

# BIO 475 - Parasitology

## Spring 2009

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Northern Arizona University

<http://www4.nau.edu/isopod>

### Lecture 6

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## History of Control Mechanisms

- 1902 Ford and Dutton, two English physicians working in The Gambia, identified one causative agent of trypanosomiasis, a parasite which they named *Trypanosoma brucei gambiense*.
- 1903 Castellani working in Uganda observed the parasite in the cerebrospinal fluid of one of his patients. In the same year, the tsetse fly was recognized by David Bruce as being the vector of the parasite.



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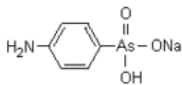
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## History of Control Mechanisms



- 1906 Ayres Kopke introduced an arsenic compound, Atoxyl, for the treatment of the disease.
- 1920 Jamot, a colonel in the French army working on trypanosomiasis control, observed that in the Ubangi river loop more than half of all deaths were due to sleeping sickness. The major epidemics early in the century claimed hundreds of thousands of lives. Entire populations were affected, and indeed Jamot reported that a whole ethnic group had been wiped out in northern Congo.
  - 1924 Tryparsamide, a drug still based on arsenic but less toxic than Atoxyl, was used on a wide scale in Belgian Congo and Cameroon.
  - 1930 A headline stated: "Our doctors have vanquished the tsetse fly!"

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## History of Control Mechanisms

•1932 700 patients became blind after receiving the wrong dose of Atoxyl. In response to this disaster, Professor Friedheim, a Swiss physician and chemist, developed the drug melarsoprol, the bold concept of which was a single product containing a highly toxic arsenic-based molecule and its antidote.

•1960 Melarsoprol was used systematically in cases where there was involvement of the central nervous system.

•1984 The World Health Organization (WHO) launched a programme to control trypanosomiasis.

•1993 WHO developed the central African initiative, a major project for regional approach to Sleeping Sickness in ten countries: Angola, Cameroon, Central African Republic, Congo, Gabon, Equatorial Guinea, Uganda, Sudan, Chad and Zaire.



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## Current Control Mechanisms

1. Certain drugs are effective, but only on early stages of trypanosomes.
  - a. Arsenic drugs.
  - b. Difluoromethylornithine (DFMO)
2. Elimination of *Glossina* resting sites - high vegetation.
  - a. Expensive and needs to be repeated.

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## Current Control Mechanisms



3. Insecticides
  - a. Tend to cause the development of resistant *Glossina* lineages.
1. Use of native animals! – but viewed as too costly to some.

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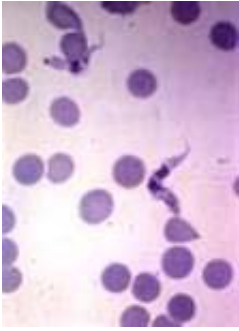
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## Other Trypanosomes



1. *T. congolense* - causes disease in cattle similar to nagana.

- a. Recognized by lack of flagellum
- b. Can be transmitted mechanically by cattle flies, *Tabanus*.

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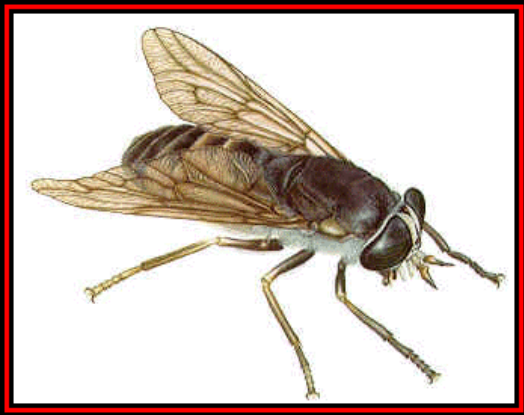
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## Other Trypanosomes



2. *T. evansi* - infects camels, horses and other ungulates.

- a. Introduced to Western Hemisphere by Spaniards.
- b. causes *surra*.



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## Other Trypanosomes

3. *T. equinum*, related to *T. evansi*

a. Mechanically transmitted by *Tabanus*.

b. Also transmitted by vampire bats - causes *murrina*.



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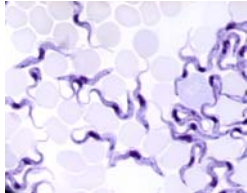
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## Other Trypanosomes



4. *T. equiperdum* - venereal disease of horses and donkeys.

a. Transmitted by coitus, causes swelling and depigmentation of genitalia

b. Fatal if untreated.

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## Section Stercoraria

1. These are trypanosomes transmitted via posterior station.

a. Transmitted mainly by blood-feeding bugs (Hemiptera).

b. Metacyclic trypomastigotes develop in hindgut of bug.

c. Usually from Family **Reduviidae**.

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## Reduviid Bugs



1. Many species defecate during feeding, host rubs feces into wound.
- a. Trypanosomes also seem to be transmitted among bugs
  - horizontal transfer.
  - 1. Cannibalism occurs.
  - 2. Possible transmission during mating.

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*Triatoma infestans*



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## Habitat of *T. infestans*



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## Important species

1. *Trypanosoma cruzi* - Chagas' Disease
  - a. Named for its discoverer, Carlos Chagas in 1910.
  - b. Discovered them in bugs first before it was recognized as a disease.



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Chagas was sent by the Director of a new Medical Institute in Rio de Janeiro in Brazil, **Oswaldo Cruz**,



to carry out an antimalaria campaign in support of the construction of a rail line in the State of Minas Gerais.



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Though Chagas was convinced he had found the vector of a human disease he did not know what that disease was. In 1909, two or three weeks after finding triatomines and a cat infected with *T. cruzi* he was called to treat a seriously ill 2 year old child named Berenice.

She was feverish, had an enlarged spleen, liver and swollen lymph nodes and her blood teemed with trypanosomes similar in morphology to those found in the marmoset. He wrote: "*Examination between cover glass and slide revealed the existence of flagellates in good number and fixing and staining of blood films made it possible to characterize the parasite's morphology and to identify it with Schizotrypanum cruzi*".



Aged 2 (1908) with Carlos Chagas

•Berenice died in 1975 (aged 69)  
—not of Chagas Disease

•Isolates cultured from her in 1957 and 1973 indicate that she was infected multiple times

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After he had been there for one year, a railroad engineer, named Cantarino Mota, told him about blood sucking bugs in the local huts, which were called "barbeiros" or "kissing bugs" due to their behavior of biting sleeping people on the face. Chagas then became interested in seeing if this bug could be transmitting some disease to humans or animals.

He examined the hindgut contents of a bloodsucking triatomine bug (*Panstrongylus megistus*), and found numerous flagellates which resembled stages of a trypanosome he has described previously from a marmoset. Chagas sent infected triatomine bugs to the Institute in Rio where they were allowed to bite monkeys and after 30 days large numbers of trypanosomes were found in the peripheral blood. Subsequently, it was found that other animals i.e. rabbits, pigs, dogs and other monkeys, could also be infected.



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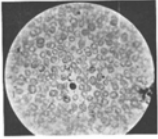
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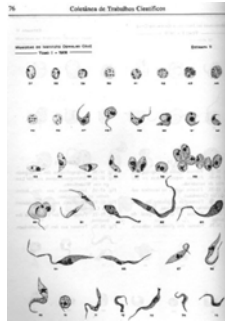
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In 1911 Chagas described the dividing forms in heart muscle. The connection between human disease and the blood-sucking bug had been made. In 1912 Chagas found that the armadillo was a reservoir host.



An original micrograph of *T. cruzi* in human blood



Drawings of the types of cells he found

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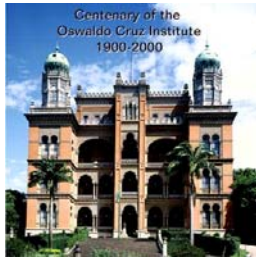
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The species name was given to honor the scientist, Oswaldo Cruz, who was the mentor of Carlos Chagas and the Director of the Oswaldo Cruz Institute where Chagas worked. The disease is named after Chagas. At 29 years of age, Carlos Chagas had described the agent, the vectors, clinical symptoms in humans and animals, and the existence of a new disease.

### Oswaldo Cruz



Unfortunately he made some enemies, including Charles Donovan and a German microbiologist named Krause. Krause denounced Chagas' findings and for 20 years his work was forgotten.

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## *Trypanosoma cruzi*



- a. Chagas thought they underwent schizogony in lungs.
- b. A form of division that results in multiple trypanosomes.

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## *T. cruzi* Infections

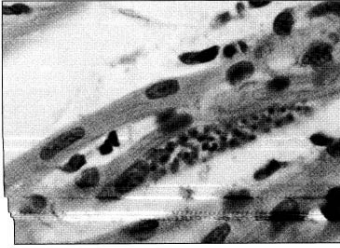


Figure 5.9  
*Trypanosoma cruzi* pseudocyst in cardiac muscle. (×780)

- b. Repeated binary fission causes cells to lyse – infect other cells.
  - 1. Often with intermediate forms interstitially - promastigotes and epimastigotes.

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## *Trypanosoma cruzi*

- b. Chagas named them *Schizotrypanum cruzi*.
  - 1. This is probably the appropriate name by priority.
  - 2. They have an amastigote stage in tissues.
- a. This is part of their unusual pathology

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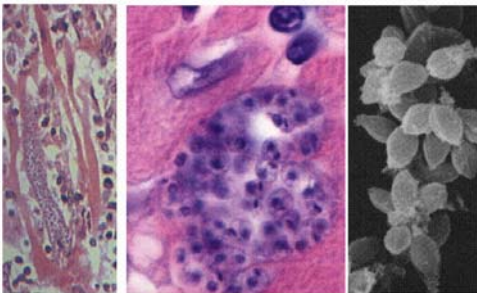
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## *T. cruzi* Amastigote



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## *T. cruzi* Transmission



1. Infected bugs feed and introduce metacyclic trypanosomes in feces.
2. Reproduction of trypanosomes occurs in subcutaneous cells.
  - a. often causes a local swelling or *chagoma*
  - b. local lymph gland swelling.

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## *T. cruzi* Transmission

### Romaña's Sign



2. Can also occur though eye as well.
  - a. Distinctive conjunctivitis is called *Romana's sign*.

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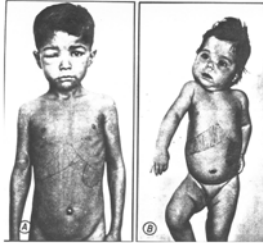
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### Romaña's Sign



### Hepatosplenomegaly



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## *T. cruzi* Life Cycle

1. Trypomastigotes enter blood, are picked up by feeding bug
  - a. In bug gut, become *short epimastigotes*.
  - b. Replicate to become *slender epimastigotes*.

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## *T. cruzi* Life Cycle

2. Later (8-10 days) become *metacyclic trypomastigotes*.
3. Become infective from other bites.

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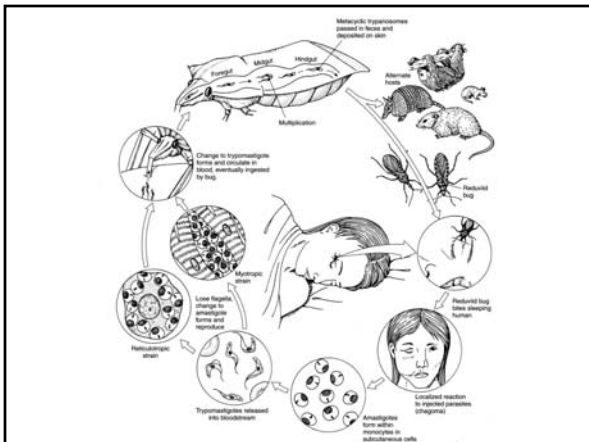
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## ***T. cruzi* Infections**

1. Trypanosomes are initially abundant in blood but then are rapidly cleared by immune system.
  - a. Cryptic infections diagnosed by allowing cleared bugs to feed and examining them.
  - b. When seen in blood, tryps have an "S" or "U" shape.

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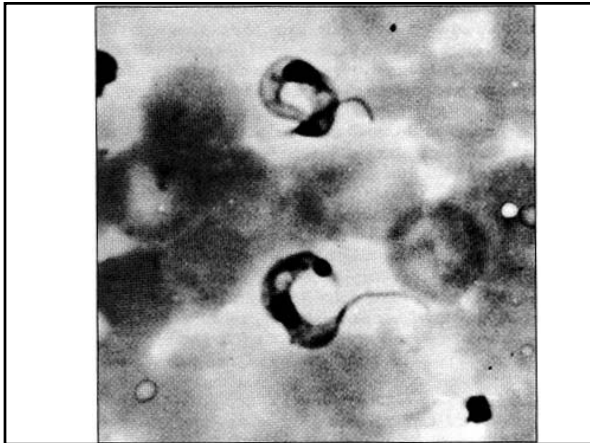
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## The ACUTE phase of *Trypanosoma cruzi* infection

1. Romana's Sign
2. Fever
3. Hepatosplenomegaly
4. Trypomastigotes in Blood
5. Lasts 2-8 weeks
6. 10% Mortality

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## *T. cruzi* Infections

2. Contrary to Chagas, do not replicate until they enter cells of spleen or smooth/cardiac muscle.
  - a. Then form *amastigote* forms - pseudocysts *full* of amastigotes

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## *T. cruzi* Infections

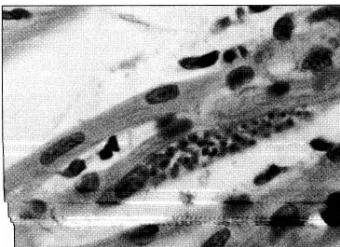


Figure 5.9  
*Trypanosoma cruzi* pseudocyst in cardiac muscle. (×780)

- b. Repeated binary fission causes cells to lyse – infect other cells.
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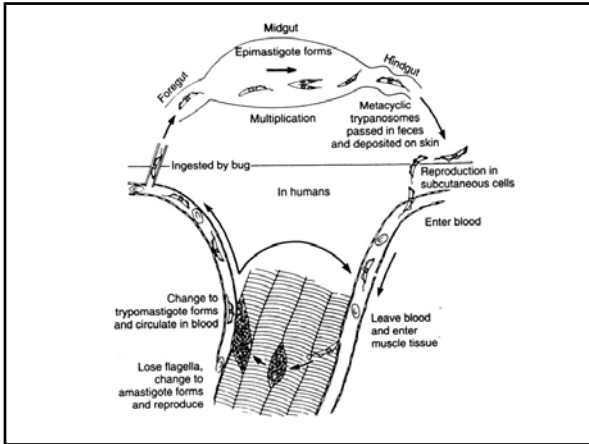
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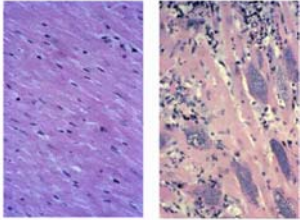
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### The INDETERMINATE phase

1. No parasite evident in blood
2. Amastigote nests in muscle tissue
3. Anti-*T. cruzi* IgG present



Normal Heart

Chagasic Heart

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### CHRONIC phase of Chagas Disease

1. Nerve Degeneration
2. Cardiomyopathy (80%)
  - Heart arrhythmia and blocks
  - Heart enlargement (cardiomegaly)
  - Apical aneurism
3. Megaesophagus (25%)
4. Megacolon (30%)

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## T. cruzi: The Disease Process

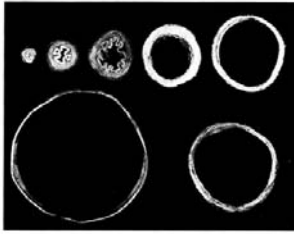


Figure 5.12  
Different stages of chagasic esophagopathy beginning with a normal organ and passing through hypertrophy and dilatation to the final megacolon.  
From M. S. R. Hut, F. Koberle, and K. Salcedo, in H. Spencer, editor, *Tropical Pathology*, 1973 Springer Verlag.

1. Is more severe in younger people.

a. Tissue destruction due to *pseudocysts*

1. Especially esophagus, colon stretched out – *megacolon*.

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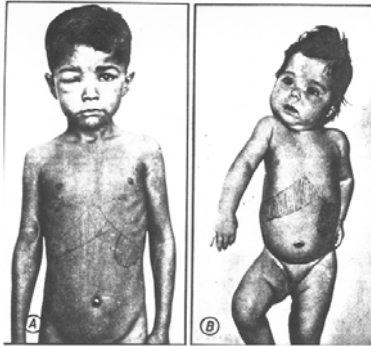
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## Hepatosplenomegaly



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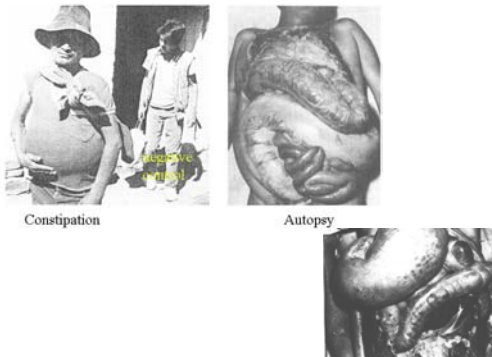
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## Megacolon



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# T. cruzi: The Disease Process

2. Heart, brain -  
loss of function.

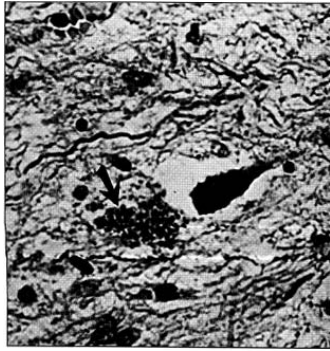


Figure 5.10  
Pseudocyst of *Trypanosoma cruzi* in brain tissue.

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## Cardiomegaly

## Apical Aneurysm

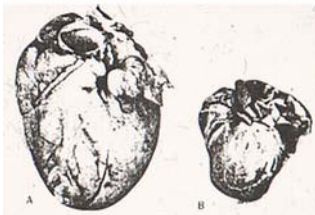


Fig. 13.11 — A — Coração de indivíduo falecido com miocardiite chagásica crônica; notar a cardiomegalia. B — Coração de indivíduo normal. (Segundo Jairo Ramos e cols.)

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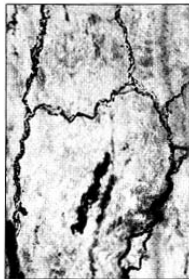
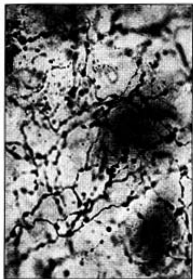
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(a)

(b)

Figure 5.11  
Diaphanized tricuspid valves with zinc-osmium impregnation of nerve fibers (dark lines). (a) Normal heart; (b) Chagas' cardiopathy with marked reduction of nerve fibers.

From M. S. R. Hutt, F. Koberle, and K. Salfelder, in H. Spencer, editor, *Tropical Pathology*, 1973 Springer Verlag.

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# T. cruzi Distribution



1. New world, where there are biting bugs a. Has been reported in Verde Valley b. Travel has caused spread to other areas of world.

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## Fecal transmission of T. cruzi



There are several genera of Reduviidae that can transmit the protozoan. *Triatoma infestans*, as its name suggests, frequently invades homes (mud and stick huts) and is responsible for what is termed the domestic transmission cycle. Other reduviids, such as *Rhodnius prolixus*, reside in rural settings or forests, they are associated with the silvatic cycle of transmission, which includes many wild animals as vertebrate hosts. As mentioned above, the common reduviid vectors *R. prolixus* and *Panstrongylus megistus* defecate during the blood meal, allowing efficient infection by the parasite.



One happy triatomid!

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Evidence that trypanosomiasis existed in the Americas is to be found in 2000 year-old mummies from Chile that show enlarged hollow viscera and cardiac fibrosis.

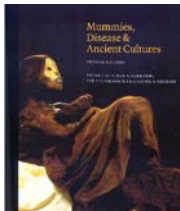


FIGURE 11.11 Mummies of the Americas

Illustrating the effects of trypanosomiasis on the mummies of ancient Chilean and Peruvian cultures.



ABOVE: A Chilean mummy. Enlarged hollow viscera with cardiac fibrosis have been attributed to *Cruzi* disease. Dr. P. Ramirez

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## T. cruzi Treatment

1. Not much available (!) although *ketoconazole* seems to work fairly well experimentally.



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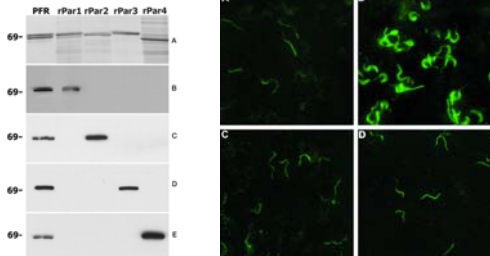
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### Development of a Vaccine against T. cruzi

Vaccines consisting of attenuated parasites, crude parasite lysates, partially purified proteins, and synthetic peptides have been tested with varying success. Some gave partial protection. Crude flagellar extracts were found to induce more than 90% protective immunity in mice and to reduce tissue lesions after a challenge infection. A series of studies from the Manning laboratory since 1995 have defined several protein components of the flagellar apparatus which appear to provide this protection.



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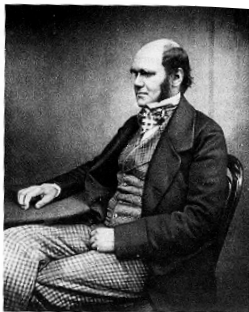
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## Infection of Charles Darwin



1. Correspondence indicates that he was bitten by a wheel bug while in Tierra del Fuego.

2. He reported crushing the insect in disgust after a painful bite.

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## The Voyage of the Beagle

"We slept in the village, which is a small place, surrounded by gardens, and forms the most southern part, that is cultivated, of the province of Mendoza; it is five leagues south of the capital. At night I experienced an attack (for it deserves no less a name) of the *Benchuca* (a species of *Reduvius*) the great black bug of the Pampas. It is most disgusting to feel soft, wingless insects, about an inch long, crawling over one's body. Before sucking, they are quite thin, but afterwards become round and bloated with blood, and in this state are easily crushed. They are also found in the northern parts of Chile and in Peru."

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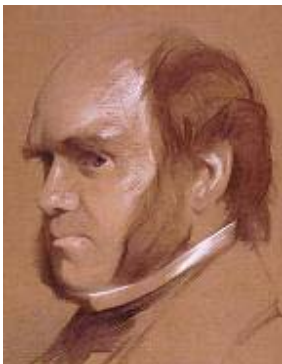
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## Infection of Charles Darwin

This insect was the triatomid, *Triatoma infestans*, of which today more than 70% of the insects in that region are infected with *T. cruzi*. Also 12% of the population in Mendoza today has antibodies against *T. cruzi*.

Darwin returned to his ship and even brought back some of these insects and fed them on the sailors. Darwin was at that time one of the most active members of the Beagle's crew. He often took long overland expeditions and was a mountain climber.



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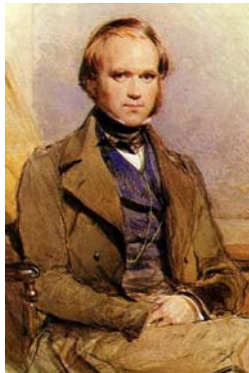
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## Infection of Charles Darwin



He returned to England in 1836 and in 1838 his health suddenly became poor. His health became progressively worse and he suffered from periodic vomiting, fatigue and flatulence. After social dinners he had violent shivering and vomiting attacks, and mainly for these reasons he gave up all social interactions.

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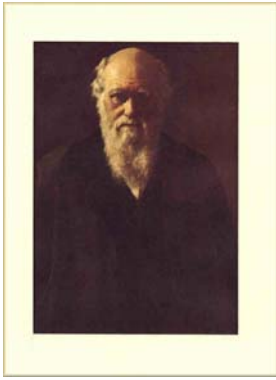
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## Infection of Charles Darwin

His diaries are full of descriptions of his mysterious illness. He wrote to the Botanist, Joseph Hooker, in 1845: "*I believe that I have not had a whole day, or rather night, without my stomach being greatly disordered, during these last three years, and most days great prostration of strength.*" In 1849 he was too ill to attend his father's funeral. He wrote: "*I was quite broken down, head swimmy, hands trembling and never a week without violent vomiting.*"



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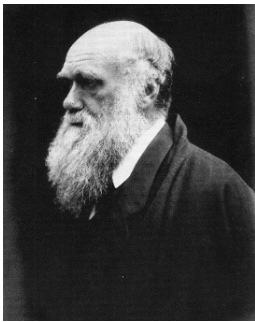
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## Infection of Charles Darwin



2. Later years of his life were marked by weakness, heart palpitations, malaise and flatulence.  
a. These are all symptoms of Chagas disease.

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## Other Stercoraria

1. *T. rangeli* - South American, similar to *T. cruzi*.  
a. Often mixed infections occur.



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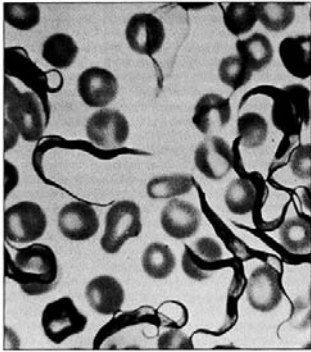
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## Other Stercoraria



2. *T. lewisi* -  
common in rodents,  
especially rats.

- a. Vectored by  
fleas.

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## Other Flagellates

1. Many of species discussed for next few  
lectures are intestinal or mucous membrane  
parasites or commensals

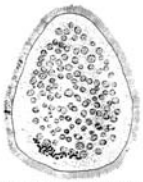


FIG. 4-9. *Opalione rostratum* (X 250). This figure shows many nuclei, some of which are in the process of division. Most of the endosporoblasts have been omitted. (From MacCall, Courtesy of the U.S. National Museum Bulletin.)

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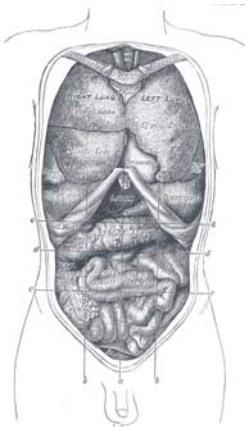
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## The Vertebrate Gut



- a. pH 1.5-8.5
- b. Enzymes and acids
- c. Variable O<sub>2</sub> levels
- d. Peristaltic action
- e. Flushing
- d. Lots of other  
commensals!

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## How Parasites May Cope

1. Attachment.
2. Changing the environment - part of their pathology.
3. Cloaking themselves to prevent detection (minor here).
4. Escape: into intestinal mucosa.

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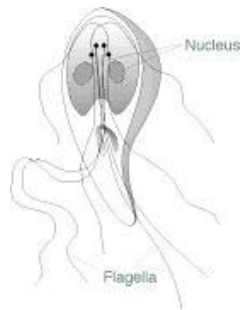
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## Phylum Retortamonada

- a. Intestinal parasites, lacking mitochondria.
- b. Three anterior flagella, one recurrent flagellum



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## *Chilomastix mesnili*

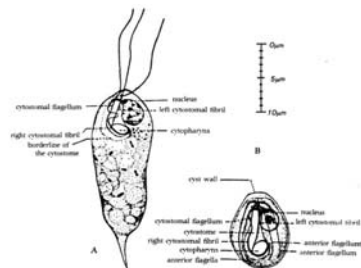


FIG. 3-8. *Chilomastix mesnili* from the guinea pig. A. Trophic flagellate, ventral side, showing body structures. B. Cyst, ventral side. (From Nic. Courtesy of the Journal of Microbiology.)

1. Pyriform gut parasite of humans (other characters in lab and book).
- a. Actively feeding form is *trophozoite*.

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## *Chilomastix mesnili*

b. Usually diagnosed by cysts that form in lower intestine.

c. Considered nonpathogenic, causes mild diarrhea.

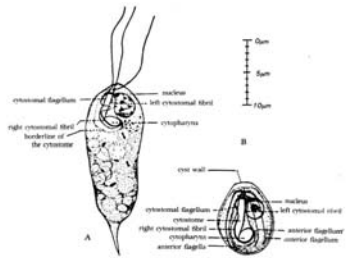


FIG. 3-9. *Chilomastix mesnili* from the guinea pig. A. Trophozoite flagellum, ventral side, showing both structures. B. Cyst, ventral side. (From Ste. Courtesy of the Journal of Microbiology.)

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