1.8 Basic Classification of Medically Important Parasites

I. Protozoa

1. *Amoebae*

- ***Non- free living pathogenic amoebae.***

- *Entamoeba histolytica*
  - ***Free living Pathogenic amoeba***
- Acanthamoeba species
- *Naegleria fowleri*
- *Balamuthia mandrillaries*
- *Sappinia diploidea*

- ***Non – free living non- pathogenic amoebae***

- *Entamoba hartimani*
- *Entamoeba gingivalis*
- *Entamoba coli*
- *Entamoba dispar*

- *Iodamoeba butschili*
- *Endolimax nana*

Cont…

2. <u>Flagellates</u>

A). Intestinal flagellates

- ✓ *Gardia lamblia*
- ✓ *Chilomastics mesenili*
- ✓ *Trichomonas huminis*
- ✓ *Enteromonas huminis*
- ✓ *Diantamoeba fragilis*
- ✓ *Retortamonas intestinalis*

B). Oral flagellates

- *Trichomonas tenax*

- ***C) Genital flagellates***

  - *Trichomonas vaginalis*

D). Blood and tissue Flagellates

- Trypanosoma species
- Leishmania species

Cont…

3. Ciliates

- *Balantiduim coli*

4. Coccidia

A) Blood and tissue coccidian

- ❖ Plasmodium species
- ❖ *Toxoplasma gondii*

B) intestinal coccidian

* *Isopora belli*
* *cryptosporuin parvum*
* *Cyclopora cayetanensis*

II. Helminths

- Multicellular worms
- Do not normally multiply in human host

1. Nematodes (Round worms)

A. Intestinal nematodes

- *Ascaris lumbricoides* (large roundworm)
- *Enterobuis Vermicularis* (thread worm or pinworm)
- *Trichurs trichiuria* (whipworm)
- *Strongloides stercoralis*
- *Ancylostoma duodenale* (hook worm)
- *Necator americanus* (hook worm)

Cont…

B. Filarial and other tissue nematodes

- *Wuchereria bancrafti*
- *Loa loa*
- *Brugia timori*
- *Onchocerca volvulus*
- *Brugia malayi*
- *Drancunculus medinensis*
- *Trichinella spiralis*
- *Mansonella species*

2. Cestodes (Tapeworms)

- *Taenia saginata* (Beef tape worm)
- Taenia solisn (pork tape worm)
- Hymenolepis nana (Dwarf tape worm)
- Hymenolepis diminuta (Rat tape worm)
- Dipyliduin coninum (dog tape worm)
- Echinococcus granulosus(fox tape worm)
- Echinococcus multilocularis(fox tape worm)
- Diphyllobothruin latum (firsh tape worm)

Cont…

3. Trematodes (flukes)

i. Liver flukes:

- Fasciola hepatic
- Opisthorchis Viverrini
- Fasciola giagantica
- Clonorchis sinensis

ii. Intestinal flukes

- Fasciolapsis buski
- Heterophyes hetrophyes
- Metagonimus yokogauli

iii. Lung flukes.

- Paragonimus westermani
- Paragonimus heterotremus

iv. Blood flukes

- *Schistosoma haematobuim*
- *Schistosoma mansoni*
- *Schistosoma japonicum*
- *Schistosoma mekongi*
- *Schistosoma intercalatum*

1.9 Types of specimen used for parasitological examinations

- **Stool**: e.g. intestinal nematodes, cestodes, trematodes and protozoa
- **Blood**: e.g. Haemoparasites
- **Urine**: e.g. *S. hematobium, T. vaginalis & W. bancrofiti*
- **Sputum**: e.g. *P. westermani*
- **Skin fliud**:- e.g. *L. aethopica, & N. fowleri*
- **Skin snip**:- e.g. *O. volvuls*
- **Cerebro-spinal fluid:-** *Trypanosoma bruci rhodisense* and *N. fowleri*
- **Bone marrow**:- e.g. *L. donovani & T. gondii*
- **Lymphdland aspirates**:- e.g. *Trypanosoma bruci rhodisense, L. donovani & T. gondi*
- **Liver aspirate**:- e.g. *E. histolytica, L. donovani & T. gondii*
- **Speel aspirate**:- e.g. *L. donovani & T. gondii*
- **Muscle biopsy**:- e.g. *T. spiralis*
- **Rectal scraping**:- e.g. Schistosoma species
- **Duodenal aspirate**:- e.g. *G. lamblia, F. hepatica* and *S. stercoralis*
- **Bronchial biopsy:-** e.g. *P. carnii*
- **Perianal swab:-** e.g. *E. vermiclaris*
- **Oral swab:-** *E. gingivalis & T. tenax*
- **Genital discharge:**- e.g. *T. vaginalis*
- **Water:** e.g. *D. medinensis*
- **Eye swab:**- e.g.

CHAPTER TWO

Systemic parasitology -Medically Important Protozoa

Lecture outcomes

- Identify the general characteristics of Protozoans
- Describe the general morphology and cellular organization of parasitic protozoa
- Describe the locomotory and reproduction system of protozoa
- Explain the Feeding, Metabolism, and Excretion in parasitic protozoa.
- Describe the classification of parasitic protozoa
- Describe the general characteristics of the five main groups and any sub-groups of the parasitic protozoa

OUTLINE

PROTOZOA

- General Introduction to protozoa
  - Definition
  - Diversity and Medical importance of protozoa
- General morphology protozoa
  - Size, Shape etc
- Cellular structure and organization
- Basic properties
  - Locomotion
  - Reproduction
  - Feeding, Metabolism, Excretion and Encystment
- Classification
- General characteristics of the five main groups

2.1 What are Protozoa?

- Proto = first , zoa = animals
- **single-celled eukaryotic organisms**
- Vary in size (1-2000 mm)

= Unknown until the invention of the microscope in 1675

= First recognized by antony van leewenhoek in 1676

= He describe it as little animal or animacula

Antonio van Leeuwenhoek
the father of protozoology?







Cont…

- **Protozoan diversity**

= protozoa are extremely diverse organisms and found in a variety of niches

= >200,000 named species

= Most species are free-living

= Some are beneficial to mankind by:

  = being part of the food chain

  = serving as experimental subjects

  = biological sewage treatment

= Few are adapted to a parasitic life

= all plant and animal species have at least one protozoan parasite

= **~10,000 are parasites** in a wide range of hosts

  = Vertebrate

  = invertebrate

  = Plants

= **~20 human pathogens**

= Adapted to life in a wide range of sites within the host

Ecological Niches in the Human Body:

- **Skin:** Leishmania
- **Eye:** Acanthamoeba
- **Mouth:** Amoebae and flagellates (usually non-pathogenic)
- **Gut:** Giardia, Entamoeba (and invasion to liver), Cryptosporidium, Isospora, Balantidium
- **G.U. tract:** Trichomonas
- **Bloodstream:** Plasmodium, Trypanosoma
- **Spleen:** Leishmania
- **Liver:** Leishmania, Entamoeba
- **Muscle:** Trypanosoma cruzi
- **CNS:** Trypanosoma, Naegleria, Toxoplasma, Plasmodium

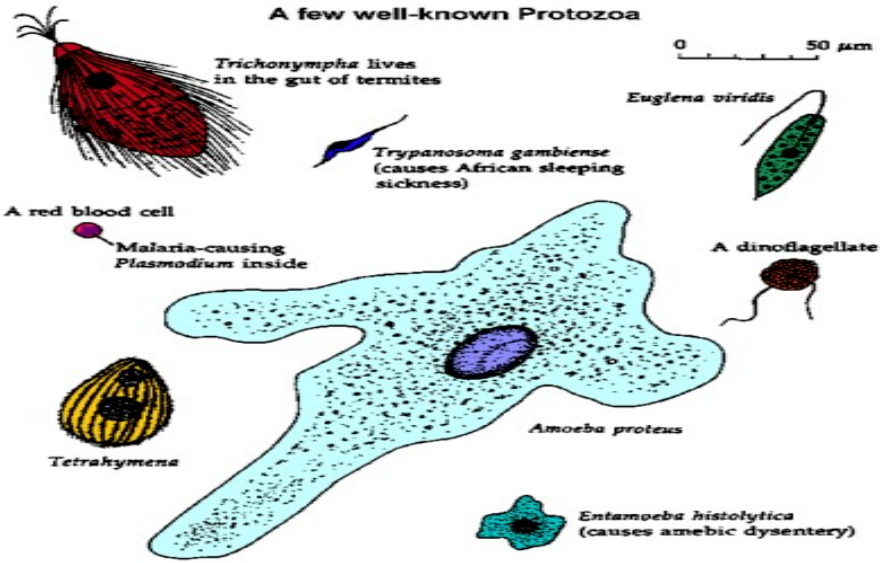
Importance of protozoa

- Medical importance
  - Cause of more sickness and death, than any other disease-causing organisms
  - reduced working capacity
  - Loss of productivity
- Veterinary importance
  - Loss of productivity
  - Death
  - reduced meat and milk production,
  - reduced reproductive potential
  - reduced working capacity
  - Zoonoses

General morphology

- Protozoa exhibit a wide variety of morphologies
- **Size: range from 1 to 150um/ 200um**
- The smaller members, 1-10um (ex: *Leishmania,Toxoplasma)*
- The largest member is the ciliate (*Balantidium coli)*

- Shape:
  - ✓ No single shape that represent all
  - ✓ Ranges from amorphous shapeless amoeba to relatively rigid forms



Structure

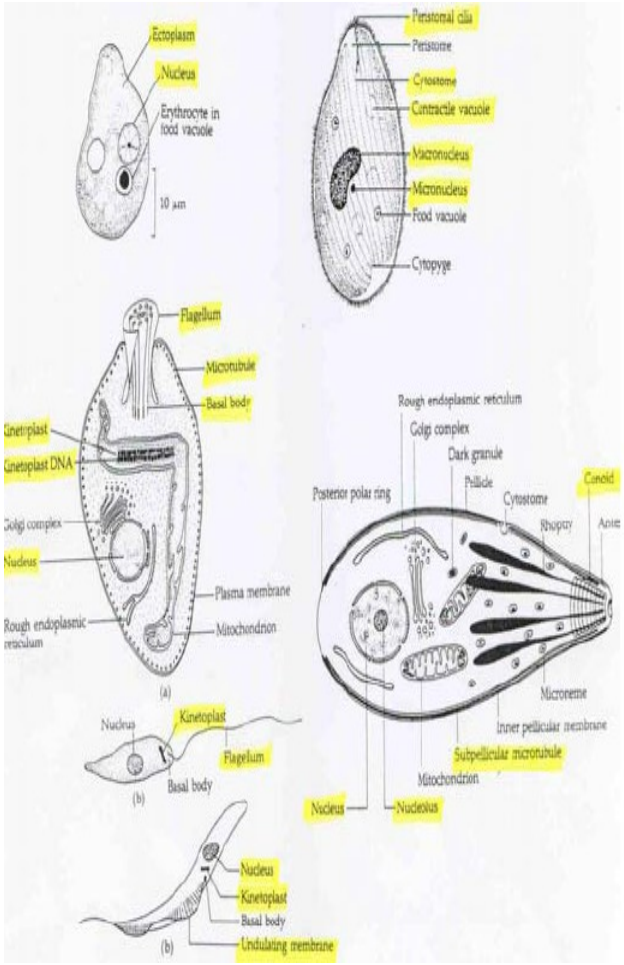
- Protozoa have relatively complex and specialized internal structure which perform:
  - The functions of locomotion, metabolism, and reproduction.
  - The same physiological functions performed by many cells in a more complex organism

Basic structure

1. Cell membrane
2. Cytoplasm
   - ectoplasm
   - endoplasm
3. Nucleus: two kinds of nuclei:
   - vesicular;
   - compact

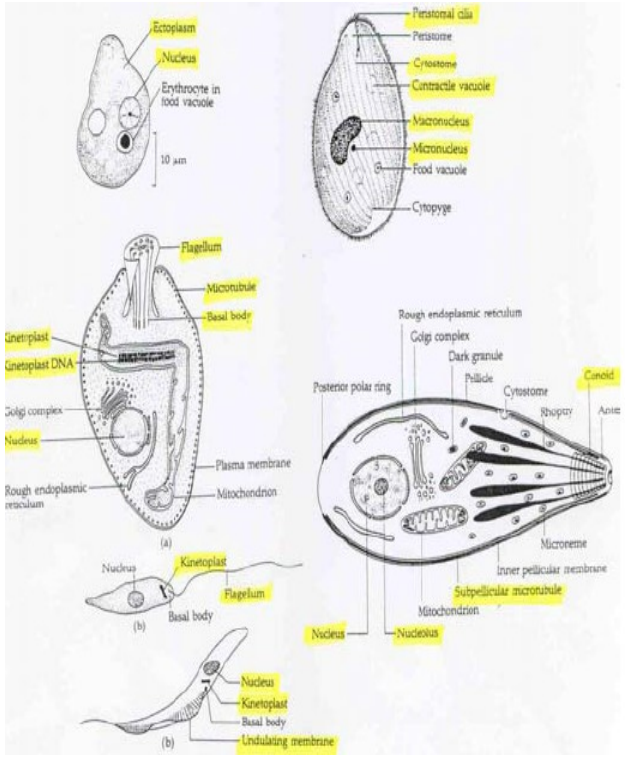
Cell membrane

- Thin membrane called plasmalemma
- Also called pellicle (ciliates) or ectoderm or outer membrane
- Microtubules line outer membrane (subpellicular microtubules



Cytoplasm

- Ectoplasm=outer transparent layer
  - Locomotion,
  - protection
  - Sensation,
  - Excretion Ingestion of food etc
- Endoplasm= inner granular layer
  - Nutrition
  - Reproduction etc



Nucleus

- Typically eukaryotic
- Most easily identifiable structure in protozoan cell
  - Single/multiple (similar in size/function or not)
    - Eg, G lambilia, cilliate
  - Contains karyosome & chromatin granules

• Nucleus

- Two morphologically distinct nuclei:
  - Vesicular
    - with a clearly defined internal space
    - scattered chromatin,
    - Karyosome
      - nucleoli-like body
      - contains DNA in apicomplexans
      - but lacks DNA in parasitic amoebas
    - Most protozoa of humans exhibit the vesicular type.
  - Compact
    - appears to be a solid mass
    - densely packed chromatin

Other structure

- Axostyle:
  - Supporting structure embedded along the longitudinal axis on the cytoplasm (some flagellates).

- Costa:
  - Rodlike structure that runs along base of undulating membrane.

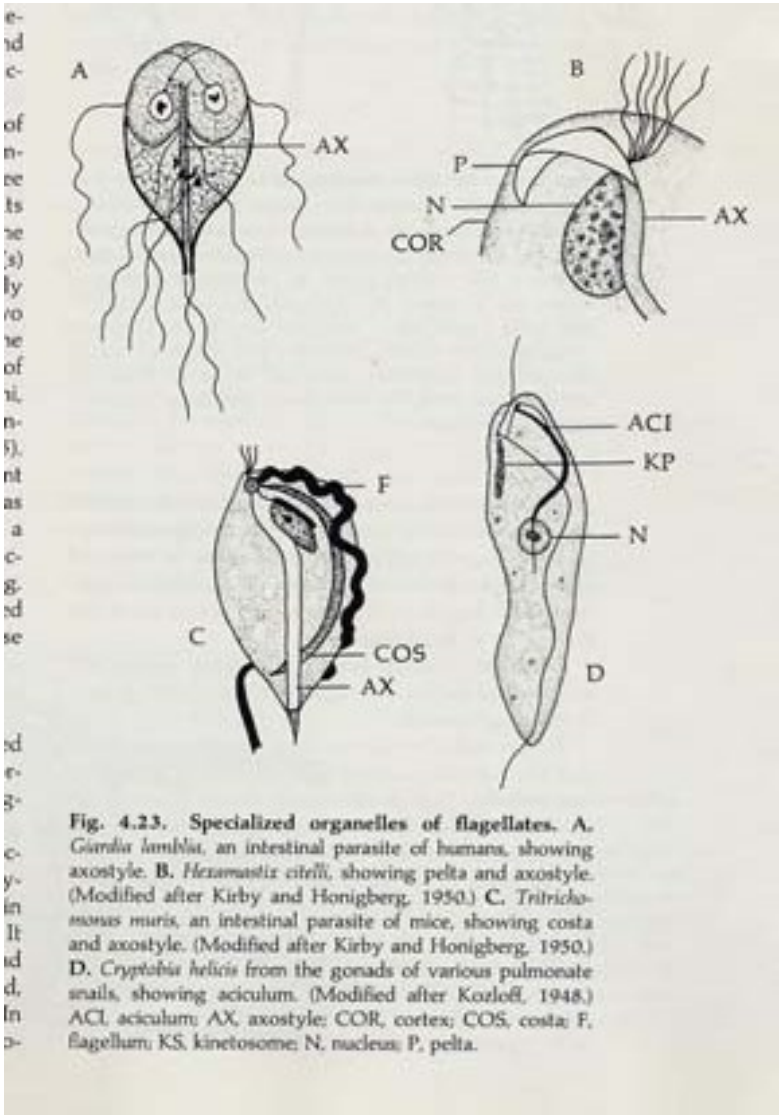


Fig. 4.23. Specialized organelles of flagellates. A. *Giardia lamblia*, an intestinal parasite of humans, showing axostyle. **B.** *Hexamastix citrili*, showing pelta and axostyle. (Modified after Kirby and Honigberg, 1950.) **C.** *Trichomonas muris*, an intestinal parasite of mice, showing costa and axostyle. (Modified after Kirby and Honigberg, 1950.) **D.** *Cryptobia helicis* from the gonads of various pulmonate snails, showing aciculum. (Modified after Kozloff, 1948.) ACI, aciculum; AX, axostyle; COR, cortex; COS, costa; F, flagellum; KS, kinetosome; N, nucleus; P, pelta.

Other structure

- Pelta:
  - crescent shaped membrane in certain flagellates (*Trichomonas)*
- Cytostome or cell mouth for
  - ingesting fluids (flagella pocke
  - may serve same function)
- Contractile vacuoles,
  - Presumably for osmoregulatio
  - (Often seen in sarcodinas and ciliates)

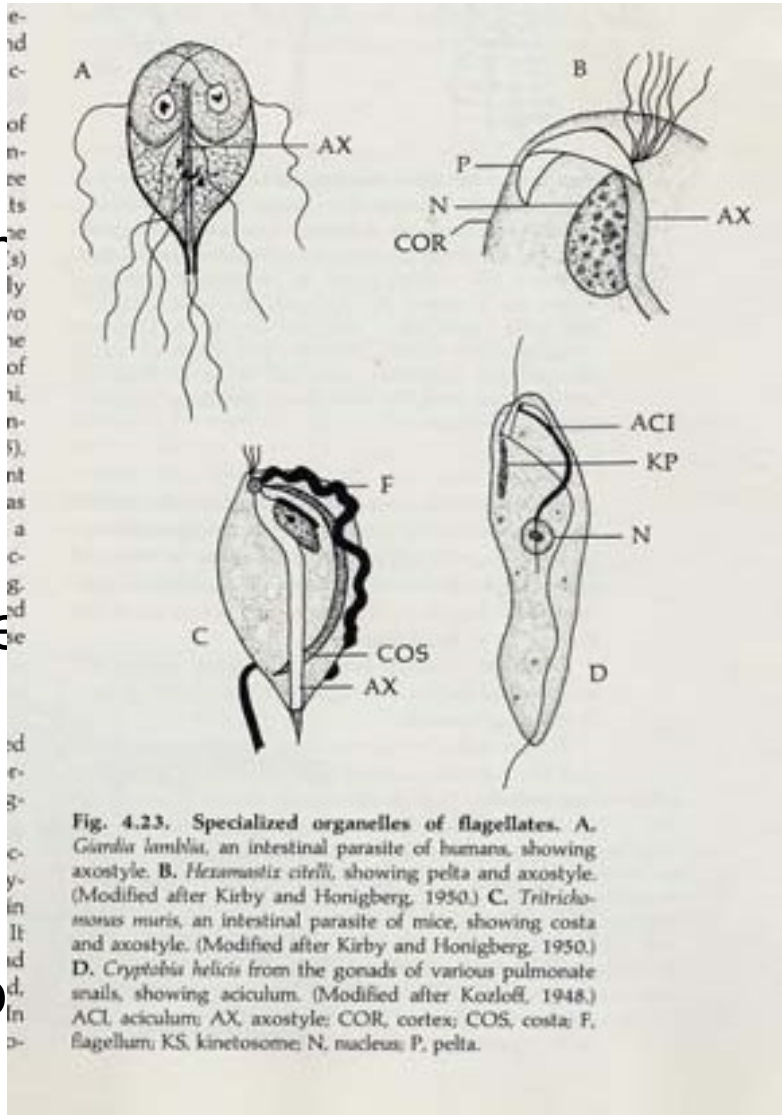
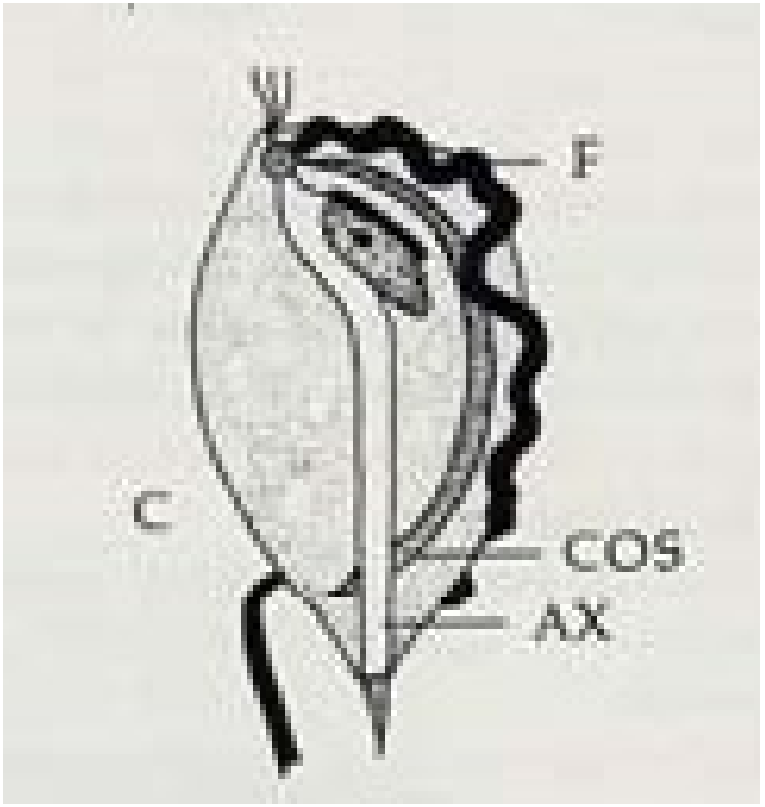


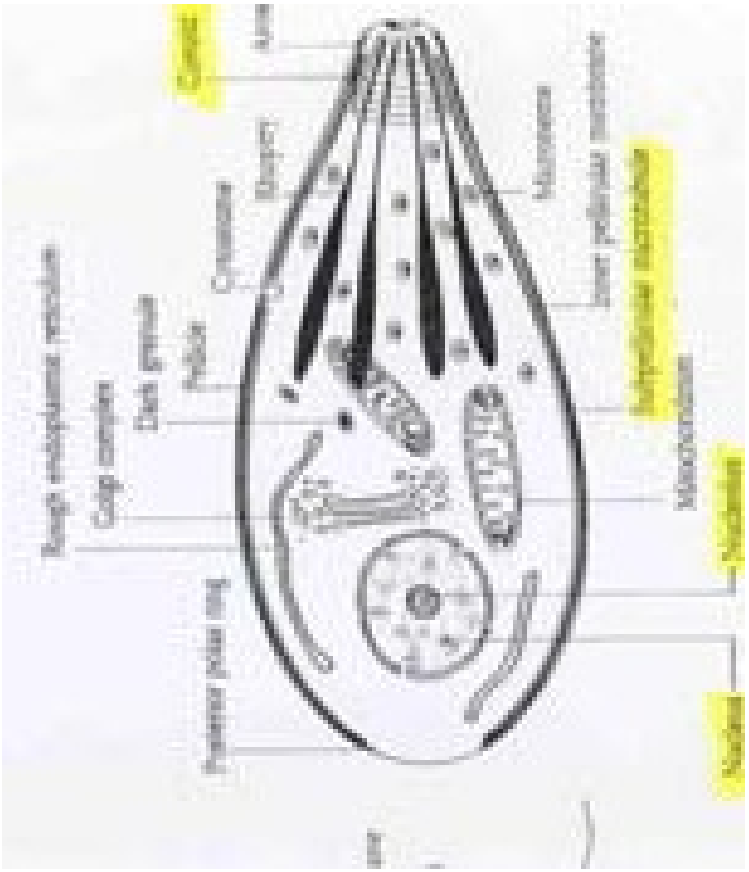
Fig. 4.23. Specialized organelles of flagellates. A. *Giardia lamblia*, an intestinal parasite of humans, showing axostyle. **B.** *Hexamastix citrilli*, showing pelta and axostyle. (Modified after Kirby and Honigberg, 1950.) **C.** *Trichomonas muris*, an intestinal parasite of mice, showing costa and axostyle. (Modified after Kirby and Honigberg, 1950.) **D.** *Cryptobia helicis* from the gonads of various pulmonate snails, showing aciculum. (Modified after Kozloff, 1948.) ACI, aciculum; AX, axostyle; COR, cortex; COS, costa; F, flagellum; KS, kinetosome; N, nucleus; P, pelta.

- Other organelles

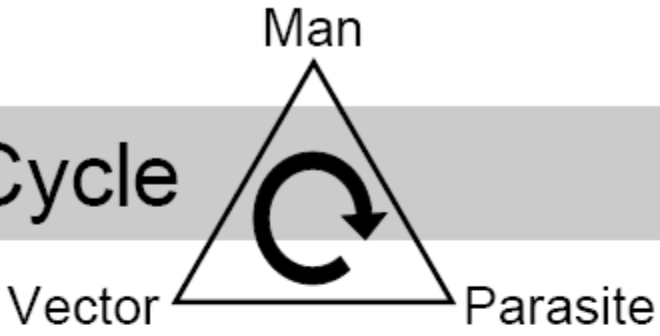
Undulating membrane

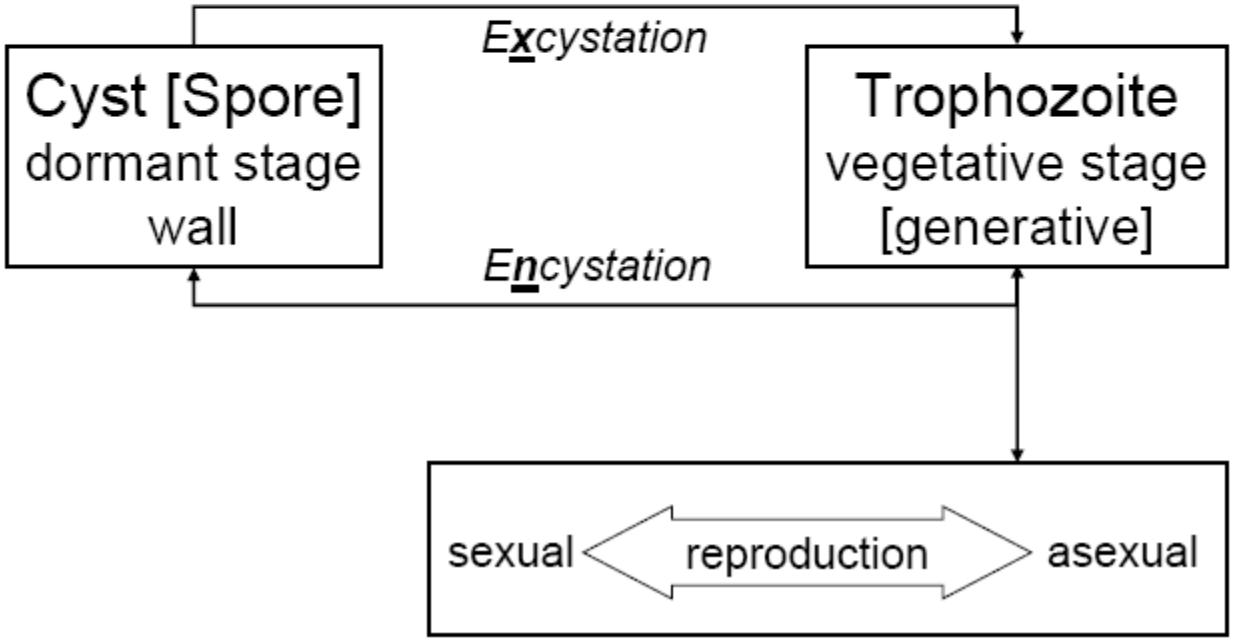


Apical complex



Protozoan Life Cycle





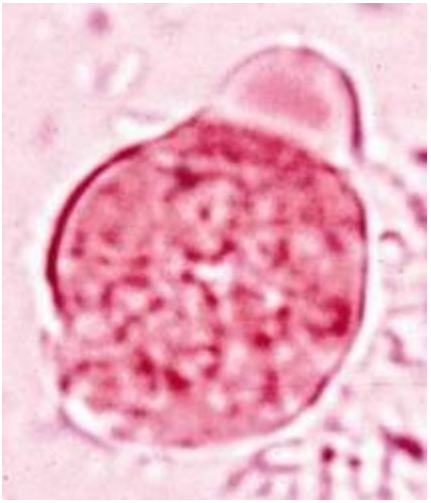
Basic properties

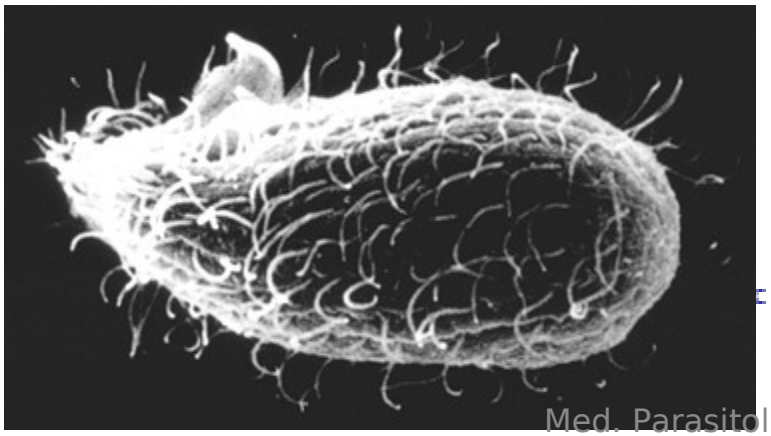


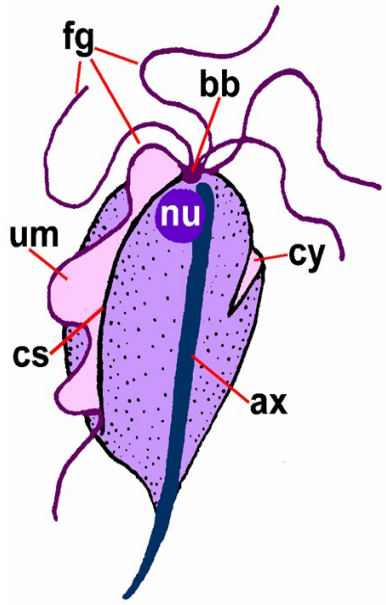
Structures that mediate locomotion or Movement

- Pseudopodia
- Cilia
- Flagella
- Gliding



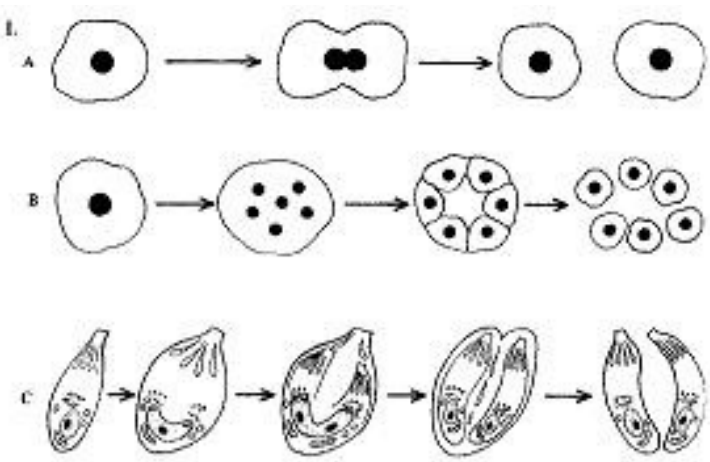






General Protozoan Reproduction

Asexual Reproductions

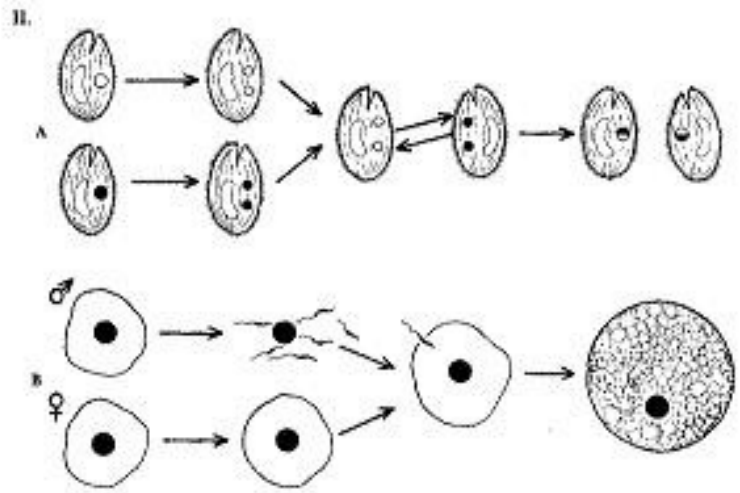


Binary Fisión

Múltiple Fisión

Endodiogenia

Sexual Reproductions



Conjugation

Singamy

Feeding mechanisms

- Most parasitic protozoa are heterotrophic Procuring nutrients mostly bacteria
  - **ingest particulates (phagotrophy)**
  - Phagocytosis
    - Engulfment of solid material
    - **predation on bacteria or other protozoa**
  - Pinocytosis
    - Invagination of membrane surrounding liquids

Feeding mechanisms

- Peristome or cytostomal feeding
  - Food is ingested at a definite site, using a specialized feeding structure
- Diffusion =**absorb solutes (osmotrophy)** **t**hrough

  cell membrane
  - Food may be retained in special intracellular reserves, or vacuoles.
- Some protozoan are photosynthetic (autotrophy) or combination

Excretion mechanisms

- Undigested particles and wastes are extruded at the cell surface by mechanisms that are the reverse of those used in ingestion
  - diffusion (primary mechanism)
  - contractile vacuole (osmo-regulation?)

Respiration/Metabolism

- Both aerobic (Malaria) and anaerobic respiration (dysenteric amoeba)
- Most parasitic protozoa are facultative anaerobes
- They can live in reduced oxygen environments (facultative or aerotolerant anaerobes; e.g. Amoeba).
- in few parasitic spp studied, metabolic paths similar to all eukaryotes (bad for drug development

Protozoa life cycle stages/terms

Most protozoa exist as **Vagetative/Resístanse forms**:

1. **Vagetative forms**: trophzotes, zoitos
   - stage of active feeding and reproduction, &
   - commonly the pathogenic protozoan form.
   - Formed by asexual fission (mitosis), or
   - also by gamete fusion in the Sporozoans.

Protozoa life...

2. **Resístanse forms**: cyst/oocys

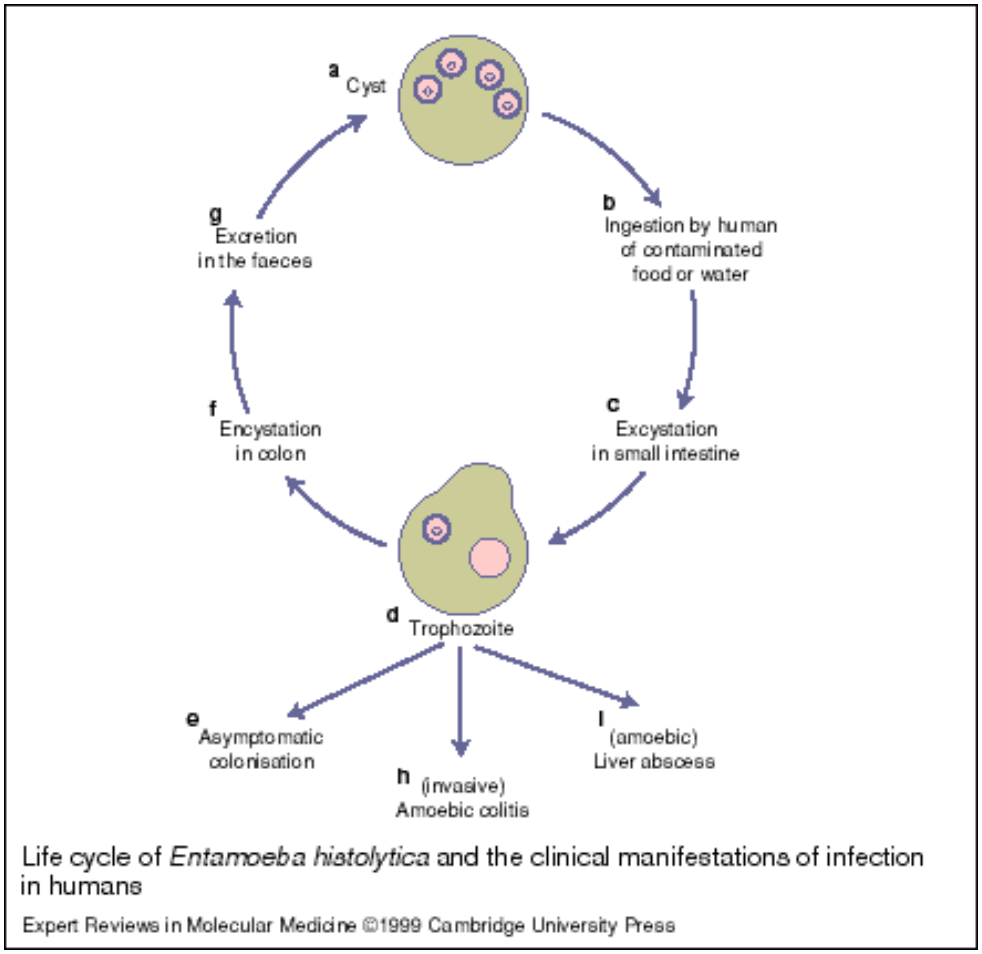
- Stage of inactivity
- Most resisiant stage = hard to kill.
- Multiplication occurs within cysts of some protozoa = "excystation" releases multiples, ex. *Entamoeba, giardia.*

Variations of the basic life cycle on the following slide occur in members of the non-sporozoan classes.

Protozoa life cycle

Standard non-Sporozoan intestinal life cycle

- Variations of the basic life cycle occur in members of the sporozoan classes.



Life cycle of *Entamoeba histolytica* and the clinical manifestations of infection in humans

Expert Reviews in Molecular Medicine ©1999 Cambridge University Press

Sporozoa life cycle stages/terms

Oocyst:

- results from fusion of gametes in the Sporozoa.
- Infective stage in most cases:
- passed in host feces in case of intestinal protozoans, or
- in blood in gut of the mosquito vector in the case of *Plasmodium.*
- Sporozoites form in oocyst in host via mitosis (asexual fission)..

Sporozoa life cycle stages/terms

Sporozoite:

- special term for trophozoite in Sporozoans.
- Formed by mitosis within oocyst & infects new host cells;
  - intestinal mucosa cells in intestinal protozoans, or
  - hepatocytes in case of *Plasmodium.*
- Mitosis (“schizogeny”) of these cells results in formation of merozoites.

Sporozoa life cycle stages/terms

Merozoite:

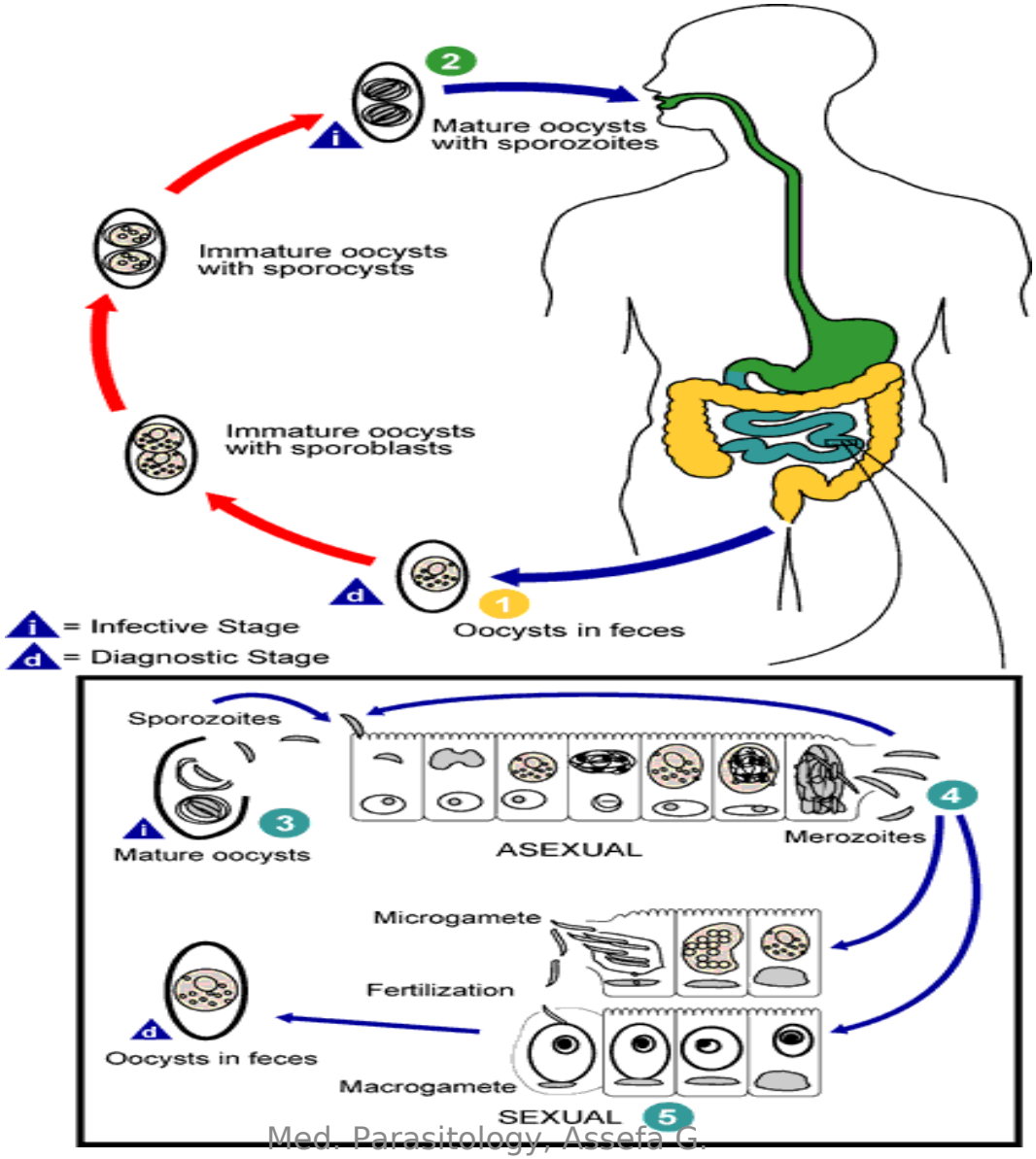
- results from mitotic fission (schizogeny) of sporozoites.
- May infect same cell type as sporozoite or a different host cell:
  - RBCs causing clinical malaria in the case of *Plasmodium.* …
- can also go through meiosis to form gametes.

Sporozoa life cycle stages/terms

Gametocyte:

- result of merozoite cell fission via Meiosis.
- Fusion of these results in formation of oocyst

Standard ??? intestinal Sporozoa life cycle



Encystation

- Many protozoa form cysts – round cytoplasmic mass surrounded by a rigid or semi-rigid cyst wall secreted by organism.
- Function:
  1. Protect against unfavorable external environmental conditions.
  2. Are the site for morphogenesis and nuclear division.
  3. Are the infective or transmissible form of parasite.

Conditions for encystment:

- loss of food source
- Desiccation
- increase in tonicity (salts)
- change in O2 concentration
  - change in pH
  - temperature change

Summary

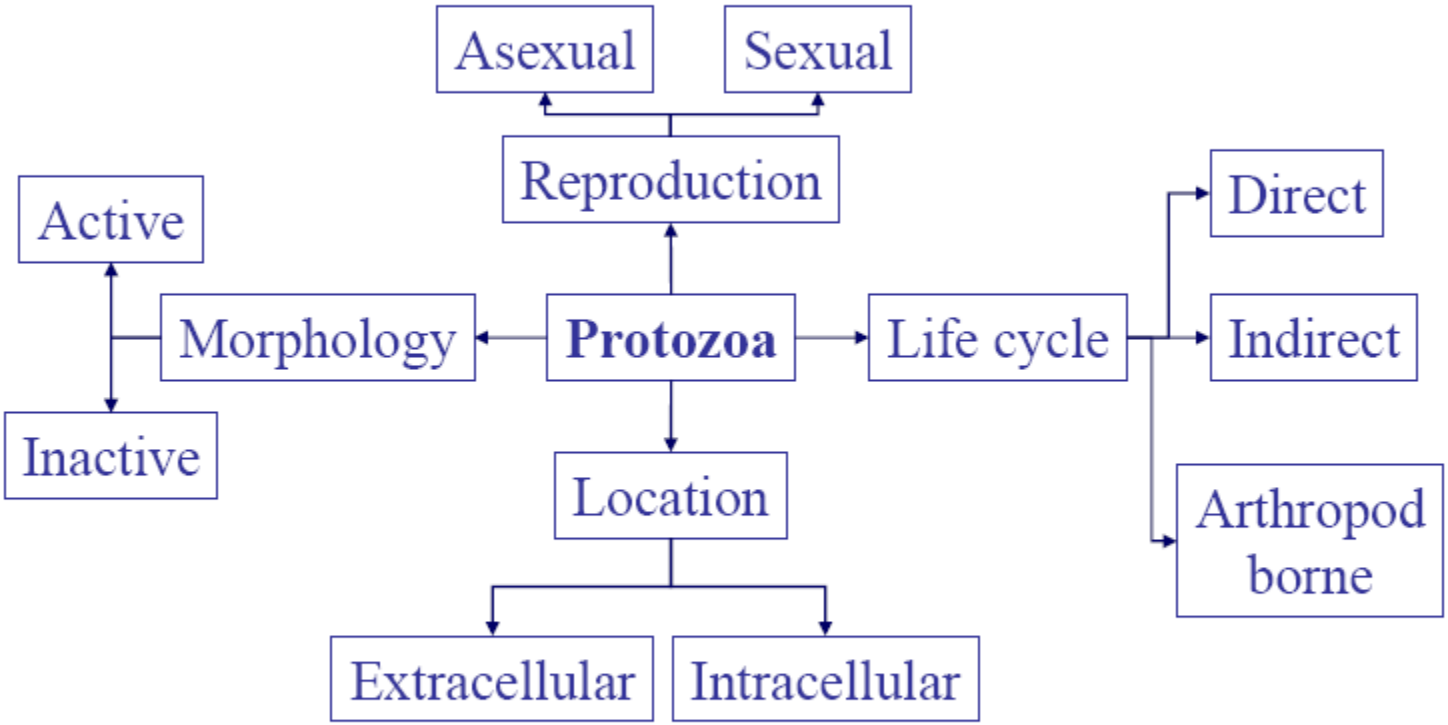
Protozoa

- unicellular eukaryote organisms
- kingdom Protista
- extremely diverse and found in a variety of niches
- Have complex internal structure
- several modes of reproduction
- Cause of severe sickness and death
- No universal agreement on classification

  there are many systems & frequent

  changes

Ways of thinking about parasitic protozoa



Parasitic protozoa 12

- Medically important protozoa
  - Amoeba
  - Flagellates
  - Ciliates
  - Coccidia
  - Sporozoa
  - Microsporidia

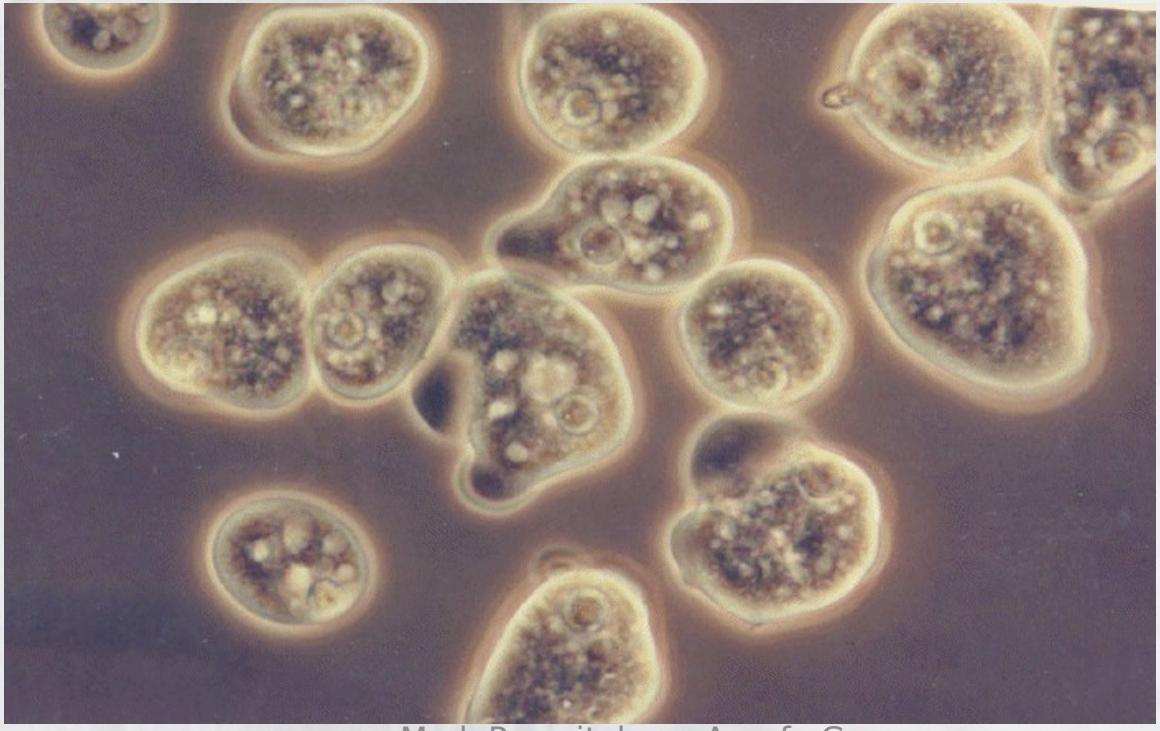
2.2 Classification of medically important protozoans

2.2.1 Class- Rhizopoda /Amoeba/- Sarcodina

General characteristics of amoeba

- They consists of shapeless mass of moving cytoplasm
- Clear ectoplasm
- Granular endoplasm
- Have organ of locomotion pseudopodia/false feet
- Digested food stored as glycogen bodies and chromatoid bars.
- Produce by simple binary fission and has simple life cycle.
- All are parasites of large intestine except – Entamoeba gingivalis which live in oral cavity.
- All exist as trophozoite and cyst, except E. gingivalis which require direct transfer of

A. Enteric Amebiasis and free- living ameba infections



INTRODUCTION

- ***E. histolytica*** *is a pseudopod-forming non flagellate protozoan* parasite.
- E. histolytica is the only pathogenic amoeba among all intestinal amoebae, infecting perhaps 10% of world's population
- cause amebic colitis and liver abscess
- No sexual forms of the parasite have been identified

- ***Entamoeba histolytica*** *has been reclassified into three* genetically distinct but morphologically identical species:

  - The invasive pathogen *E. histolytica*

  - *The noninvasive parasites E. moshkovskii and E. dispar.*

- Report= Cyst of *E. histolytica/ dispar*

Trophozoite (active form



- Size: 10-40 micrometers in diameter, some are above 60 micrometers.

- Pseudopodium(ectopalsmic rotrusion):Broad or finger-like in form, thrust out quickly.
- **ectoplasm formed first**

  endoplasm flows slowly into it.

- Motility is progressive and directional.

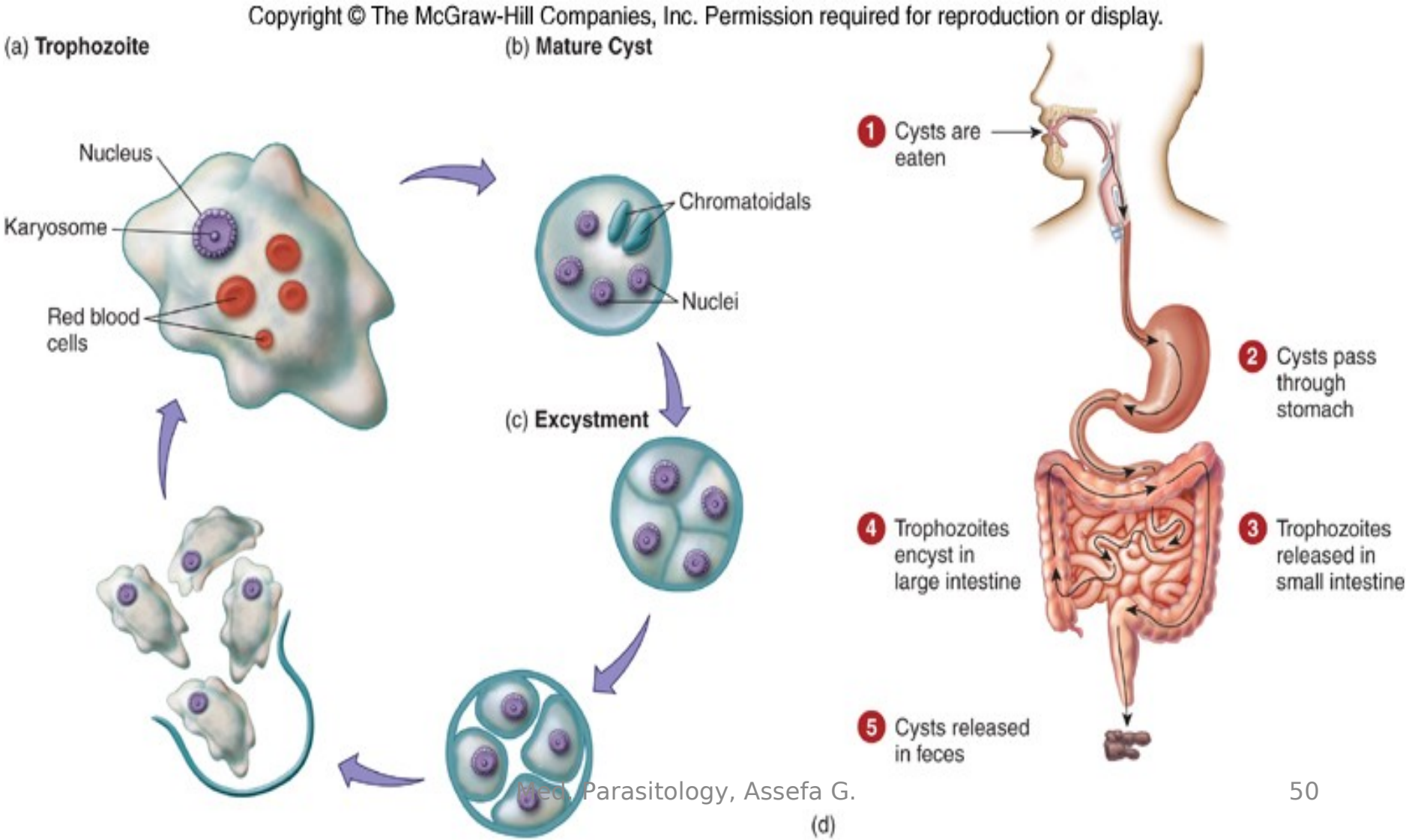
Cyst (non-motile)

- 10-20 mocrometers in size,spherical in shape

1-2 nuclei (immature cyst); 4 nuclei (mature cyst-infective stage).

- Inclusions:(become smaller and smaller as the cyst ages) ,glycogen vacuole appears as a clear space; food reservoir chromatoid body dark blue rods or dots
- Nucleus of E. histolytica are useful in differentiation of the pathogenic amoeba from other non-pathogenic amoebae.

Life Cycle


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(a) Trophozoite
(b) Mature Cyst
Nucleus
Karyosome
Red blood cells
Chromatoidals
Nuclei
(c) Excystment
(d)
1 Cysts are eaten
2 Cysts pass through stomach
3 Trophozoites released in small intestine
4 Trophozoites encyst in large intestine
5 Cysts released in feces

Epidemilogy

- E.histolitica infection are worldwide distribution
- But more prevalent in the tropics and subtropics.
- The high incidence of amoebiasis is due to both natural factors and poor sanitation.
- This disease is transmitted by the food, vegetable, fruit and drinking water contaminated by the stool with 4 nuclei cysts

Pathogenic mechanism

- The pathogenic materials of E. histolytica include lectin, amoebic pore forming and protein dissolving enzyme

- The lectin guides the trophozoite to adhere to the intestinal epithelia, neutrophils and red blood cells

- The trophozoite invade the mucosa by producing tiny **pinpoint lesions at** the site of entry, spread into the submucosa and produce typical **flask-shaped ulcer**.

- The open of the ulcer toward intestinal lumen looks like a crater

Clinical Manifestation

(1)Intestinal amoebiasis

- Amoebic dysentery is the most common form of amoebiasis

- The acute case discharges **unformed feces several times per day** with the pain in right inferior part of the abdomen, nausea, low fever around 38℃, and fatigue.

- The jam-like stool with foul smell, in which there are great number of trophozoites and Charcot-Leyden crystals

typical flask-shaped ulcer of intestinal amebiasis



Clinical Manifestation…

2. **Amibic colitis**

- Patients with **amebic colitis** typically present with a history over several weeks of gradual

  of **abdominal pain and tenderness, diarrhea, and bloody stools**

- Differentiate between other infectious causes including *Shigella, Salmonella, Campylobacter, and* enteroinvasive and enterohemorrhagic *Escherichia coli*

- Unusual manifestations of amebic colitis include
  - acute necrotizing colitis,
  - ameboma (granulation tissue in colonic lumen mimicking colonic cancer in appearance),
  - cutaneous amebiasis,
  - rectovaginal fistulas
- A single stool examination for parasites is insensitive

Dysentery and Colitis……

Table 1: Symptoms and Signs of Amebic Colitis

Symptoms and Signs	Percentage
Gradual onset	Most
History of symptoms > 1 wk	Most
Diarrhea	94–100%
Dysentery	94–100 %
Abdominal pain	12–80 %
Weight loss	44 %
Fever > 38°C	10 %
Heme (+) stools	100 %
Male-female ratio	1:1

Extra-intestinal amoebiasis

Liver Abscess

- The most common extra-intestinal amoebiasis is the liver abscess due to the parasite getting into the liver through the **portal vein system**

- Signs of amebic liver abscess include right upper quadrant pain, fever of 38.5°C to 39.5°C, leukocytosis

- have a palpable mass

Extra-intestinal amoebiasis.....

- The abscess is single and in the right lobe of

  the liver 80% of the time(Why?)

- The **lack of bacterial infection in the abscess fluid** is most helpful in ruling out a pyogenic abscess

Table 2 *Symptoms and Signs of Amebic Liver Abscess*

Symptoms and Signs	**Range**
History of symptoms >4 wk	21%–51%
Fever	**85%–90%**
Abdominal tenderness	**84%–90%**
Hepatomegaly	30%–50%
Jaundice	6%–10%
Diarrhea	20%–33%
Weight loss	33%–50%
Cough	10%–30%
Male-female ratio	9:1

liver containing amebic abscess



Other cases

- **Amoebic abscess of pulmonary**: chest pain, cough, jam-like sputum, the shadow of the abscess can be seen on X-ray.

- **Intestinal perforation**: acute abdominal pain, high fever, septic shock.

- **Amoebic abscess of brain**: headache, nausea, vomiting, delirum, coma.

Other cases

- **Amoebic vaginitis:** burning sensation, foul leucorrhoea

- **Amoebic urithritis:** irritability of the bladder--frequent micturition(urination)

- **Skin fistulas** of anus or liver portion.

Rx

- Metronidazole is recommended for acute amoebic dysentery.

How does it acts?

Prevention

- sanitary disposal of stool; preventing food, vegetable, fruit and drinking water from the contamination of cyst.
- Drinking water must be boiled
- Food and drinks must be protected from flies
- Personal hygienic and health check of food handler and waiter in the restaurant.

Non-pathogenic amoeba

- Entamoeba dispar
- Entamoeba coli
- Entamoeba hartmanni
- Entamoeba gingivalis
- Endolimax nana
- Iodamoeba butshlii
- Dientamoeba fragilis

Free-living Amebas

- ***Naegleria***
- ***Acanthamoeba* and**
- ***Balamuthia***

Free-living Amebas

Ameba	**Diseases**
Naegleria fowleri	**PAM(primary amebic meningoencephalitis)**
Acanthamoeba species	GAE(granulomatous amebic encephalitis); skin or lung lesions; amebic keratitis
Balamuthia mandrillaris	GAE; skin or lung lesions

1. Naegleria

- **Previously:** *N aerobia* and *N invadens, Naegleria australienses* and *N australiensis italica*
- The pathogenic species of *Naegleria* was named *N fowleri* after Malcolm Fowler, who first isolated the organism from a patient with primary amebic meningoencephalitis.

Structure

- **Trophozoites:** are 10 to 15 µm in diameter and produce broadly rounded lobopodia.
- **Trophozoites:** can also transform to a flagellated form.
- **Cysts :**are single-walled, spherical and 8 to 12 µm in diameter. (not found in human)

Epidemiology

- The organism is found worldwide in soil and warm fresh water. Infectious cysts may be carried in dust.

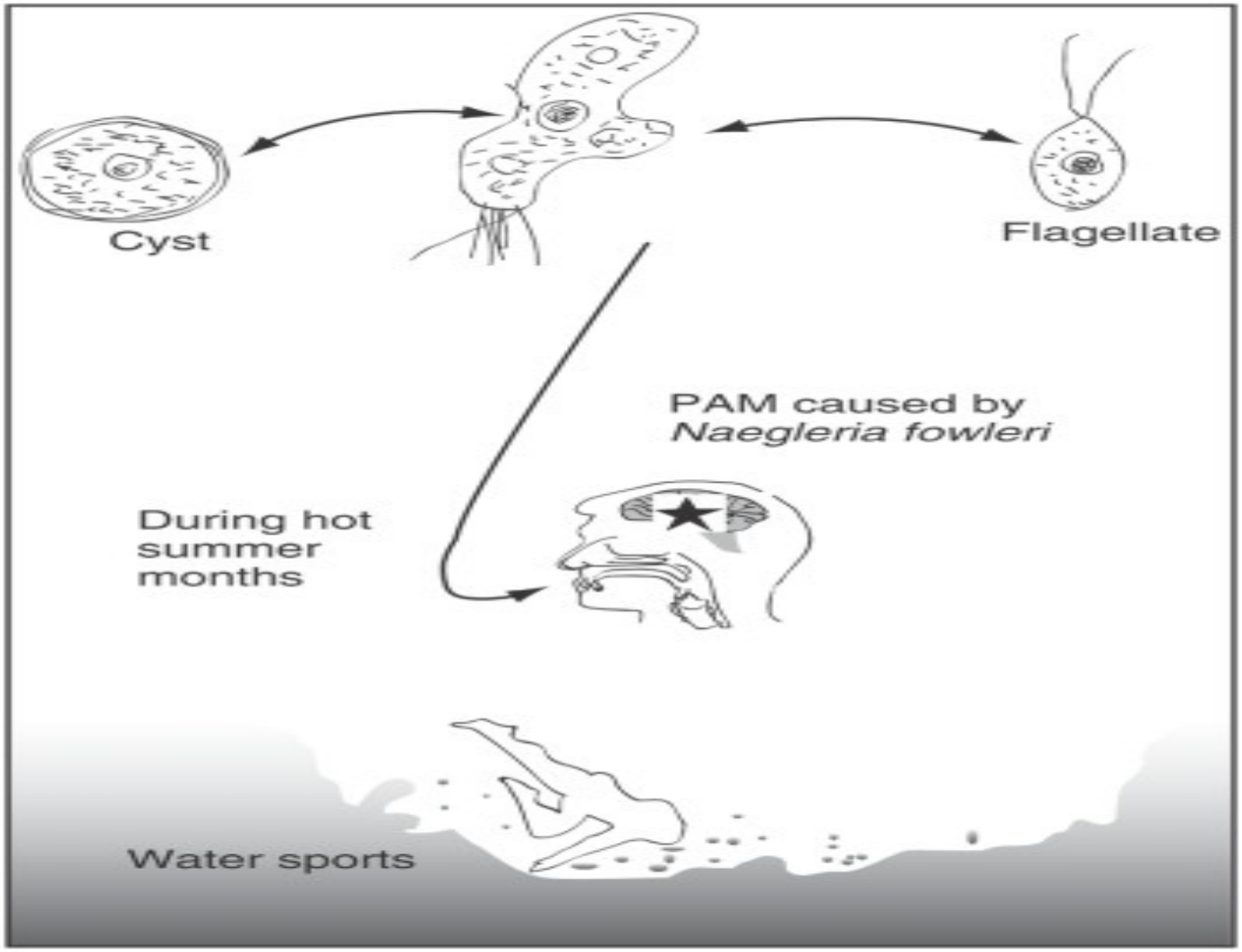
Transmission

- In almost all cases, *N fowleri(trophozoite)* enters the body by being inhaled or splashed onto the olfactory epithelium

Multiplication

- *Naegleria fowleri* is a free-living inhabitant of fresh water and soil.
- The ameboid trophozoite form reproduces by **binary fission**
- And also gives rise to the encysted and flagellated forms, which do not reproduce
- **It is thermophilic**: reproducing successfully at temperatures up to 46°C.

Life cycle



Primary amebic meningoencephalitis due to *Naegleria fowleri*.

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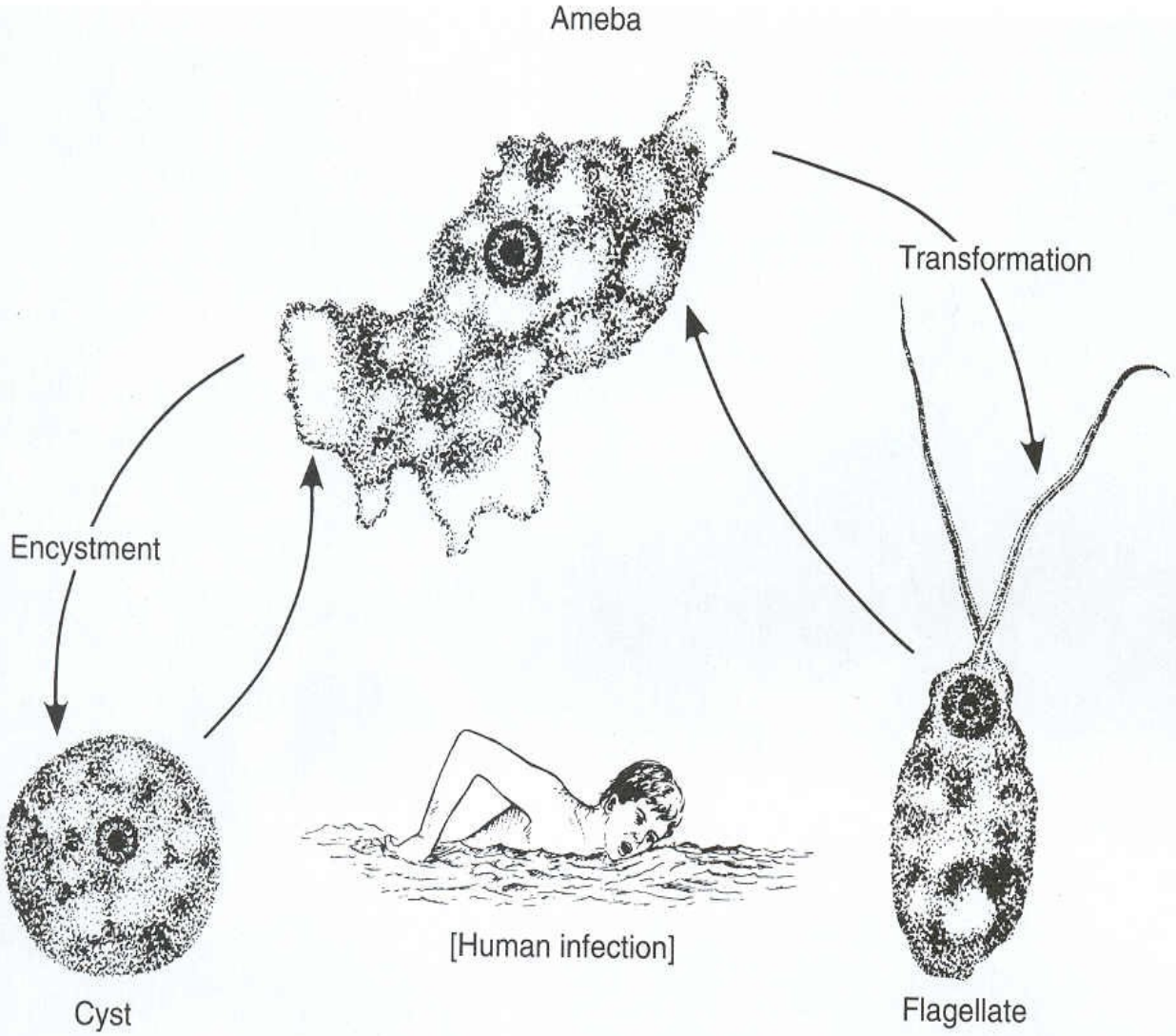


Figure 7.13 **Life cycle of *Naegleria fowleri*.**

In the laboratory, and probably in nature, transformation from ameba to flagellate is caused by depletion of nutrients (David John, personal communication).

From D. T. John, "Amebas," in *Yearbook of science and technology*. Copyright © 1986 McGraw-Hill Company, Inc., New York, NY. Reprinted by permission.

Clinical Manifestation

- *Naegleria fowleri* is the agent of **primary amebic meningoencephalitis**
- A fulminating, rapidly fatal disease.

  >150 cases of this disease have been recorded worldwide**(~300 up to2008)**
- The incubation period: 2 to 3 days to as long as 7 to 15 days, depending partly on the size of the inoculum and the virulence of the strain

- The disease appears with the sudden onset of headache, fever, nausea, vomiting, and stiff neck.

- Symptoms progress rapidly to lethargy, confusion, and coma.

- **In all of the recorded cases, the patient died within 48 to 72 hours.**

Pathogenesis

- The **sustentacular cells** of the olfactory neuroepithelium are capable of active phagocytosis
- And this appears to be the mechanism by which the amebas invade the body
- The amebas then travel up factory nerve to the brain-> olfactory bulb-> subarachnoid space-> to the rest of the **central nervous system**
- **Respiratory symptoms** :Due to hypersensitivity or allergic reactions, or may represent a subclinical infection.

Diagnosis

- **PAM cannot** be distinguished clinically from acute pyogenic or bacterial meningoencephalitides.
- **usually occurs in children and young adults in good health who have recently swum in warm water**
- **CSF:** -under a light microscope for *N fowleri*

  - Glucoese reduce and protien increase
- **Culture:** CSF, brain tissue –definitive dx

Treatment

- Amphotericin B and miconazole appear to be the drugs of choice
  - Prevention and control

- chlorinating swimming pools,
- whirlpools, and Jacuzzis,
- not diving or splashing in warm water ponds

2.Acanthamoeba/ Balamuthia mandrillaris

- *Acanthamoeba castellanii, A culbertsoni*, and other *Acanthamoeba* species as well as the recently described *B mandrillaris,* **can cause opportunistic lung and skin infections** in immunocompromised or otherwise debilitated individuals.

Epidemiology

- Pathogenic *Acanthamoeba* species and *B mandrillaris* are ubiquitous in **fresh and salt water and in soil**. Infective cysts can be carried by water or dust.

- Both cysts and trophozoites are infective

Clinical Manifestations

- **Granulomatous Amebic Encephalitis**
- GAE is a multifocal, hemorrhagic and necrotizing encephalitis caused by opportunistic free- living *Acanthamoeba* species
- Principally *A castellanii* and *A culbertsoni* or by *B mandrillaris*. The disease usually afflicts debilitated or immunosuppressed individuals
- It sets in with insidious, focal neurologic changes that mimic the clinical picture of single or multiple space-occupying brain lesions

- **Acanthamoeba Keratitis**
- Painful corneal ulcerations that fail to respond to the usual antibacterial, antiviral, and antifungal treatments may be caused by *Acanthamoeba*.
- Untreated corneal perforation and loss of the eye . **The disease is quite rare.**
- It is usually associated with contaminated contact lenses.

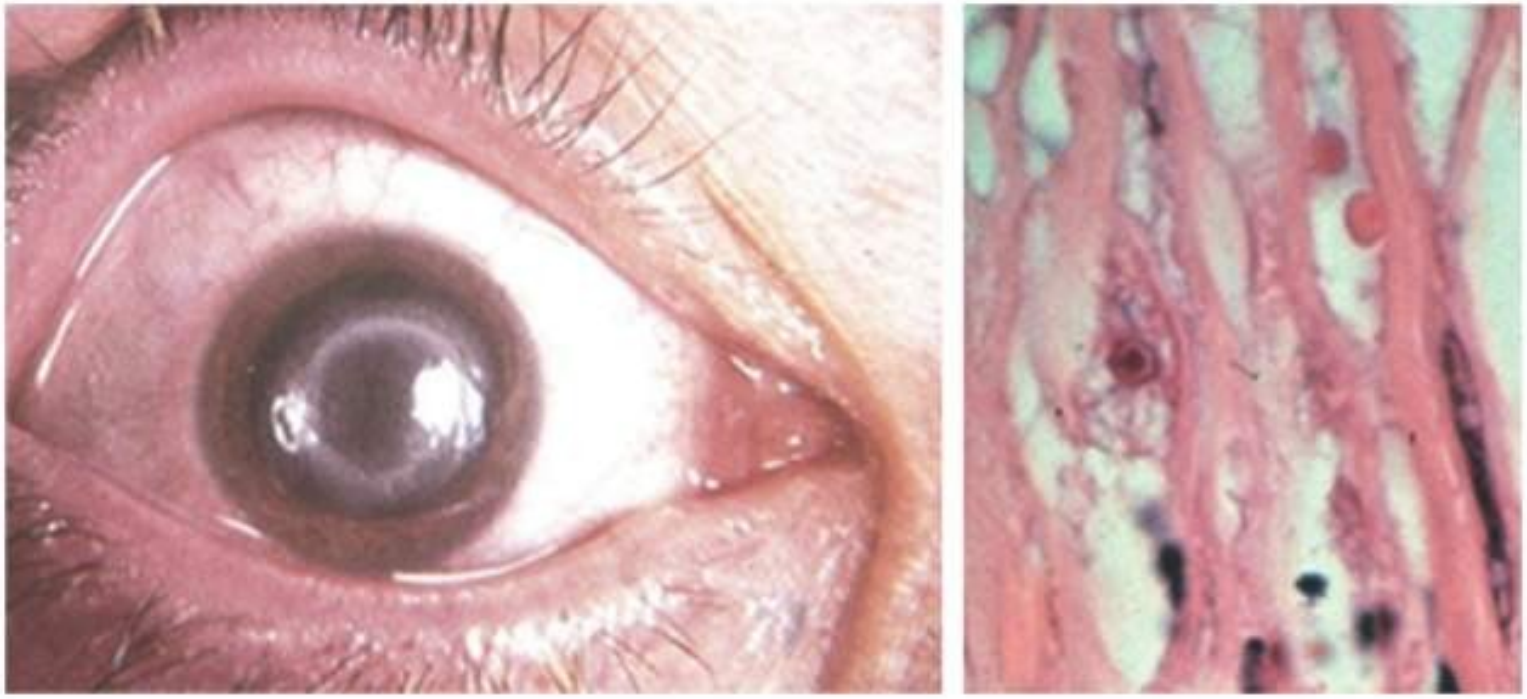


FIGURE *Acanthamoeba* keratitis. Note the concentric ring infiltrate *(A)* and a section of the cornea showing *Acanthamoeba* trophozoite in the corneal stroma *(B)*. (magnification x1000)

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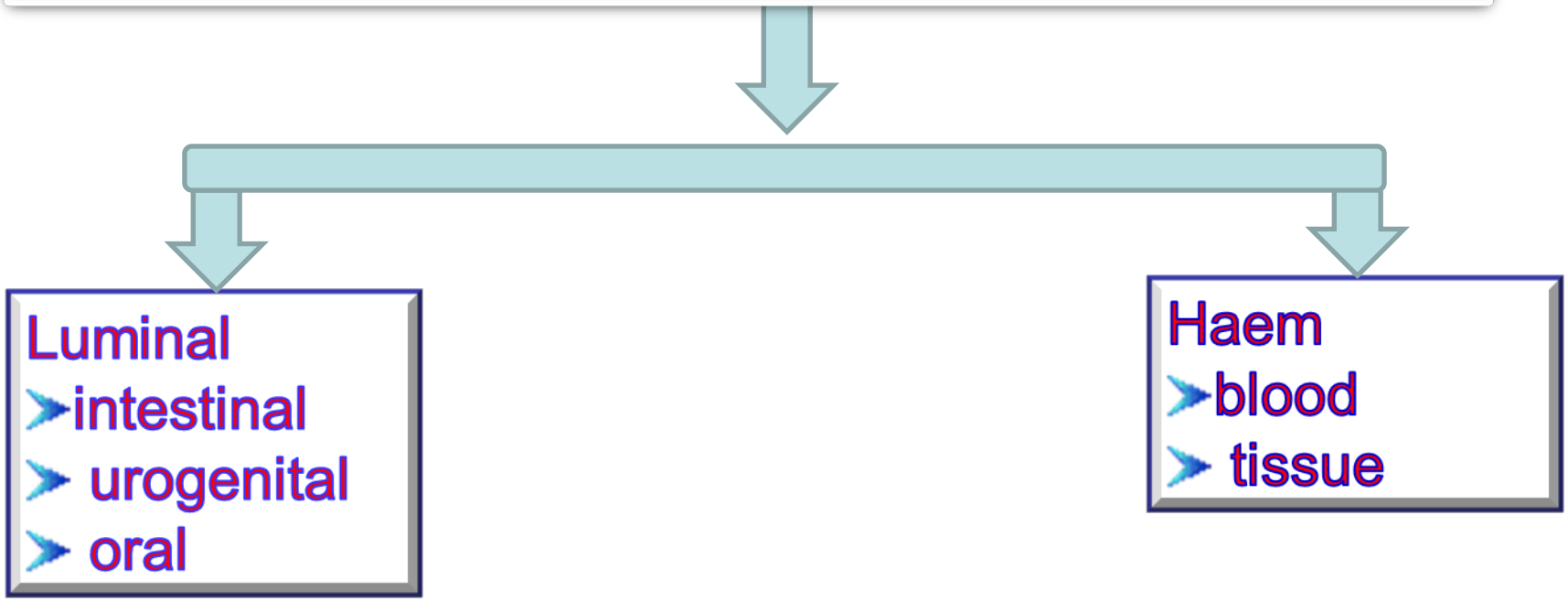
Diagnosis

- In many cases, **granulomatous amebic encephalitis** is not diagnosed until after or, at best, shortly before death
- Brain tissue
- skin biopsy
- scrapings of the corneal ulceration
- To examine for trophozoites and cysts

Rx , prevention and control

- Brolene (propamidine isethionate) and dibromopropamidine have been reported to be effective
- Lenses should be cleaned properly, using commercial rather than home-made saline, and should be disinfected with a chemical or (preferably) a thermal system. Lenses should be removed before swimming.
- Since the amebas are ubiquitous, preventive measures to reduce contact are difficult

2.2.2 CLASS- ZOOMASTIGOPHORA (FLAGELLATES)



Objectives

- Upon completion of this sub topic you will be able to:
  - Explain transmission, life cycle, prevention and control of G*iardia lamblia, T. hominis* ,T.vaginalis, blood and tissue flagellates (leshimaniasis and trypanosomiasis)
  - Discuses epidemiology and clinical feature of Giardiasis, Vaginitis, leshimaniasis and trypanosomiasis
  - Distinguish the infective and diagnostic stages of *Giardia lamblia,* T.vaginalis, blood and tissue flagellates

I. Luminal (intestinal,urogenital& oral) flagellates

- Intestinal Flagellates:
  - ***Giardian lamblia***
  - *Dientamoeba fragilis*
  - *Chilomastix mesnili*
  - *Trichomonas hominis*
  - *Enteromonas hominis*
  - *Retortamonas intestinalis*
- Urogenital flagellates
  - ***Trichomonas vaginalis***
- Oral flagellates
  - *Trichomonas tenax*

Luminal flagellates...

- **General characteristis**
  - Usually posses 2-6 flagella(~ 8 flagella.)
  - Inhabit the intestinal, urogenital and oral cavity
  - Direct life cycle/no biological vector
  - All have trophozoites and cyst stage except the Trichomonas species
  - All are comensal/ non-pathogenic/except G.lamlia and T. vaginalis

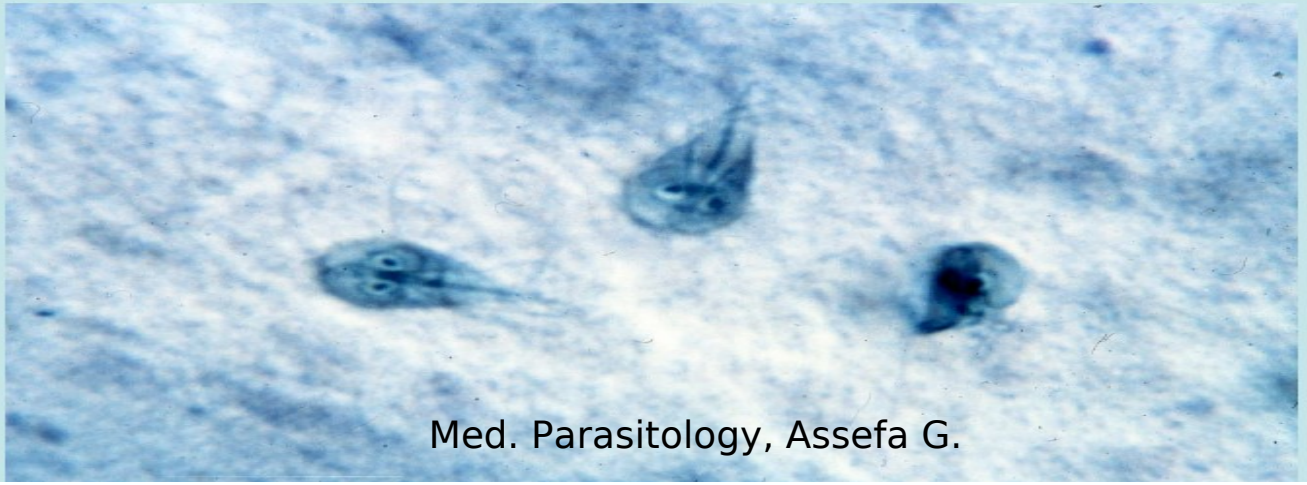
Epidimology

- worldwide distribution
  - higher prevalence in tropical or developing countries (20%)
  - 1-6% in temperate countries
- most common and easly recognized protozoa in stools
  - ~200 million cases/yr
- giardiasis
  - often asymptomatic
  - acute or chronic diarrhea

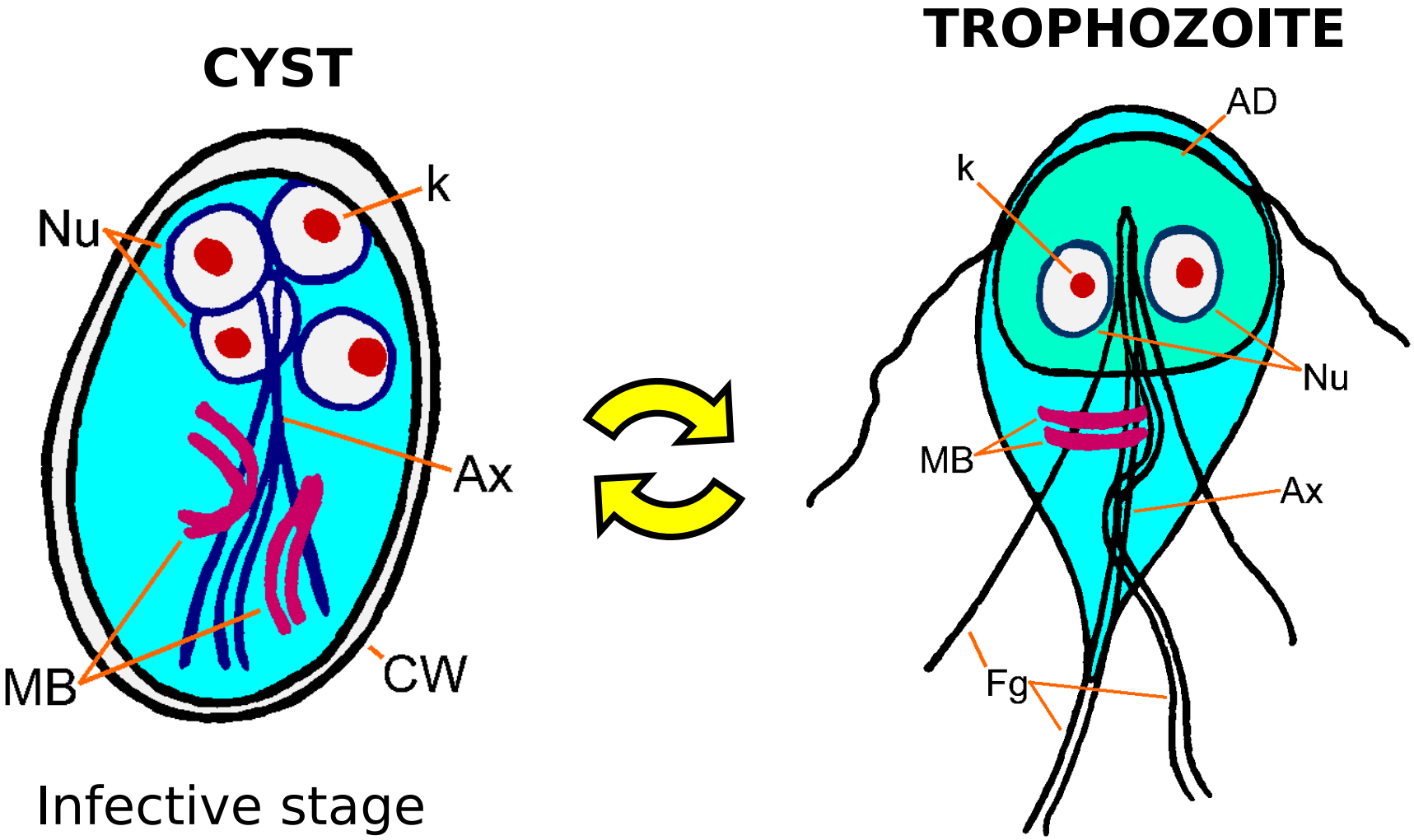
Anton van Leeuwenhoek



This is what he sees in his own stool sample



Morphology

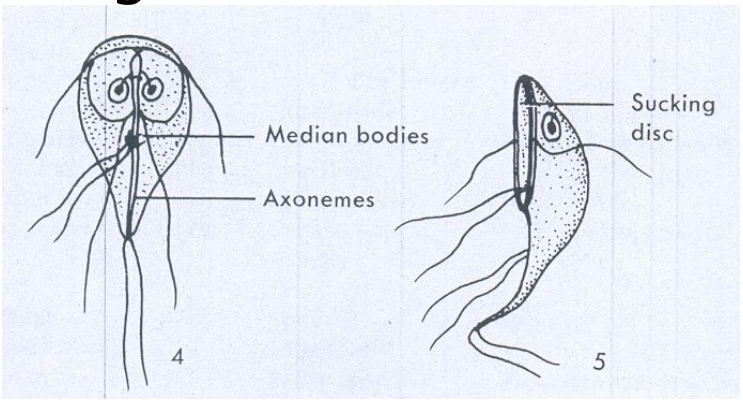


Infective stage passed in feces

Replicative stage inhabiting sm. intestine

A. *Giardia lamblia*

Trophozoite stage



Typical Characteristics:

Size range: 8-20 um

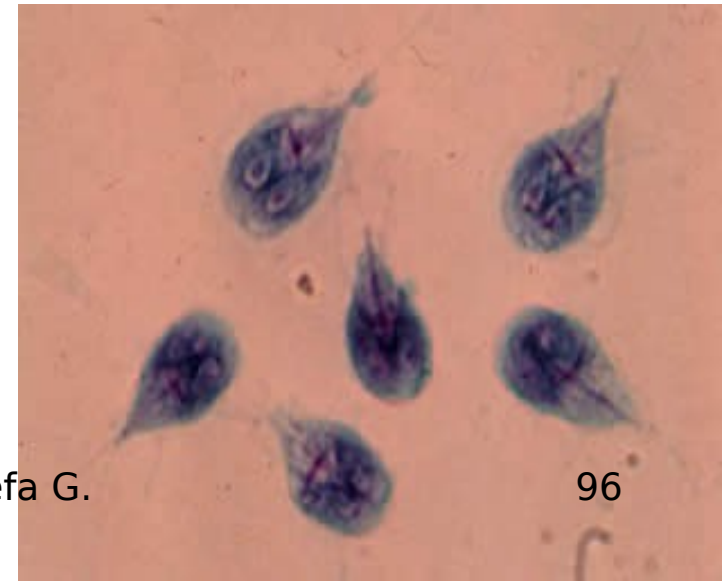
Shape: Pear/teardrop

Motility : “Falling leaf”

Appearance: Bilaterally symmetrical

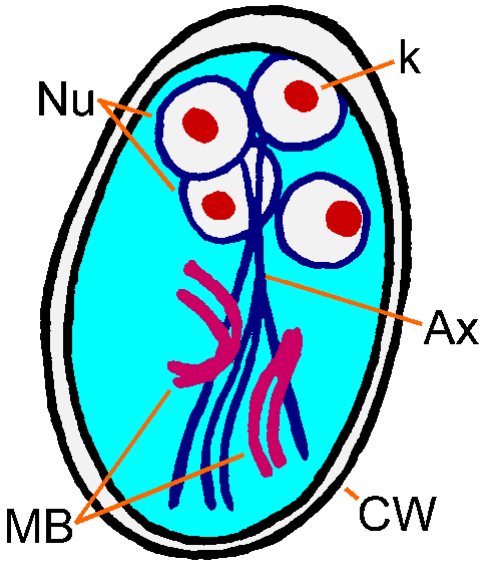
Nuclei: Two ovoidal-shaped, each with a large Karyosome
No peripheral chromatin

Flagella: Four pairs arising from the ventral side :
One pair anterior end
One pair posterior end
Two pairs central laterally

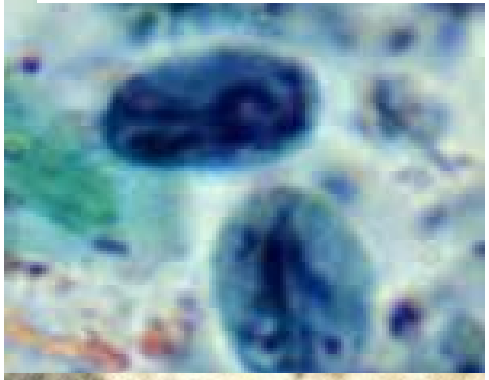


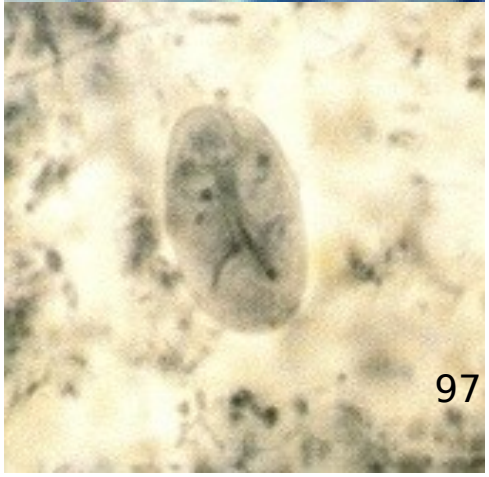
Giardia lamblia

Cyst



- **Typical Characteristics:**
- **Size range:** 11-14 um
- **Shape:** Ovoid ellipsoidal or may appear round
- **Nuclei**: -2-4 located at one end
  young cyst 2 nuclei,
  mature cyst 4 nuclei
  - Central Karyosome
  - No peripheral chromatin





- Transmission
  - Giardia is transmitted via the cyst stage

1- ingestion of faecally contaminated food

or water with cyst

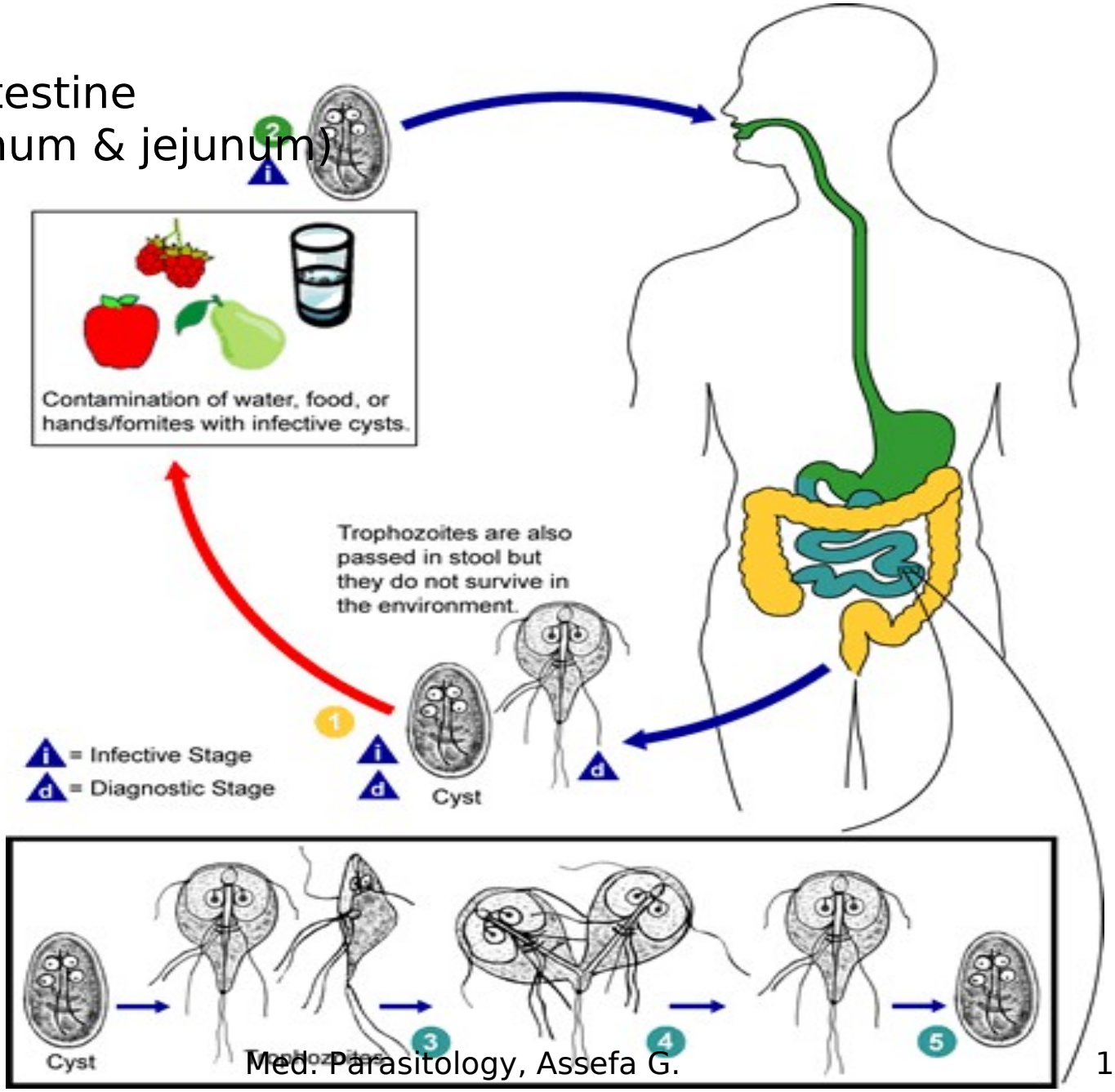
2- sexually among homosexual (feco-oral contact)

Fecal-Oral Transmission Factors

- Poor personal hygiene
  - Children (eg, day care centers)
  - Food handlers
- Developing countries
  - Poor sanitation
  - Endemic
- Water-borne epidemics
- Male homosexuality
  - Oral-anal contact

Habitat

Small intestine
(duodunum & jejunum)



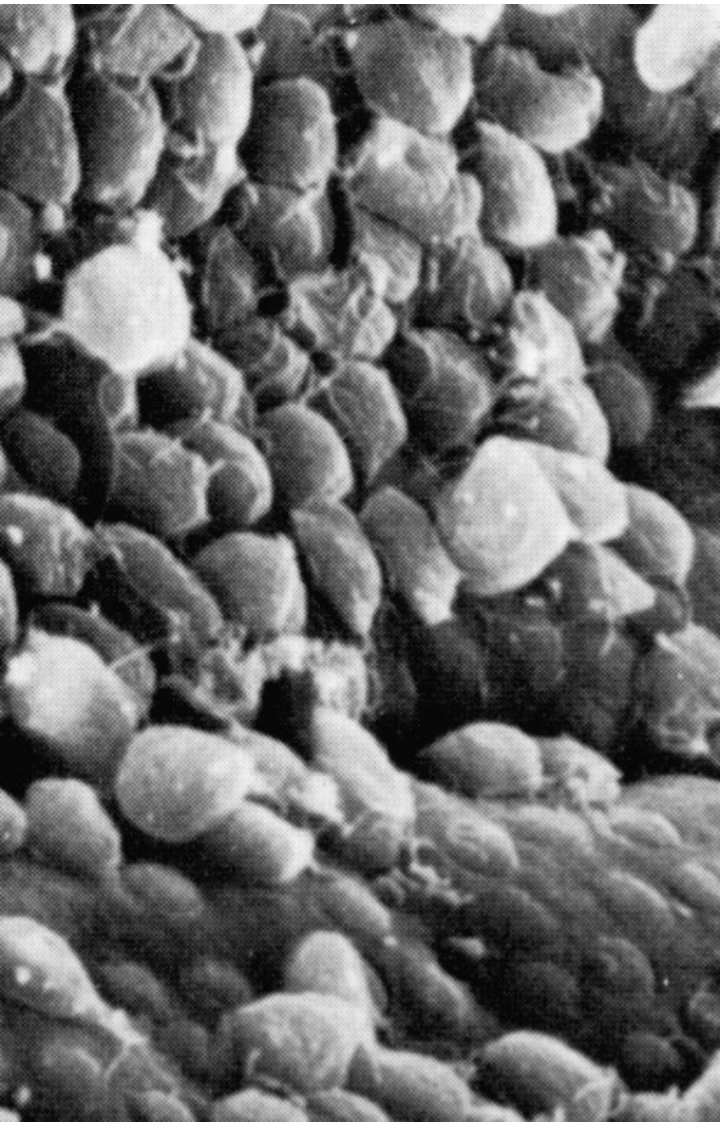
Life Cycle

1. Life cycle is direct - no intermediate host.
2. When cyst is ingested, the organisms escape from the cyst in the duodenum.
3. Attach via adhesive disk to microvillar surface of epithelium of upper two-thirds of the small intestine.
4. They divide by longitudinal fission.
5. Encystment occurs in lower intestinal tract.
6. Freshly passed cysts are infective
7. Trophozoites are found in diarrheic stools; cysts are found in formed stools.

- Cyst survive
  - cool, moist conditions
    - for long periods
  - in cold water:
    - 2 months at 8°C
    - 1month at 21°C
- some cysts can survive –13°C for 2 weeks
- relatively resistant to chlorination, particularly if the water is cold
- the amount of chlorine in drinking water is not sufficient to kill *G. lamblia*

- Cysts are susceptible to:
  - desiccation and direct sunlight
- Cysts and trophozoites are susceptible to:
  - 1% sodium hypochlorite
  - ammonium disinfectants.
- They can also be killed by boiling for at least 1 minute.

Pathogenesis



- Epithelial damage
  - Cellular infiltration
- Malabsorption develops
- Enzyme deficiencies
  - Lactase (lactose intolerance)

Possible Mechanisms

- **mechanical irritation**
- **obstruction of absorption**

Laboratory Diagnosis:

- Macroscopic
  - Stool:
    - is usually offensive, bulky, pale, mucoid (fatty), diarrheic (watery) but
    - there is no blood in the stool.
- Microscopy
- Immunological methods
- Molecular methods

- Microscopy
  1. Finding the trophozoite stages in fresh diarrhoeic stool
     - wet mounts or stained
     - Identity or confirm in stained smeer
  2. Finding the trophozoite stages in duodenal aspirate
  3. Finding cyst stage in formed stool.
     - wet mounts or stained
     - Concentration of cyst
       - » Formol-ether/zink-sulphate
  - trophozoites and cysts are excreted irregularly
  - Collect stool at 3 non-consecutive days

What is the purpose of normal saline in stool examination?

Treatment

Drug of Choice
- metronidazole (Flagyl)
- 750 mg/tid/5d
- >90% cure rate

Alternatives
- tinidazole (single dose)
- paromomycin (pregnancy)
- quinicrine
- furazolidone

Control

- avoid fecal-oral transmission
- improve personal hygiene
  - especially institutions
- treat asymptomatic carriers
  - eg, family members
- health education
  - hand-washing
  - sanitation
  - food handling
- protect water supply

How to get rid of *Giardia* in water?

* boiling (at least 1 minute)
* filtration (1µm pores)
* chemical treatment (Iodine, …)

Summary

- **Giardia lamblia:**
  - First discovered by Leeuwenhoek 1697
  - 2nd most frequent intestinal Protozoon
  - exist as both trophozoite, cyst
  - Reproduce by binary fission
  - Have fecal-oral transmission
  - Colonize duodenum, small intestine but no invasion
  - Less harmful, but chronic damage to intestinal wall can lead to malabsorption syndrome
  - Similar lifecycle to E.histolytica, but reservoir hosts may play role in transmission.
  - Diagnosis commonly by finding trophozoites and cyst in stool

B. *Trichomonas hominis*

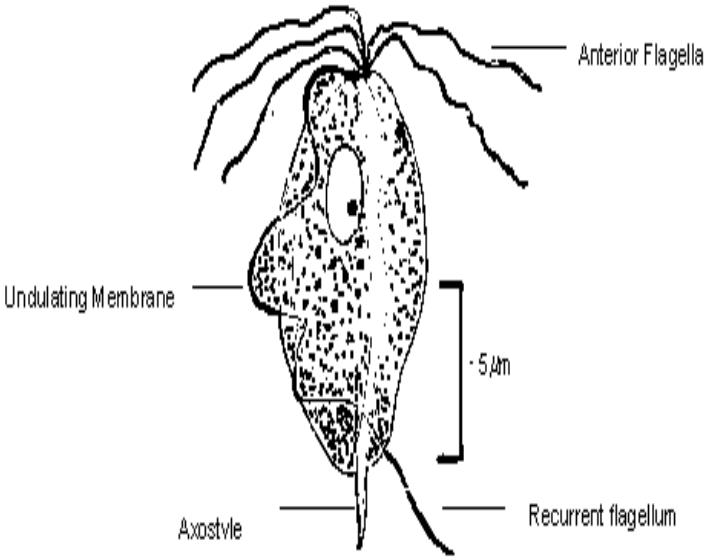
- ***Distribution**:*
  - probably the most common intestinal flagellates next to *Giardia lamblia*
- ***Habitat:*** Large intestine.
- ***Morphology:*** has trophozoite stage only

How It pass the HCl acid in stomach?

Trophozoite

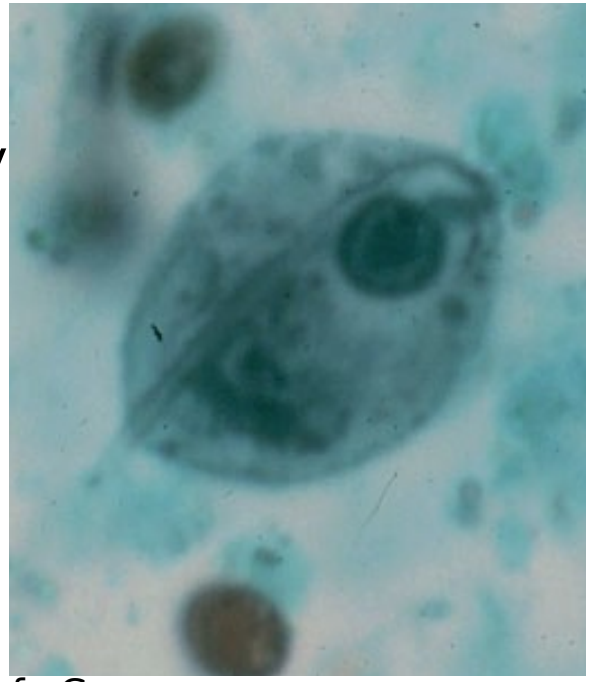
unstained

- Size: 10-15μm, pyriform (oval with two pointed poles)
- 4 anterior flagella(3-5)
- Motility: whirls and turns (jerk in all directions, seeming to vibrate.
- Undulating membrane and costa reach full length of the body.
- Axostyle extend beyond the body



Stained

- Single nucleus
- Conspicuous cytostome
- semi- rigid axostyle



C. Other Flagellates Found in Human Feces

- *Chilomastix mesnili*
- *Pentatrichomonas hominis*
  - formerly called *Trichomonas hominis*
- *Enteromonas hominis*
- *Retortamonas intestinalis*
- *Dientamoeba fragilis*
  - no flagella

D. *Trichomonas vaginalis* (Urogenital flagellates)

- General feature
  - Inhabit the urogenital tract of male and female
  - Has only trophozoite stage
  - Most frequent STD pathogen
  - longitudinal binary fission

TRICHOMONADS

Human *Trichomonas* Species	
T. tenax	oral cavity
*T. hominis**	intestine
T. vaginalis	uro-genital

*aka: *Pentatrichomonas*

Trichomonas vaginalis

Distribution:- **World wide .**

Habitat:- **uro-genital tract**

females: vagina

males: urethra, prostate,

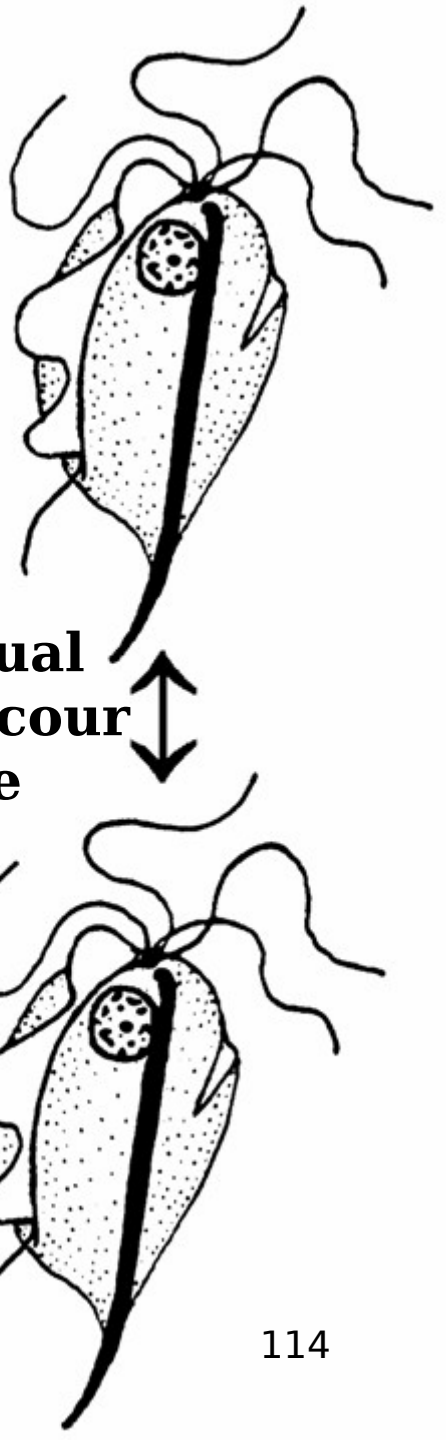
Transmission*:* trophozoite stage transmitted during sexual intercourse

-non-sexual contact possible

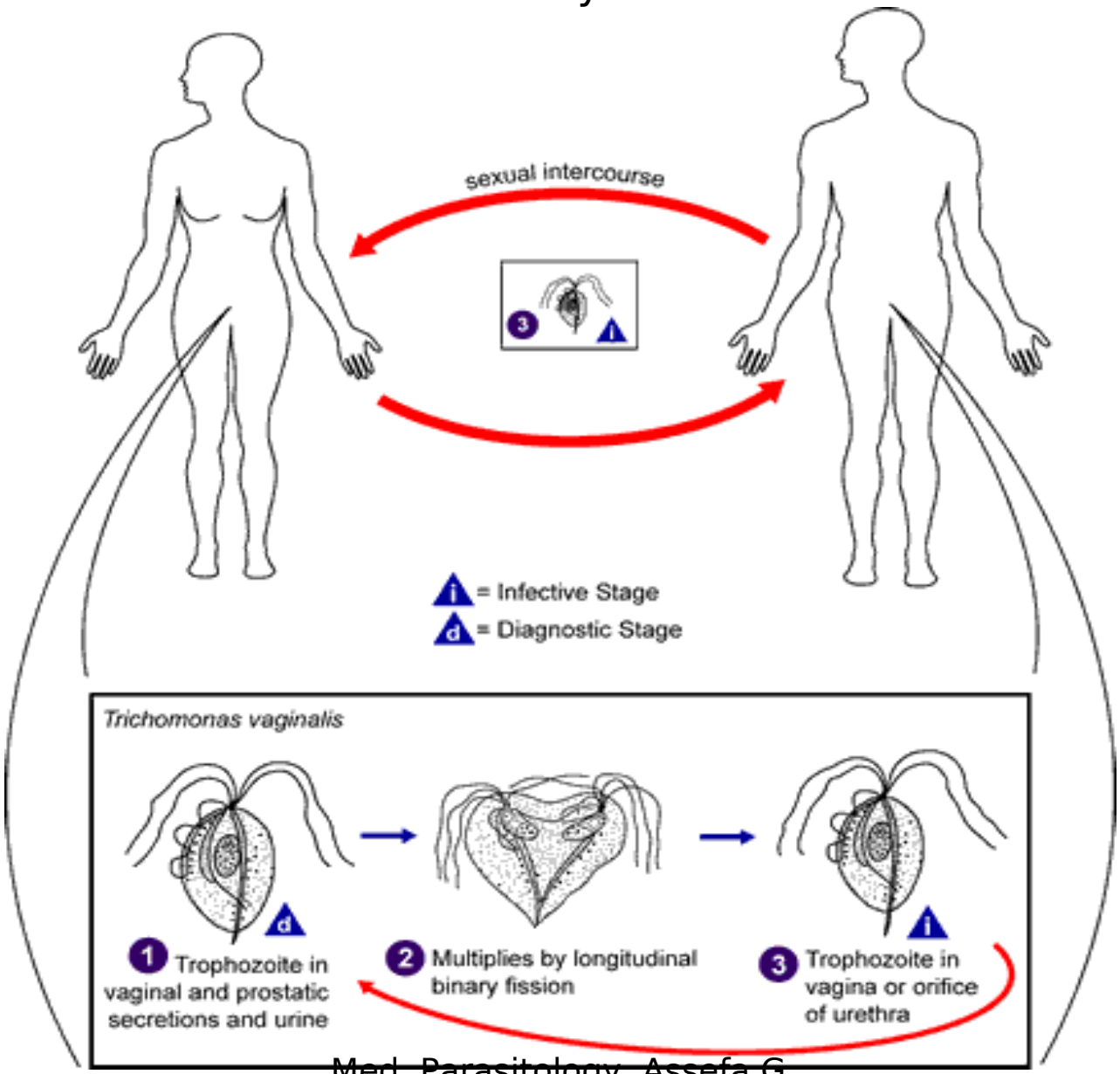
-common STD

- co-infection w/other STDs
- more prevalent in at risk groups

- both sexes equally susceptible
- symptoms more common in females



Life cycle


sexual intercourse
i = Infective Stage
d = Diagnostic Stage
Trichomonas vaginalis
1 Trophozoite in vaginal and prostatic secretions and urine
2 Multiplies by longitudinal binary fission
3 Trophozoite in vagina or orifice of urethra

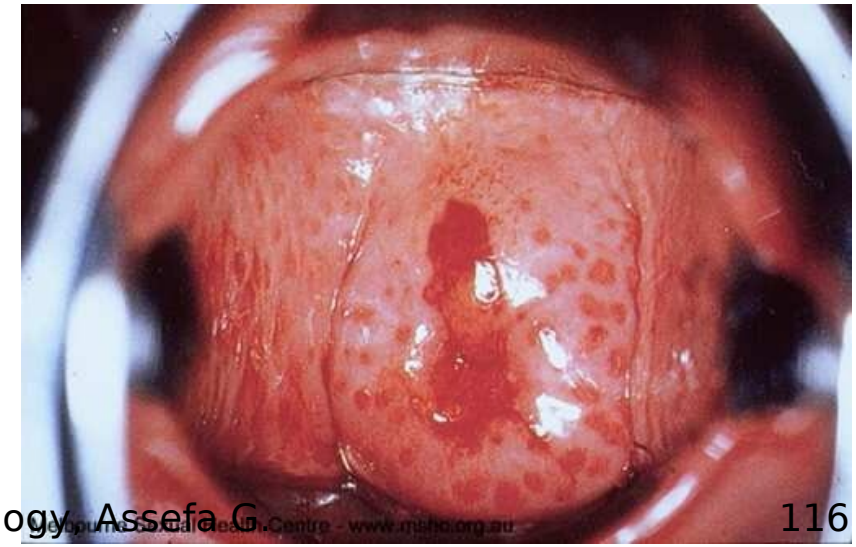
Pathology

Trichomonas vaginitis





- Inflammation
- Erosion
- Discharge
- itching, burning
- urethritis, dysuria
- dermatitis



Clinical manifestations

In females:

- ranges from asymptomatic, to mild or moderate irritation, to extreme vaginitis
  - 50-75% abnormal discharge (frothy, yellowish or greenish)
  - 25-50% pruritis
  - 50% painful coitus
- onset or exacerbation often associated with menstruation or pregnancy
- vaginal erythema, (~2%)

- **How *Trichomonas vaginalis survive inside the reproductive organ of women during* menstruation?**

Clinical manifestations

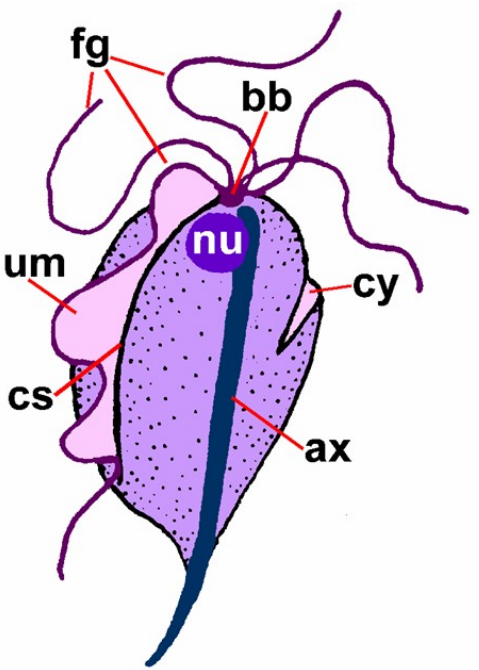
In males:

- 50-90% are asymptomatic
- Mild dysuria or pruritus
- Minor urethral discharge

Laboratory diagnosis

- Finding the trophozoit in smear of vaginal or uretheral discharge
  - Wet mount
  - Giemsa/field stained
  - Acridine orange stained
- Occasionally in urine sediment of men and women
- in vitro culture in Diamond's medium

***Morphology*: Has trophozoite stage only**



Trophozoite

- Size 15-25 by 5-12μm, is the largest *Trichomonas*.
- Shape: pyriform
- Motility: Jerky (on-spot), non-directional motility .
- undulating membrane :Short extending along two third of the body.
- In Giemsa/field stain
- Nucleus: Single
- Flagella: 3-5 anterior free flagella
- Axostyle- extends beyond the body
- U.m bordred by flagella
- In acridne orange: flagellates fluoresce orange among green pus cell



TREATMENT

- metronidazole (Flagyl)
  - 250 mg (3/d) for 5-7 days
  - single 2 g dose
- Tinidazole

simultaneous treatment of partner! (85-90% cure rate) Why?

PREVENTION

- **limit # of sexual partners**
- **condoms**

Summary

- Most frequent STD pathogen
- Pathogenesis:
- Females: Vaginitis, pruritis; strawberry cercix.
- Males: Urethritis; prostatovesiculitis.
- endemic problem in honosexual communities.
- Inhabit the Genitourinary tract
- Has no intermediate host and reservoir host.
- Infective form: trophozoite No cyst form
- Mode of infection is through sexual contact.
- Laboratory identification: finding the Trophozoites in vaginal and urethral discharges; prostate exudates.

II. Blood and Tissue flagllates

- Belonging to the family Trypomastidae
- six genera but only two of them are responsible to cause disease to man
  - Genus *Leishmania*
  - Genus *Trypanosoma*

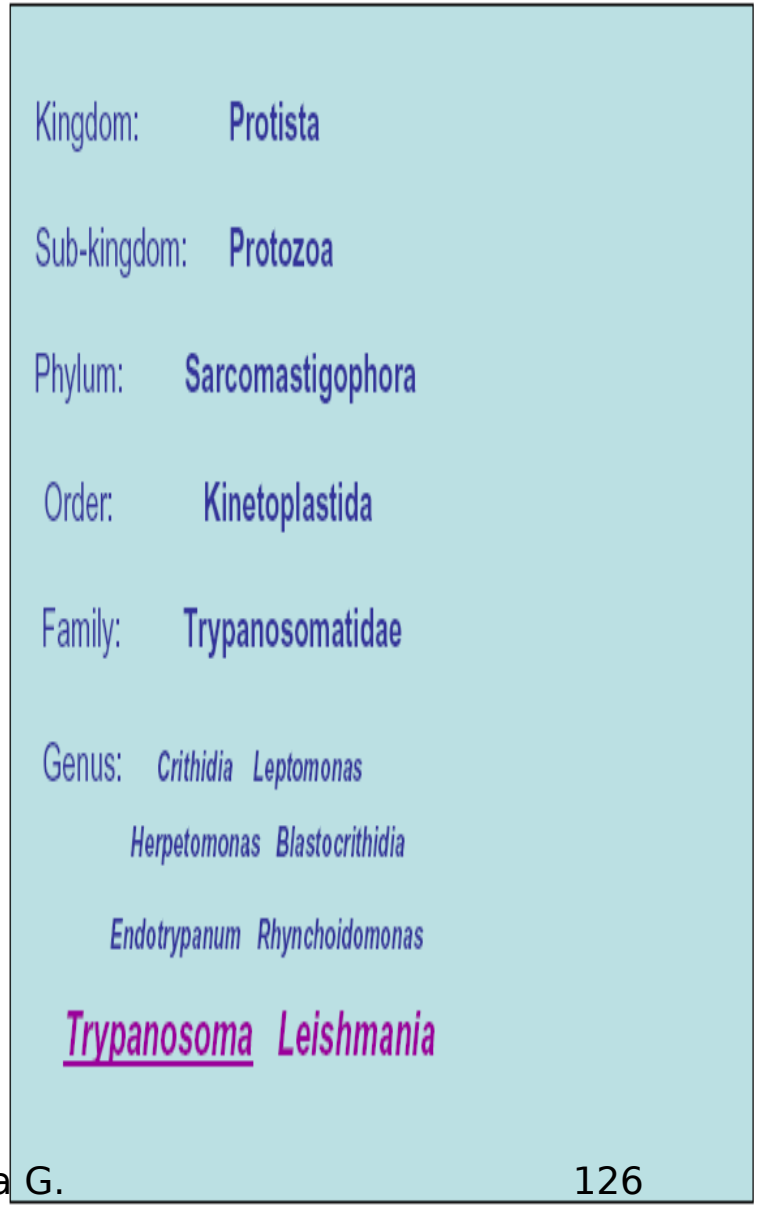
General Characteristics

- Reproduces by simple longitudinal binary fission
- Transmission occurs through biological insect vectors as intermediate hosts & human as definitive host
- The species are morphologically indistinguishable, but they can be differentiated on the basis of their
  - clinical features, geographical distribution, serologic tests, immunological tests, etc.
- The different developmental forms are differentiated on the basis of
  - Presence or absence of free flagellum
  - Presence or absence of undulating membrane
  - Position of the kinetoplast relative to the nucleus

A. Trypanosomes Species

- General Feature

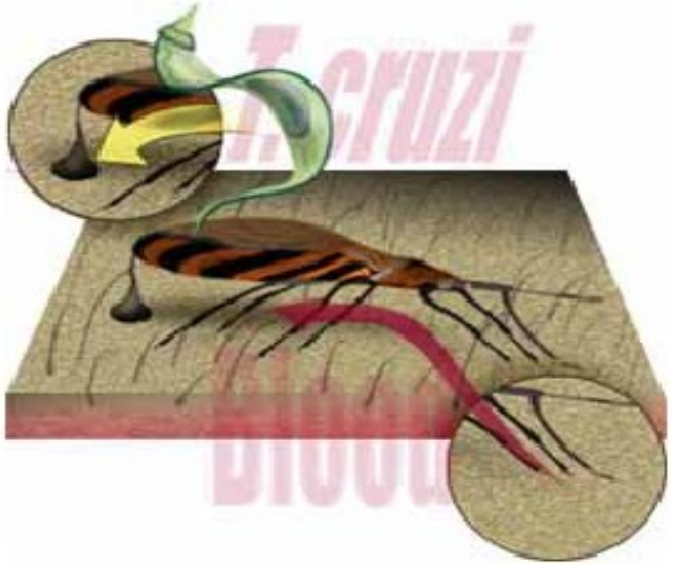
1. They occur both in flagellates and non-flagellate forms
2. Reproduce by simple longitudinal binary fission
3. The infective stage is always the vegetative form
4. Transmission occurs by the bite of biological vectors as an intermediate host
   - Vector:- tsetse fly, kissing bug



Genus trypanosoma

Classified in to two groups based on the type of development in the insect vector and mode of transmission

Stercorarian: parasites develop in the posterior part of the vector and are transmitted through release of parasites with feces during feeding. (*T. cruzi*)



Salivarian: parasites develop in the anterior part of the insect vector and are transmitted by injection with saliva during feeding **(African trypanosomes)**



Genus Trypanosoma

- Two distinct forms occur in humans
  - African Trypanosomiasis
    - Trypanosoma brucei gambiense
    - Trypanosoma brucei rhodesiense
    - Transmited by the Tsetse fly
  - American Trypanosomiasis
    - Trypanosoma cruzi
    - Transmited by the tritomine bug

Human (HAT) and animal (AAT) African trypanosomiasis

Animal trypanosomiasis causes a negative economic, social and nutritional impact.



Human trypanosomiasis remains a serious threat



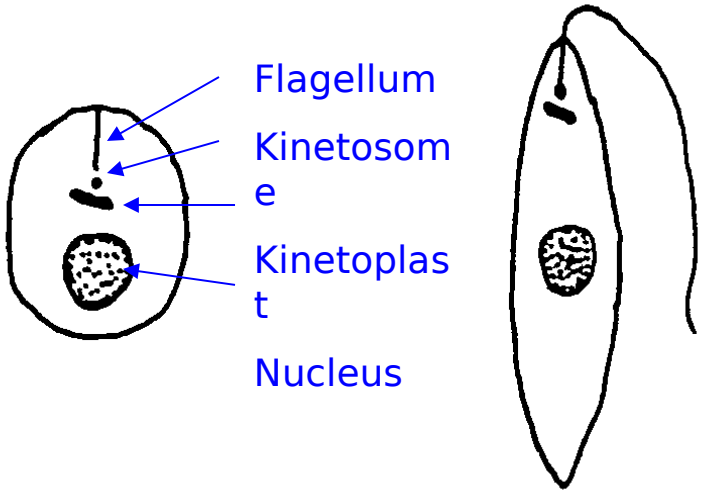




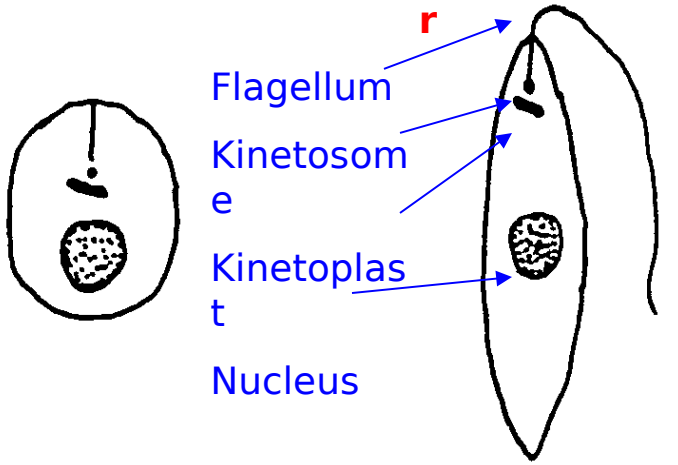
Human African Sleeping Sickness

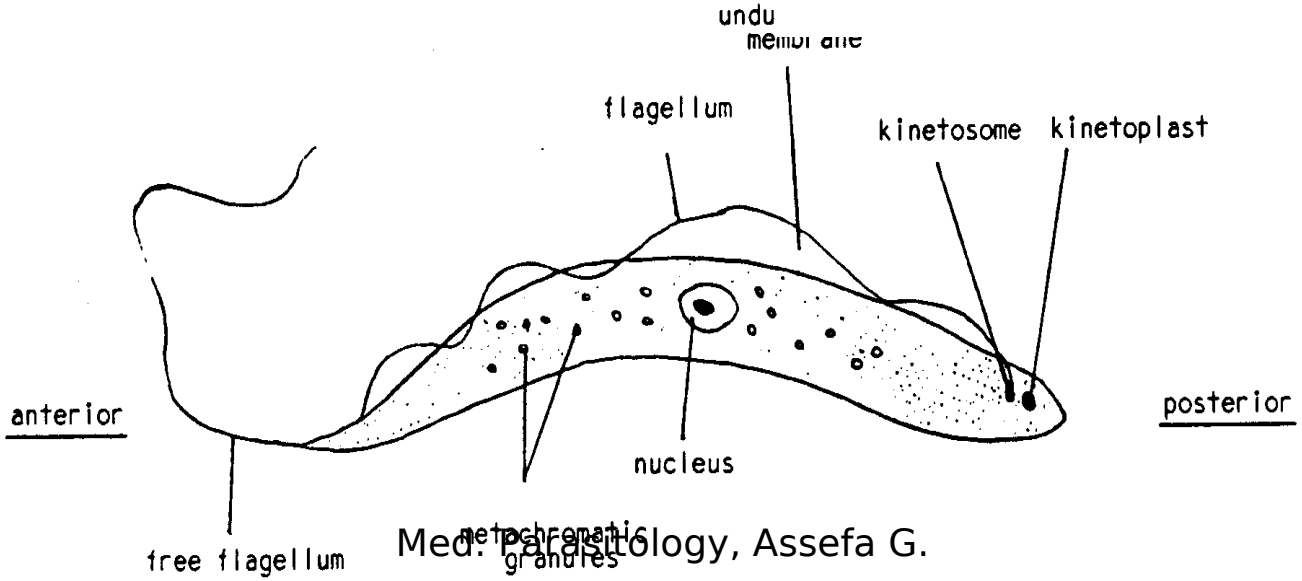
- It is caused by the flagellate protozoan, Trypanosoma brucei
- exists in 2 morphologically identical subspecies:
  - Trypanosoma brucei gambiense
    - Disease: West African or Gambian African trypanosomiasis
    - Geographical Dist: Central & West Africa
  - Trypanosoma brucei rhodesiense
    - Disease: East African or Rhodesian African trypanosomiasis
    - Geographical Dist : Central and East Africa

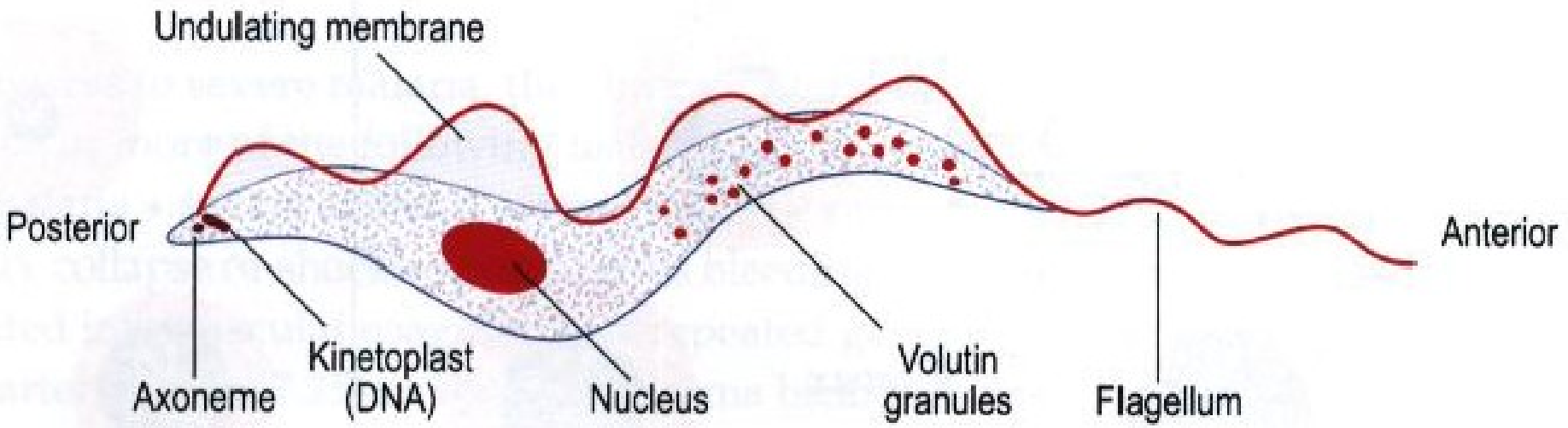
AMASTIGOTE PROMASTIGOTE



AMASTIGOTE PROMASTIGOTE

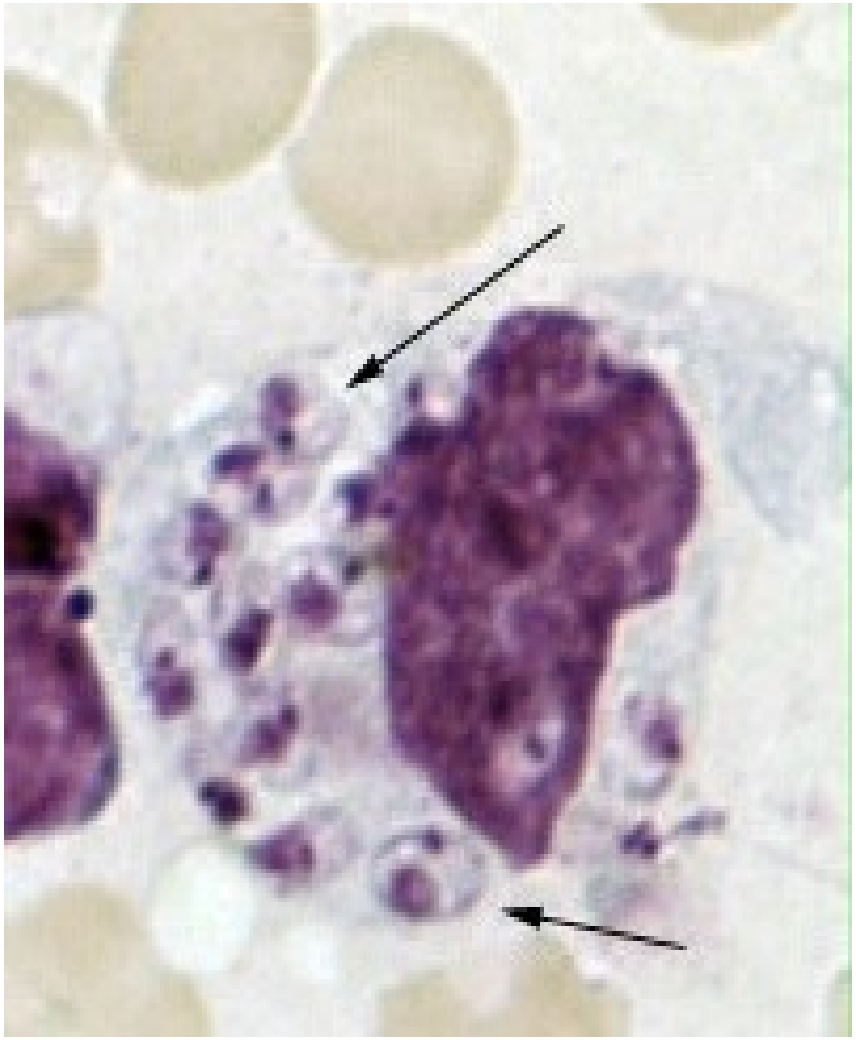


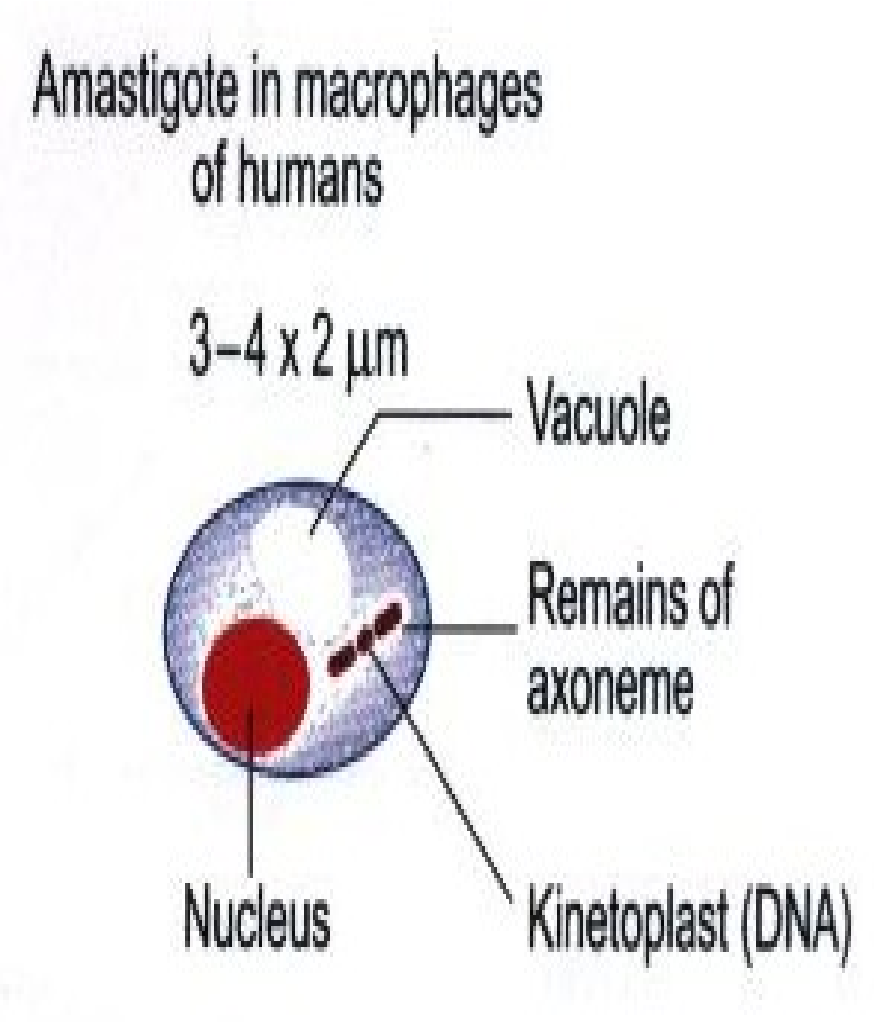



Undulating membrane
Posterior
Anterior
Axoneme
Kinetoplast
(DNA)
Nucleus
Volutin
granules
Flagellum

The following are the the main developmental forms

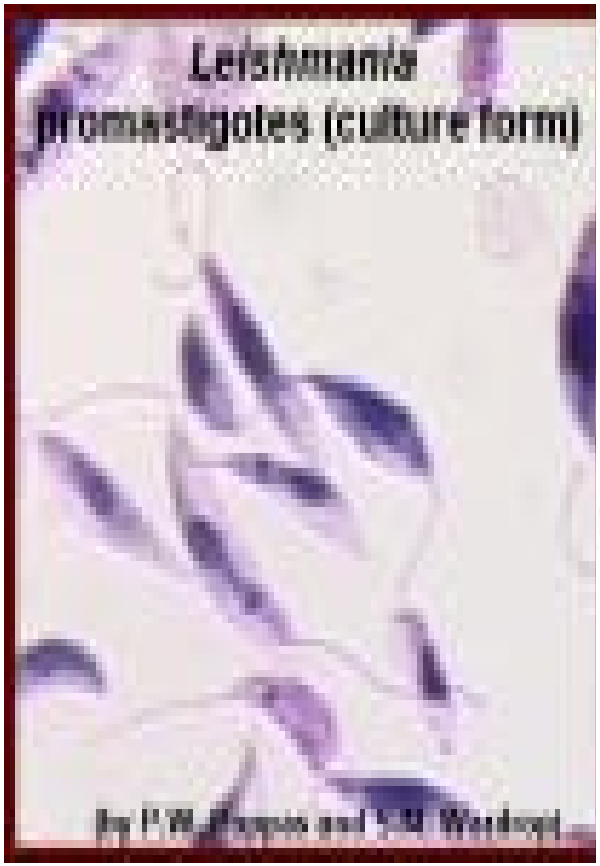
1. **Amastigote (Leishmanial form)**
   - Rounded body, central nucleus and eccentric kinetoplast visible
   - no free flagellum,
   - No undulating membrane,
   - The only intracellular forms of all leishmania species and *Trypanosome cruzi*.

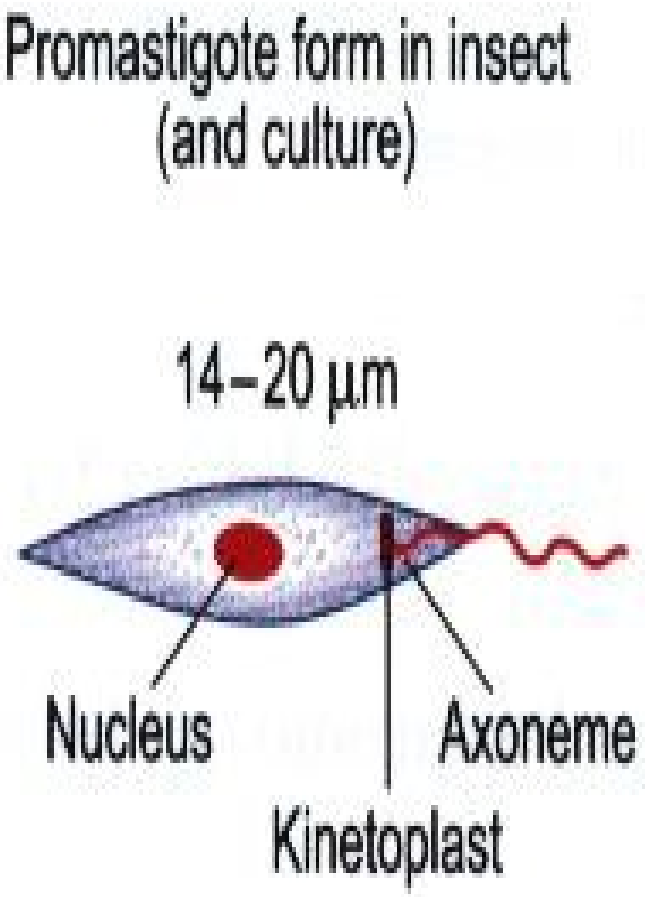



Amastigote in macrophages of humans
3–4 x 2 µm
Vacuole
Remains of axoneme
Nucleus
Kinetoplast (DNA)

2. Promastigote (Leptomonad form)

- Elongated body, central nucleus, anterior kinetoplast
- Single anterior flagellum arises from kinetoplast
- Found in the invertebrate host, and in culture media (of all *Leishmania* species) and in man for *Tryponosoma cruzi*

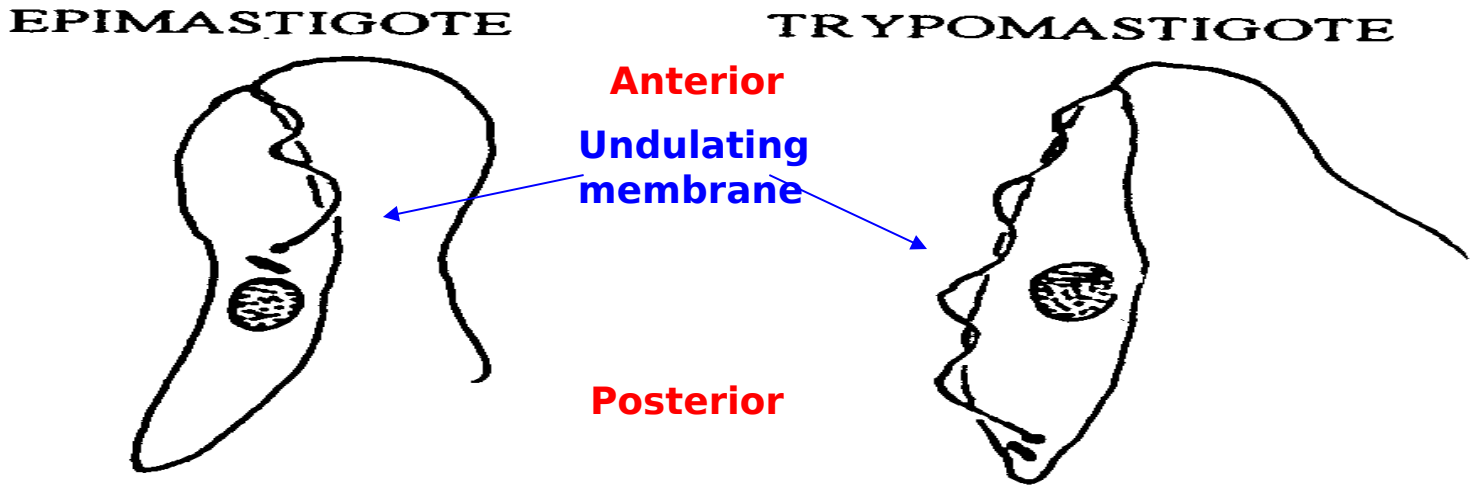

Leishmania
promastigotes (culture form)

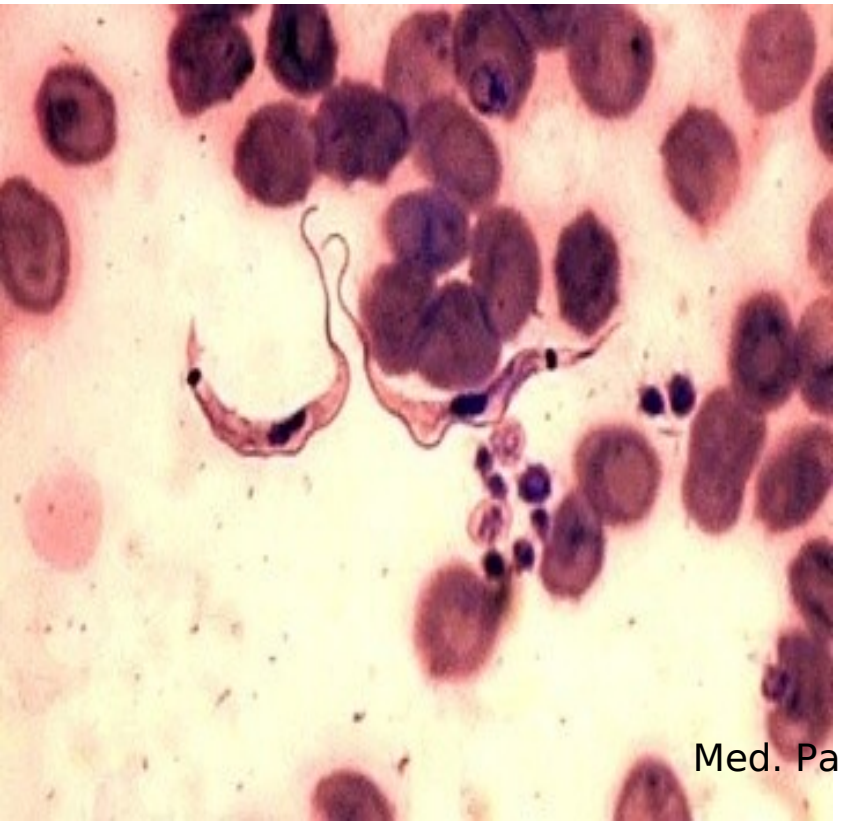

Promastigote form in insect
(and culture)
14–20 µm
Nucleus
Axoneme
Kinetoplast



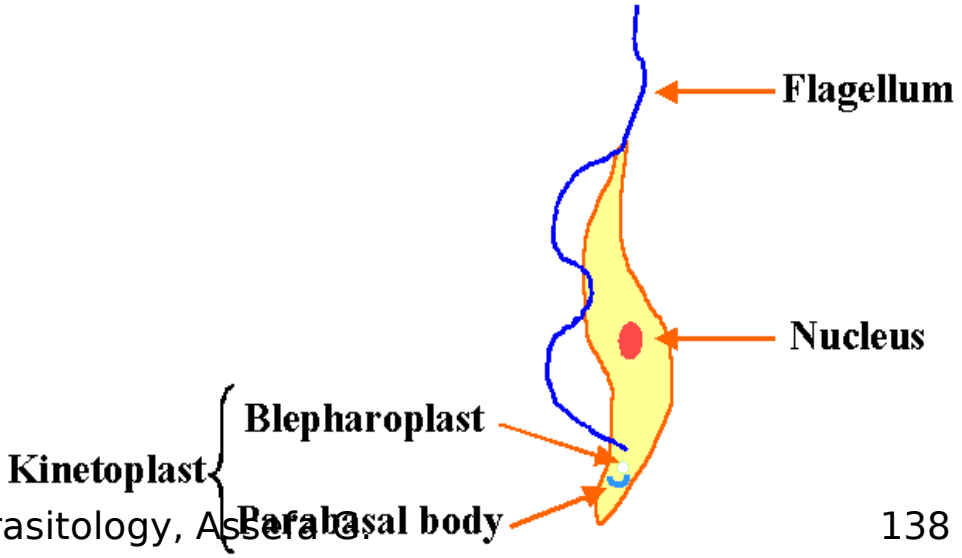
3.Epimastigote /crithidial/ forms

- Elongated body, single free flagellum, single nucleus
- Has undulating membrane,
- kinetoplast is just anterior to the nucleus
- found in the invertebrate host and in culture media (of *Trypanosome* species)





Trypomastigote



4. **Trypomastigote**

- Pleomorphic, it can be as “U” or “C” shaped,
- Central nucleus, posterior kinetoplast
- single Flagellum arises posteriorly
- Has undulating membrane
- found in the peripheral blood of vertebrates and is the diagnostic stage of *Trypanosome* species.

5. Metacyclic Trypomastigote /Trypanosomal/ Forms.

- Morphologically similar to trypomastigote stage but it is short and stumpy

- Final developmental stage in the gut of the insect vectors

- Infective stage of *Trypanosomes* species

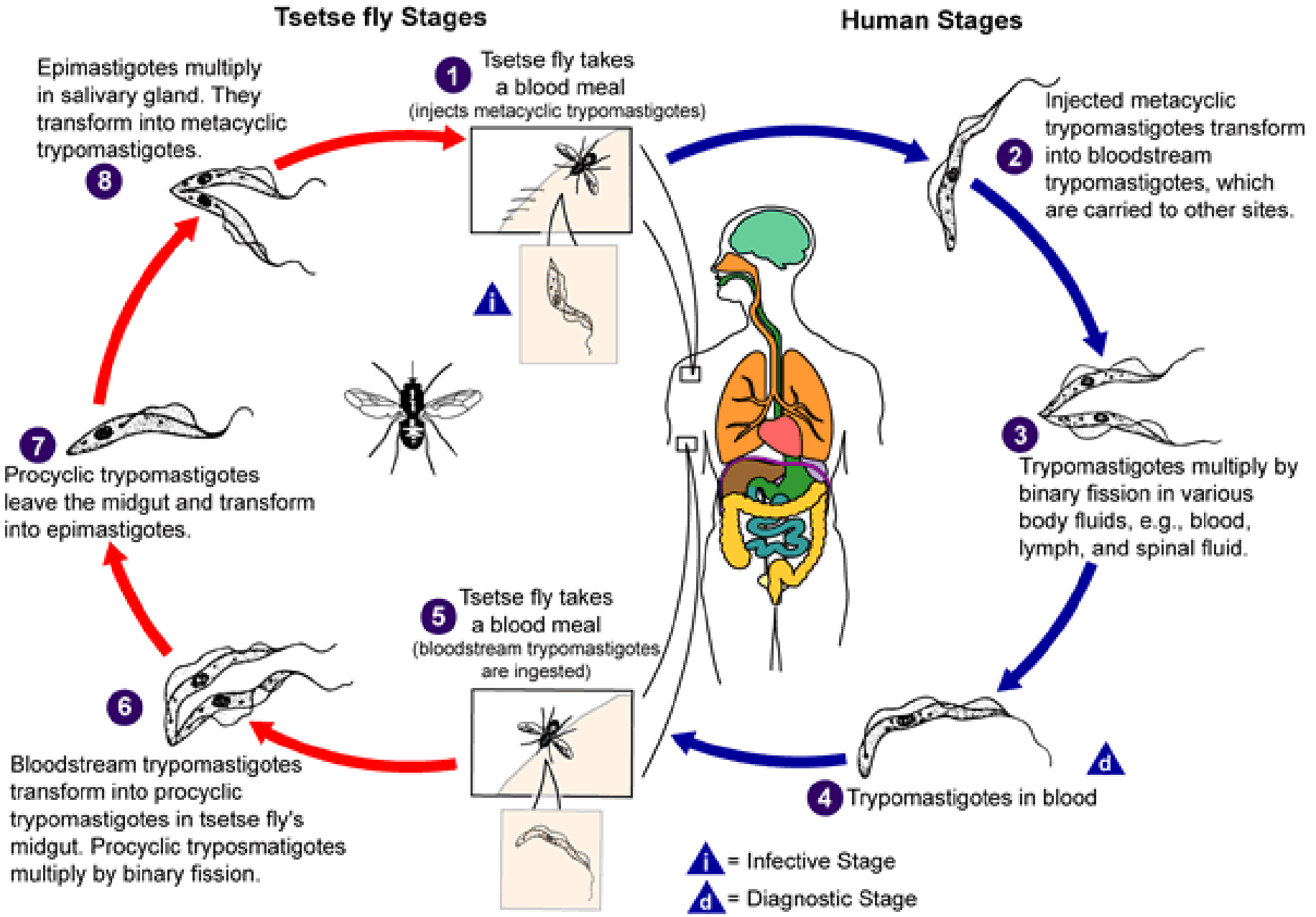
Distribution

- African typanosomiasis occurs in tsetse fly areas of sub-Saharan Africa (14^0 and 20^0 south)
- Tsetse fly occurs only in Africa
- *T.b. gambinese:* - Found in western central Africa, West Africa, extending from Senegal across to Sudan down to angola.
- *T.b. rhodesiense:*- Found in east Africa, central Africa, southern Africa, from Ethiopia down to Botswana
- *Distribution* of both species over laps in the region of great lakes Ethiopia. T.b rhodesiense is found around Gambela along Baro, Gilo (Akobo rivers) ,Gamegofa, Kefa (Maji) Welaga (Anger Didesa valley)

Transmission

- The disease is transmitted by the bite of tsetse fly of genus glossina (both male and female tsetse fly)
- **Other means of transmission**

- Blood transfusion (fresh blood)
- Congenital transmission – rare (T.b. gambiense)


Tsetse fly Stages
Human Stages
Epimastigotes multiply in salivary gland. They transform into metacyclic trypomastigotes.
8
1 Tsetse fly takes a blood meal
(injects metacyclic trypomastigotes)
i
Injected metacyclic trypomastigotes transform into bloodstream trypomastigotes, which are carried to other sites.
2
3
Trypomastigotes multiply by binary fission in various body fluids, e.g., blood, lymph, and spinal fluid.
7
Procyclic trypomastigotes leave the midgut and transform into epimastigotes.
5 Tsetse fly takes a blood meal
(bloodstream trypomastigotes are ingested)
6
Bloodstream trypomastigotes transform into procyclic trypomastigotes in tsetse fly's midgut. Procyclic tryposmatigotes multiply by binary fission.
d
4 Trypomastigotes in blood
i = Infective Stage
d = Diagnostic Stage

Pathology and clinical features

- **Clinical features**

- Development of pain full swelling called *chancre*
- Irregular fever with shivering
- Sweating
- Persistent head ache
- Pain in neck, shoulders and calves
- Swollen lymph gland

Cont…

- **Pathology**

- Splenomegally
- Oedema of the eyelids and face
- Sleeplessness
- Meningoencephalitis
- Myocardial problem
- Scratching of skin
- Hematological change
  - fall in haemoglobin
  - Reduction of platelet number

Treatment

- Treatment should be started as soon as possible and is based on the infected person's symptoms and laboratory results.

- The drug regimen depends on the infecting species and the stage of infection.

- Pentamidine isethionate* and suramin are the drugs of choice to treat the hemolymphatic stage of West and East African Trypanosomiasis, respectively.

Laboratory Diagnosis

1. Examination of blood for trypanosomes
2. Examination of aspirate from enlarged lymph glands for trypanosomes gambiense trypanosomiasis
3. Examination of CSF for trypanosomes
4. Examination of chancre fluid in early stage
5. Serology for antibodies e.g. ELISA, IFA, card agglutination tests, etc
6. Other supportive test

- Hemoglobin decrease
- Reticulocyte increases
- ESR increases
- Total and differential WBC (leukocytosis with monocytosis)
- Urine analysis for - protein, Cells, Casts

Prevention and control

1. Detecting and treating human infections at an early stage
2. Identification of infected reservoir hosts
3. Vector control insecticide and using insecticide impregnated bed net
4. Sitting human settlement away from tsetse fly infected areas
5. Spraying vehicles with insecticide as they enter and leave tsetse fly infested areas.
6. Health education

B. Leishmaniasis Species

- Causative agent of Leishmaniasis are obligate intracellular protozoa of the genus ***Leishmania***

- They reside as intracellular **amastigotes** within macrophages in mammals and as extracellular **promastigotes** in the gut of their insect vectors, phlebotomine sand flies

- Named after Leishman, who first described it in London in May 1903

- Human infection is caused by about 21 of 30 species that infect mammals. These include:
  - *L. donovani* complex with 3 species
    - ✓*L. donovani,*
    - ✓ *L. infantum,*
    - ✓ *L. chagasi*;
  - *L. mexicana* complex with 3 main species
    - ✓*L. mexicana*
    - ✓ *L. amazonensis*
    - ✓ *L. venezuelensis*;

- *L. braziliensis complex*
  - L *braziliensis*
  - *L. Peruviana*

- *L. Guyanensis complex*
  - *L. Guyanensis*
  - *L. panamensis*

- *L. tropica; L. major & L. aethiopica*

- Leishmaniasis can easily classified clinically as
  - **Visceral leishmaniasis**
  - **Cutaneous leishmaniasis**
  - **Mucocutaneous leishmaniasis**
  - **Diffuse cutaneous leishmaniasis**

- These different forms of the disease is caused by the different species of *Leishmania*

- **Cutaneous leishmaniasis(CL)**
  - *L. tropica*
  - *L. major*
  - *L. aethiopica*
  - *L. panamensis*
  - *L. guyanensis*
  - *L. peruviana*

- **Visceral leishmaniasis(VL)**
  - *L. donovani*
  - *L. infantum*
  - *L. Chagasi*

- **Mucocutaneous leishmaniasis(MCL)**
  - *L. panamensis*
  - *L. guyanensis*
  - *L. Brazilliensis*

- **Diffuse cutaneous leishmaniasis(DCL)**
  - *L. amzonensis*
  - *L. aethiopica*

Epidemiology

- 350 million people are at risk in 88 countries around the world
  - ✓72 of which are developing countries
- an estimated 12 million cases world wide ;1.5 to 2 million new cases occur every year
  - ✓CL form representing 50 to 75% of all new cases

- Most of the affected countries are in tropics and sub tropics
  - 90% of all VL cases occur in Bangladesh, Brazil, India, Nepal and Sudan;
  - 90% of all MCL cases occurs in Bolivia, Brazil and Peru
  - 90% of all CL cases occur in Afghanistan, Brazil, Iran, Peru, Saudi Arabia and Syria

- Geographical distribution of leishmaniasis is limited by:
  - The distribution of the sandfly,
  - Its tendency to take blood from humans
  - Its capacity to support the internal development of specific species of *leishmania*

- The incidence of leishmaniasis is increasing, mainly because of:
  - Man-made environmental changes
  - Poverty and malnutrition
  - Movement of susceptible populations into endemic areas

- **In Ethiopia**
  - Four species of *Leishmania is found*, namely,
    - *L. aethiopica,*
    - *L.major*
    - *L. tropica*
    - *L. donovani*

- **Visceral leishmaniasis (VL)**
  - Occurs mainly in arid and semiarid lowlands below 1,300 m altitude
  - Important endemic foci include
    - Gelana focus at lake abaya,
    - segen valley (Aba- Roba focus) in Knoso Wereda
    - Omo river plains
    - Metema and Humera plains in north western Ethiopia

- **Cutaneous leishmaniasis**
  - Endemic at altitudes between 1400 and 2700 m in most administrative regions
  - Prevalence rates of 5.5 – 40% were reported from villages in Shewa , Wello and G.Gofa with the highest rate in Ocholo village in G. Gofa
  - rock hyrax (*Procavia habessinica*) and tree (*Heterhyrax brucei*) hyraxes serving as reservoir host for *L. aethiopica*

Transmission and life cycle

- Common mode of transmission.
  - *Leishmania species are transmitted by female sand flies of* the genus *Lutzomyia in the Americas and Phlebotomus in* Old world
- Rarely, transmission occurs in utero or at the time of birth, as a result of blood transfusion, by direct person-to-person contact, or as a consequence of a laboratory accident.

.



Life cycle
Two Life-cycle stages

Promastigote

-**Elongated, with flagella (10-20 µm long)**

-Occur extracellularly in the

-insect midgut & in artificial culture

-Motile

Amastigote

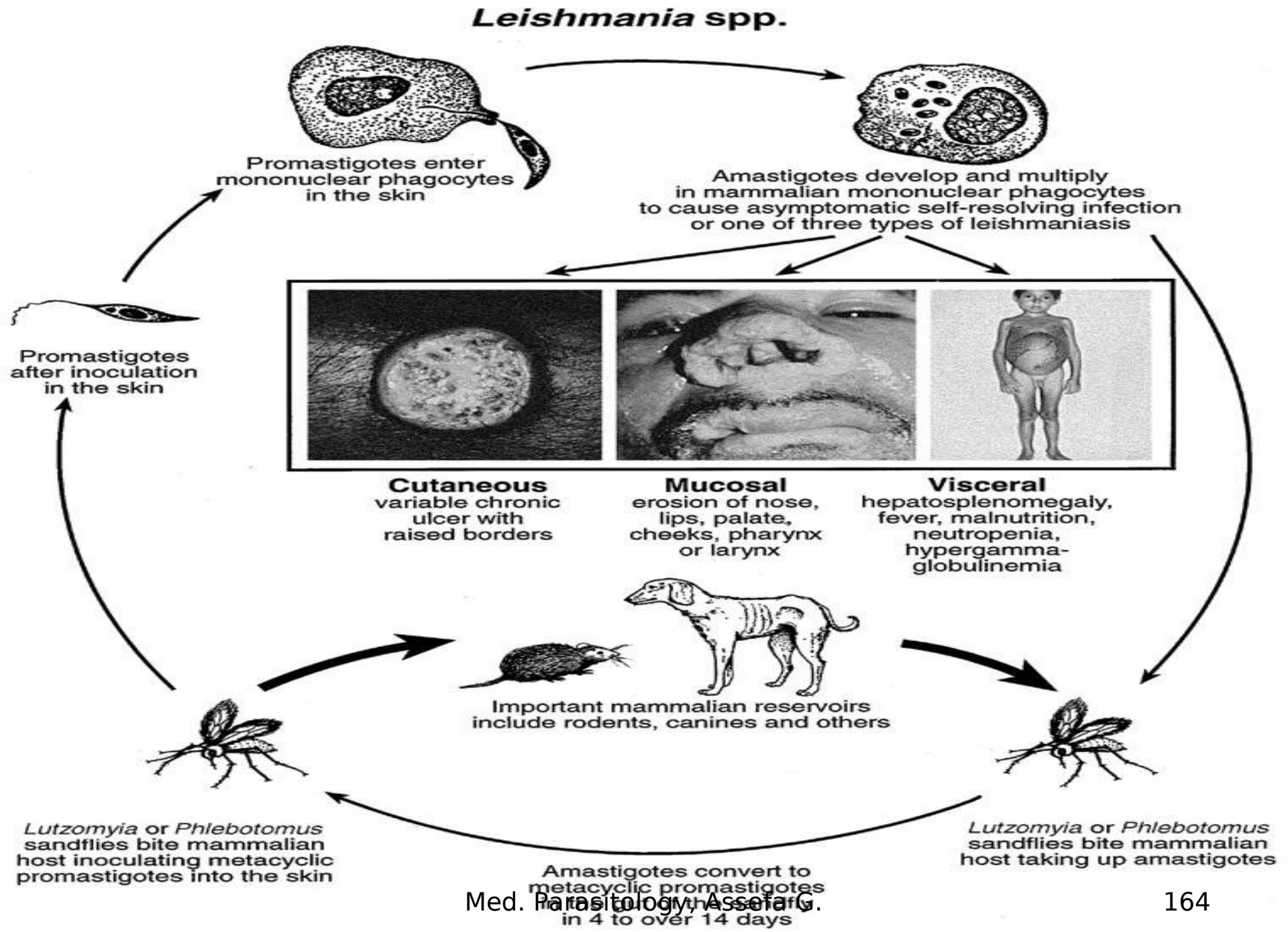
-**Round (3-7 µm diameter)**

-Occurs intracellularly,

during mammalian stage

-Non-motile

Leishmania spp.



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Mammalian Reservoir Hosts

- **Rodents**
- **Gerbils**
- **Hyraxes**
- **Bats**
- **Porcupines**
- **Opossums**
- **Sloths**
- **Primates**
- **Dogs**
- **Foxes**
- **Anteaters**

General life cycle of *Leishmania* species



- Female sandflies inject the infective stage, promastigotes, during blood meals
  - about 30 species of sand flies acts a



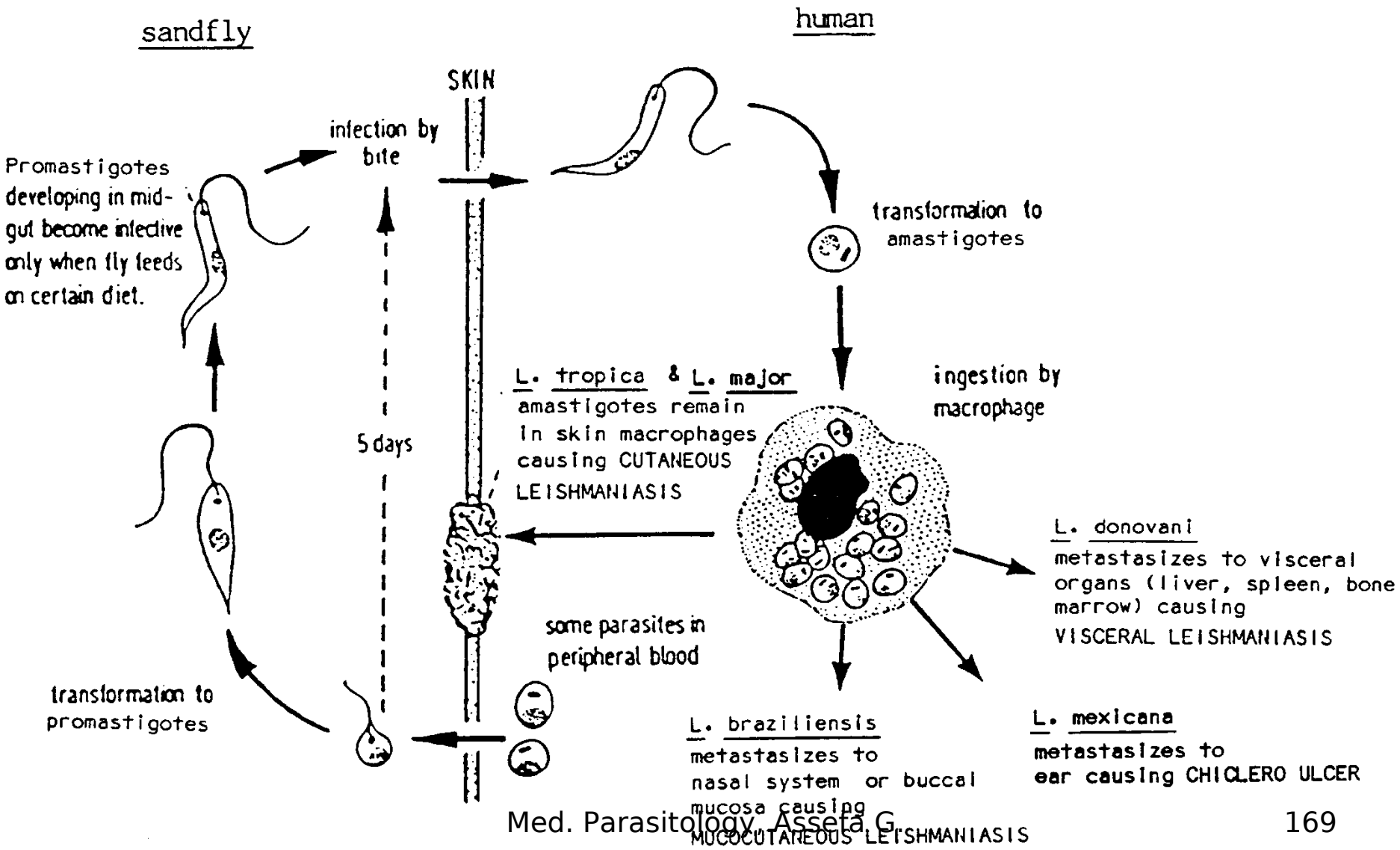
- Promastigotes are phagocytized by macrophages & transform into intracellular amastigotes form

- Amastigotes multiply by binary fission, rapture from macrophages , and infect new cells
- **In VL** the amastigotes are carried through blood circulation , then invade and multiply in the macrophages of spleen, liver, Bone marrow, lymph glands , etc.
- **In Cl , MCL** – the amasigote multiply in skin macrophages (histocytes)

- Sandflies become infected during blood meals when they ingest macrophages infected with amastigotes

- Host cell break down and releasing the amasigotes which is then transform to promastigotes

- Multiply , fill the lumen of the gut and migrate to the proboscis


sandfly
human
SKIN
infection by bite
Promastigotes developing in mid-gut become infective only when fly feeds on certain diet.
transformation to amastigotes
L. tropica & L. major amastigotes remain in skin macrophages causing CUTANEOUS LEISHMANIASIS
ingestion by macrophage
5 days
L. donovani metastasizes to visceral organs (liver, spleen, bone marrow) causing VISCERAL LEISHMANIASIS
some parasites in peripheral blood
transformation to promastigotes
L. braziliensis metastasizes to nasal system or buccal mucosa causing MUCOCUTANEOUS LEISHMANIASIS
L. mexicana metastasizes to ear causing CHICLERO ULCER

Host-Parasite Interactions

- Entrance into the host and establishment of infection by leishmanias is enhanced by saliva from the vector

- Two substances were involved
  - ✓ **maxadilin,** or maximum dilation molecule: keeps the capillary bed open
  - ✓ **SIP** or salivary immunosuppressive protein : restrains the immune system's early efforts to eliminate the parasites

Cont…

- Metacyclic promastigotes obstruct the proboscis of leishmania-infected sand flies, preventing easy aspiration of blood and stimulating the fly to probe even more.

- Sand fly saliva has been shown to enhance the infectivity of promastigotes by inhibiting the L-arginine-dependent nitric oxide killing mechanism of macrophages

Cont…

- Infective promastigotes entering the blood of the vertebrate are covered by two key molecules:
  - **protein gp 63** and
  - **lipophosphoglycan (LPG)**
    - Both mediate the uptake of promastigotes by macrophages

- The promastigotes are engulfed & form **phagosome**

Cont…

- phagosome + lysosome to form a **phagolysosome**

  - ✓ As the **promastigotes** transform into **amastigotes**, which produce compounds that counter lysosomal enzymes

  - ✓ The **gp 63** molecule inactivates proteolytic enzymes

  - ✓ **LPG** protects against other enzymes

Clinical features and pathology

- A spectrum of findings is observed within each of the three major clinical syndromes: cutaneous, mucosal, and visceral leishmaniasis

- Each of these syndromes is associated with more than one *Leishmania species, and any given species* is capable of producing more than one syndrome

Cont…

1. Cutaneous leishmaniasis (CL)

- most common form,

- The typical lesion of cutaneous leishmaniasis develops at the site where promastigotes are inoculated by an infected sand fly

- Relatively benign, self-healing skin lesions (localized or simple CL)

- The classic wet lesion of *L. major is "pizza-like," with* a raised outer border, granulating base, and overlying white, purulent exudate

- Biopsies of cutaneous lesions demonstrate a spectrum of findings . From macrophages containing intracellular amastigotes to lymphocytes with granuloma formation

- **Causative agents**

- *Leishmania tropica*
- *Leishmania major*
- *Leishmania aethiopica*
- *Leishmania mexicana*
- *Leishmania peruriana*
- *Leishmania panamensis*
- *Leishmania guyanensis*



L. aethiopica

2. Diffuse Cutaneous Leishmaniasis

- Lesion develop over large areas of the body
- resembling lepromatous leprosy
- Scaly, not ulcerated, nodules
- Chronic and painless
- Numerous parasites in lesions
- Seldom heal despite treatment

3.Mucocutaneous Leishmaniasis



- primarily *L. braziliensis* (espudia)
- two stages
  - simple skin lesion
  - 2^{o} mucosal involvement
- metastasis via blood or lymphatic systems
- can occur long after primary lesion (up to 16 years)
- frequently in naso-pharyngeal mucosae
  - junction of skin and mucosa





Cont…

***L. braziliensis* (espudia)**

- variable types and sizes of lesions
  - chronic and painless
- ulcerative type
  - rapid and extensive mutilation





Cont…

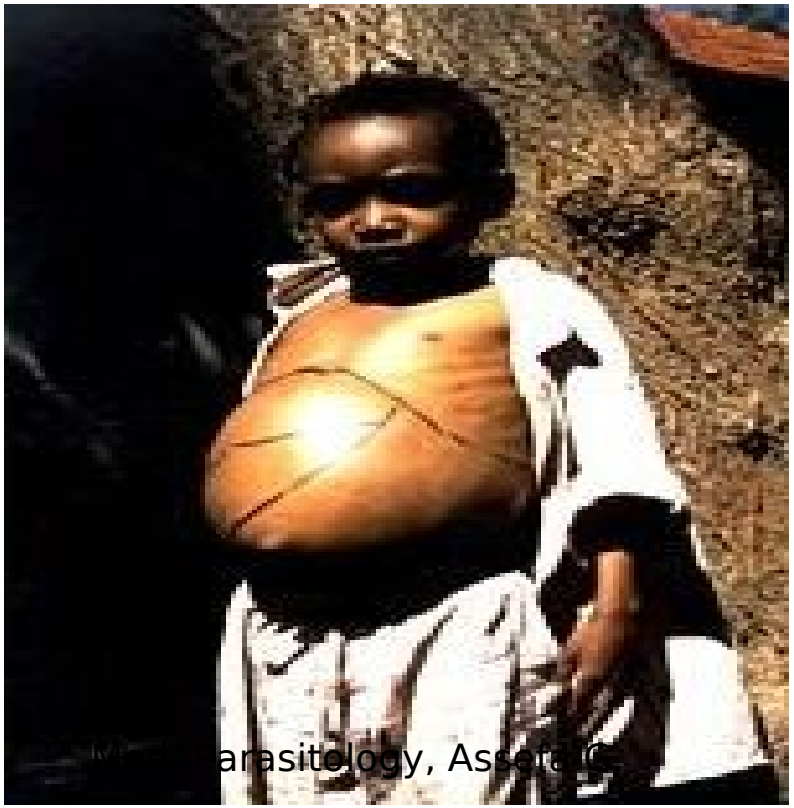
- *L. braziliensis* (espudia)
- variable types and sizes of lesions

- ulcerative type
  - rapid and extensive mutilation

- non-ulcerative type
  - local edema (upper lip)

4.Visceral Leishmaniasis (VL)

- Generalized infection of the reticuloendothelial system,
- High mortality response
- The majority of infections are in apparent and self-resolving; a subset smolder with mild symptoms



Cont…

- Minority progress to full-blown visceral leishmaniasis, which is characterized by fever, weight loss, hepatosplenomegaly, neutropenia, and hypergammaglobulinemia
- Onset is usually insidious but it can be abrupt
- Symptoms may be present for weeks to months before patients come to medical attention in rural, endemic areas
- Lab findings include :Anemia, neutropenia, thrombocytopenia,eosinopenia, and pronounced hypergammaglobulinemia ,elevated liver enzymes and bilirubin

Visceral Leishmaniasis

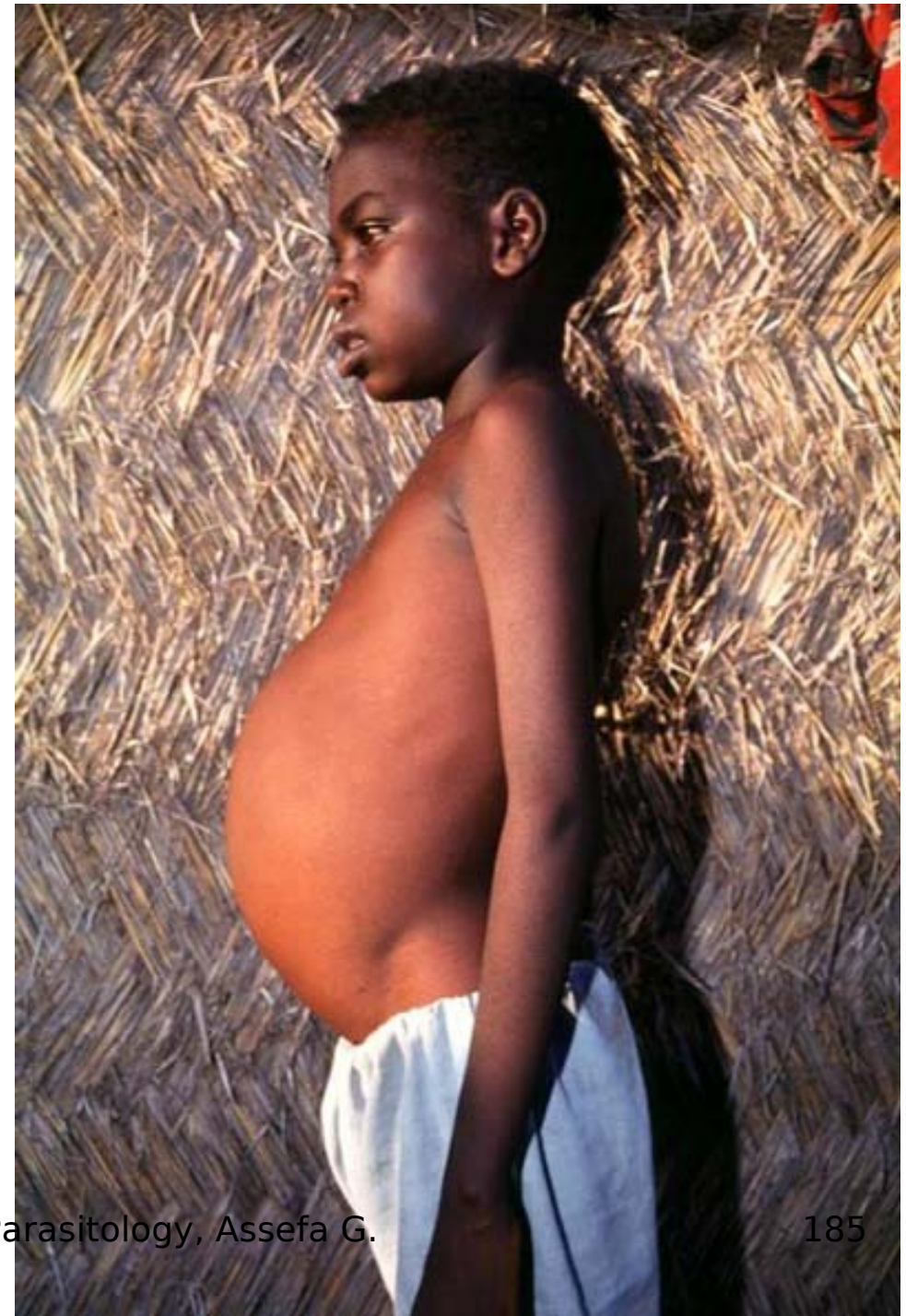
- caused by the *Leishmania donovani* complex,
  - ✓ *Leishmania donovani*
  - ✓ *L. infantum*
  - ✓ *L. chagasi*
- reticuloendothelial system affected
  - spleen, liver, bone marrow, lymph nodes
- onset is generally insidious
- progressive disease
  - 75-95% mortality if untreated
  - death generally within 2 years



Clinical Presentation

- incubation period
  - generally 2-6 months
  - can range 10 days to years
- fever, malaise, weakness
- wasting despite good appetite
- spleno- and hepatomegaly, enlarged lymph nodes
- depressed hematopoiesis
  - severe anemia
  - leucopenia
  - thrombopenia

- Profile view of a teenage boy suffering from visceral leishmaniasis. The boy exhibits splenomegaly, distended abdomen and severe muscle wasting.

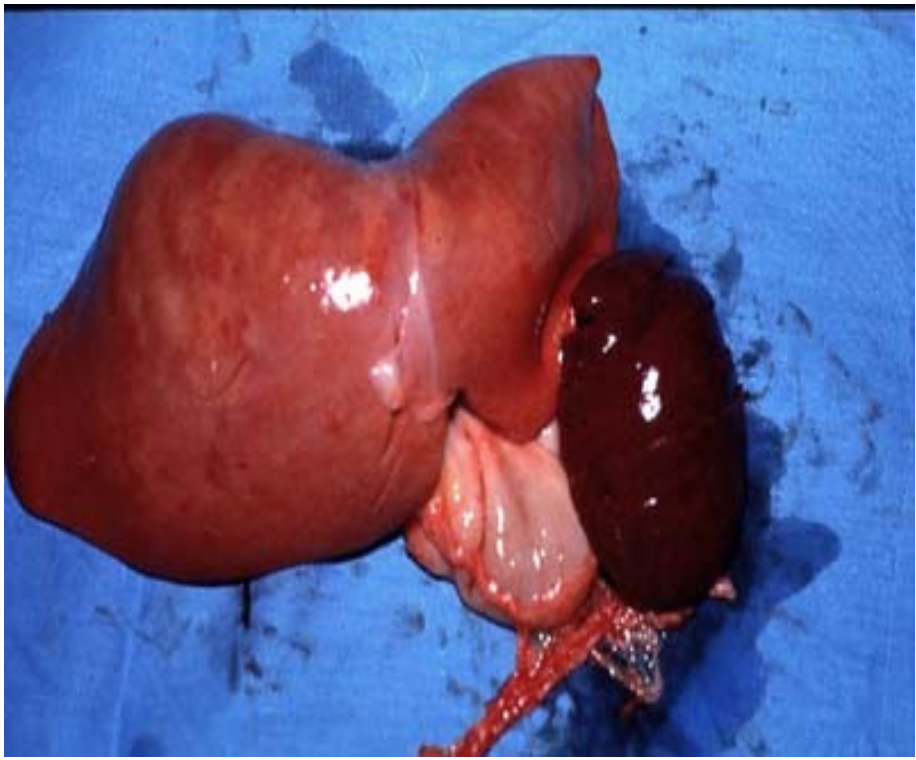


- A 12-year-old boy suffering from visceral leishmaniasis. The boy exhibits splenomegaly and severe muscle wasting





- Jaundiced hands of a visceral leishmaniasis patient



- Enlarged spleen and liver in an autopsy of an infant dying of visceral

Post Kala Azar Dermal leishmaniasis (PKDL)

- ✓ characterized by hypo pigmented and raised erythematous patches on the face, trunk of the body and limbs
- ✓ may develop in to nodules and resembles those of lepromatous leprosy, fungi infections or other skin disorders
- ✓ Occasionally there is ulceration of lips and tongue

Cont…

- ✓ occurs in 1-3% of Indian and 50% of Sudanese VL patients
- ✓ It require expensive and prolonged treatment
- ✓ easily cured with treatment (in contrast to DCL)

Leishmaniasis and HIV Infection

- Coexistence of leishmaniasis with HIV infection is a serious concern
- Leishmaniasis is spreading in several areas of the world because of the rapidly spreading epidemic AIDS
- The immune deficiency has lead to increased susceptibility to infections, including leishmaniasis

- Co-infection with HIV has lead to the spread of leishmaniasis, typically a rural disease, into urban areas

- In patients infected with HIV, leishmaniasis accelerates the onset of AIDS
  - by cumulative immunosuppression and
  - by stimulating the replication of the virus

- It may also change asymptomatic *Leishmania* infections into symptomatic infections

Diagnosis of CL, MCL, DCL

- suspected because of:
  - Geographical presence of parasite
  - History of sandfly bite
  - Skin lesion:
    - chronic, painless, ‘clean’ ulcer
    - nasopharyngeal lesions
    - nodular lesions

1. Demonstration of parasite amastigotes (scrapings, biopsy, aspirates)
2. culture from ulcer material
3. Leishmainin test

make incision in active part of lesion



scrape cells from incision



DIAGNOSIS Visceral leishmaniasis

(i) Demonstration of parasite in tissues by

- light microscopic examination of the stained specimen,
- culture
- animal inoculation

(ii) Detection of parasite DNA in tissue samples

(iii) immunodiagnosis by detection

- Antigen detection
- antibody detection

Treatment

- Sodium stibogluconate (Pentostam)
- Pentamidine isethionate
- Amphotericin B
- Cryotherapy and thermotherapy

Prevention and control

1. Early detection by serological diagnosis (VL) and treatment of infected persons

2. Personal protection from sandfly bites by:
   - Using insect replants
   - Avoiding endemic areas especially at times when sandifies are most active
   - Use of pyrethroid impregnated bed nets and curtains

3. Vector control by the use of light traps, sticky paper traps, or residual insecticide spraying of houses

4. Destruction of stray dogs and infected domestic dogs

5. Elimination and control of rodents

6. Sitting human dwellings away from the habitats of animal reservoir hosts where sandifies are known to breed