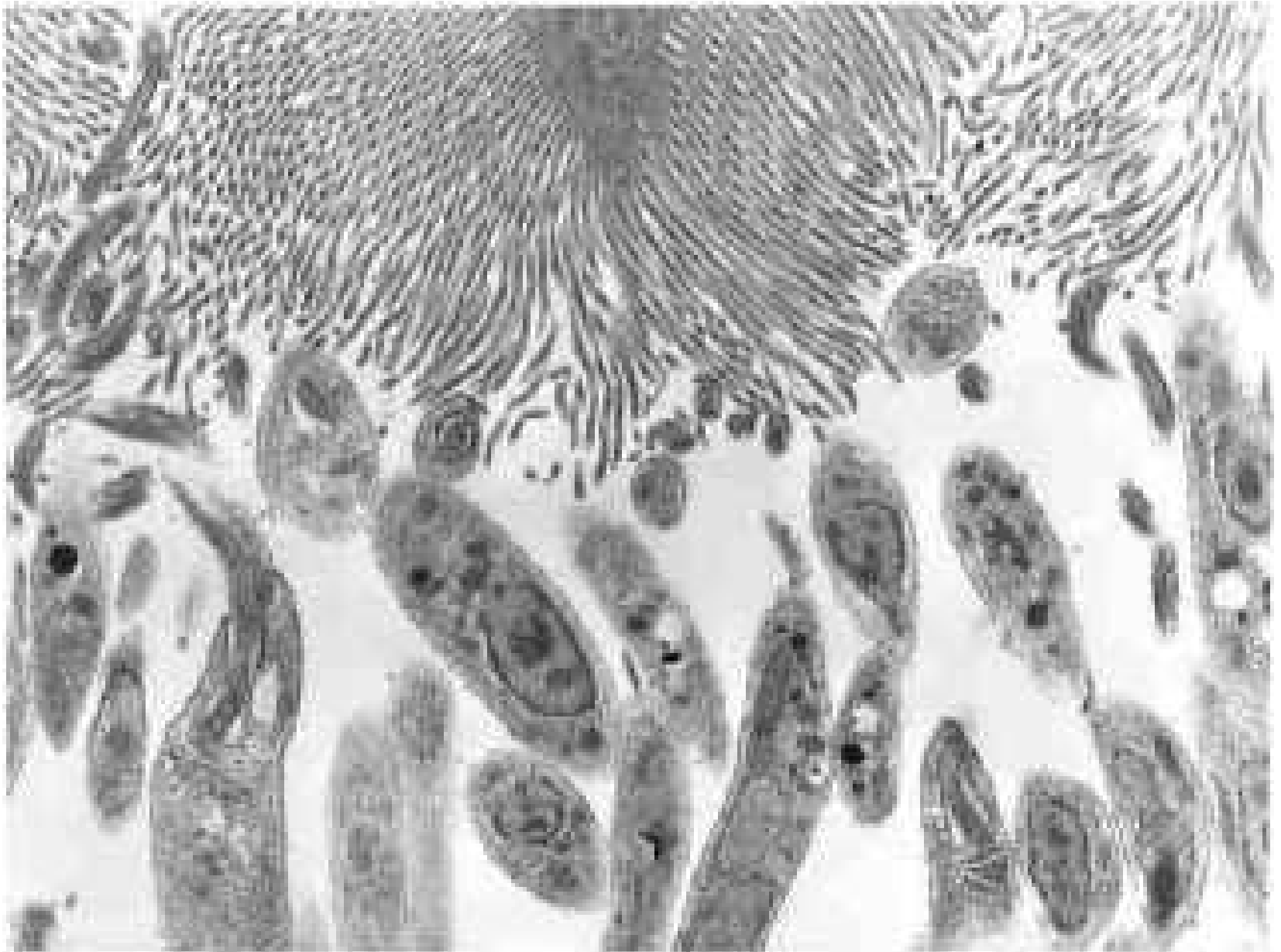


Figure 2. A cluster of midgut epithelial cells of *Phlebotomus papatasi* showing a strong expression of PpGalec (red). Actin and nuclei are stained green and blue, respectively.



## Nectomonads attached to microvillae in fly midgut

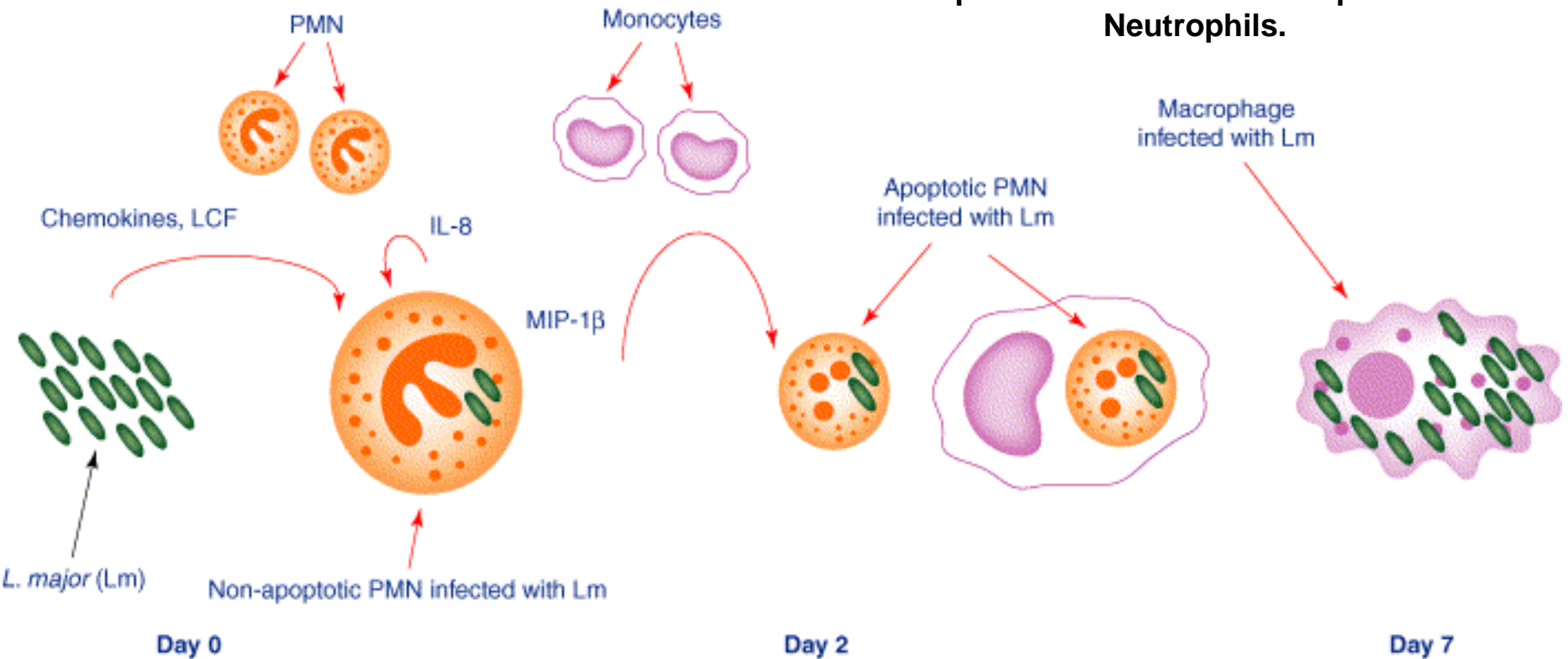


# Trojan Horse Hypothesis

Metacyclic promastigotes taken up first by Neutrophils (PMN's)

A method for silent delivery of parasites to macrophages

LPG not required for targeting, but is required for maintenance of parasites in Neutrophils.

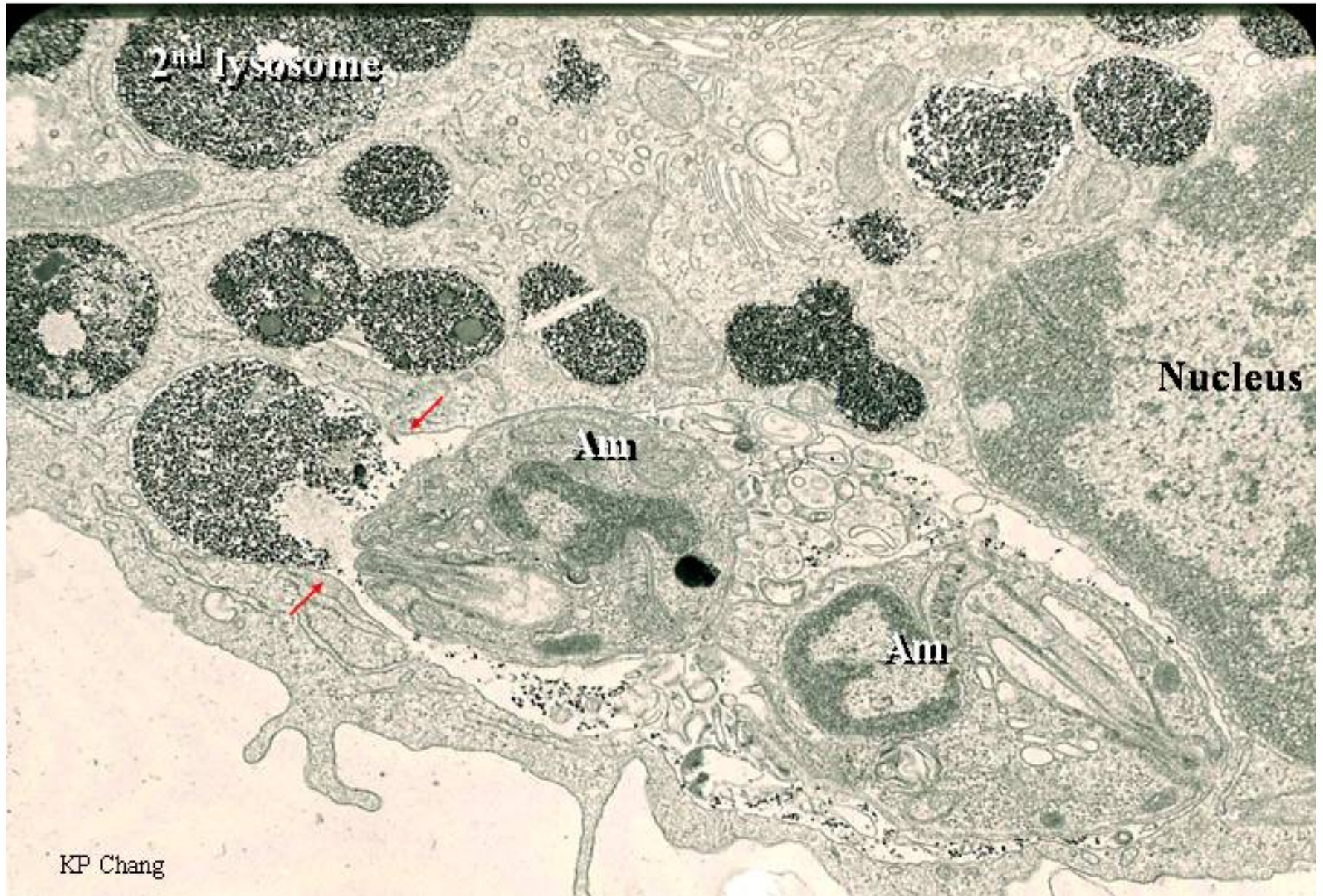


# Cell Biology of Macrophages

Promastigotes are taken up by phagocytosis of apoptotic neutrophils and the parasites differentiate into amastigotes inside the phagosome. This is a very hostile environment where cells taken up by the macrophage are normally destroyed by several mechanisms:

- **Oxidative burst** - Phagocytosis of a foreign body activates an NAD(P)H oxidase in the plasma membrane. This enzyme transfers protons to molecular oxygen, forming the highly reactive superoxide and hydroxyl radicals at the site of engulfment. These radicals react with the pathogen's phospholipid membrane and also with its macromolecules
- **Acidification** - After fusion of the phagosome with the endosome, the vesicle is acidified by a proton ATPase. The low pH (4.5-5.0) causes denaturation of proteins, which become susceptible to acid hydrolases.
- **Digestion** - The endosome fuses with primary lysosomes, and acid hydrolases are released which degrade DNA, RNA, proteins and carbohydrates.

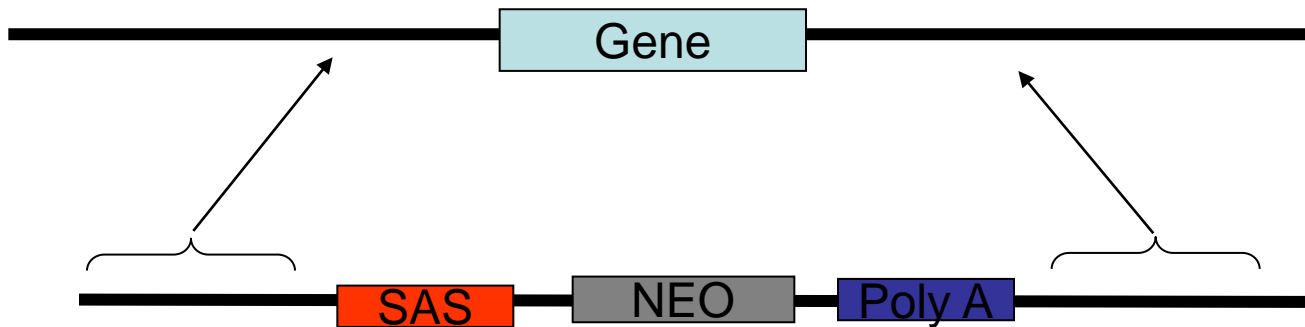
# Phagosome-lysosome fusion in *L. donovani* infection of macrophage



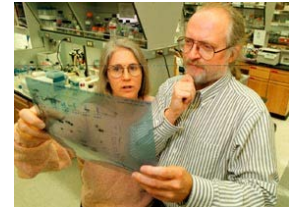
# Transformation of Leishmania

Plasmids can replicate as episome  
or  
Plasmids can recombine with genomic DNA

For expression of integrated resistance gene  
Only need an upstream Splice Acceptor Sequence (SAS)  
and a downstream Polyadenylation signal



Selection of transformants is performed by plating on agar and selecting for drug resistance.

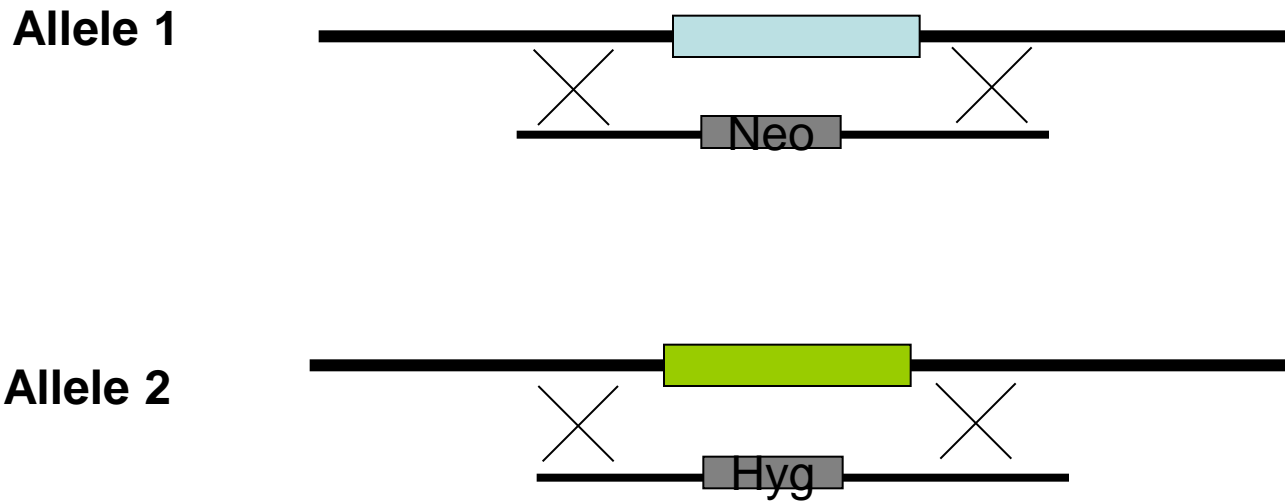


Steve Beverley



Electroporation

# Double gene knockout by homologous recombination



**Drug resistance markers available for *Leishmania*:**

- Neomycin (Neo) R**
- Hygromycin (Hygro) R**
- Phleomycin (Ble) R**
- Puromycin R**
- Blasticidin R**