TIKRIT UNIVERSITY
COLLEGE OF PHARMACY

MICROBIOLOGYII
MEDICALPARASITOLOGY

MEDICAL PARASITOLOGY

PRESENTED BY
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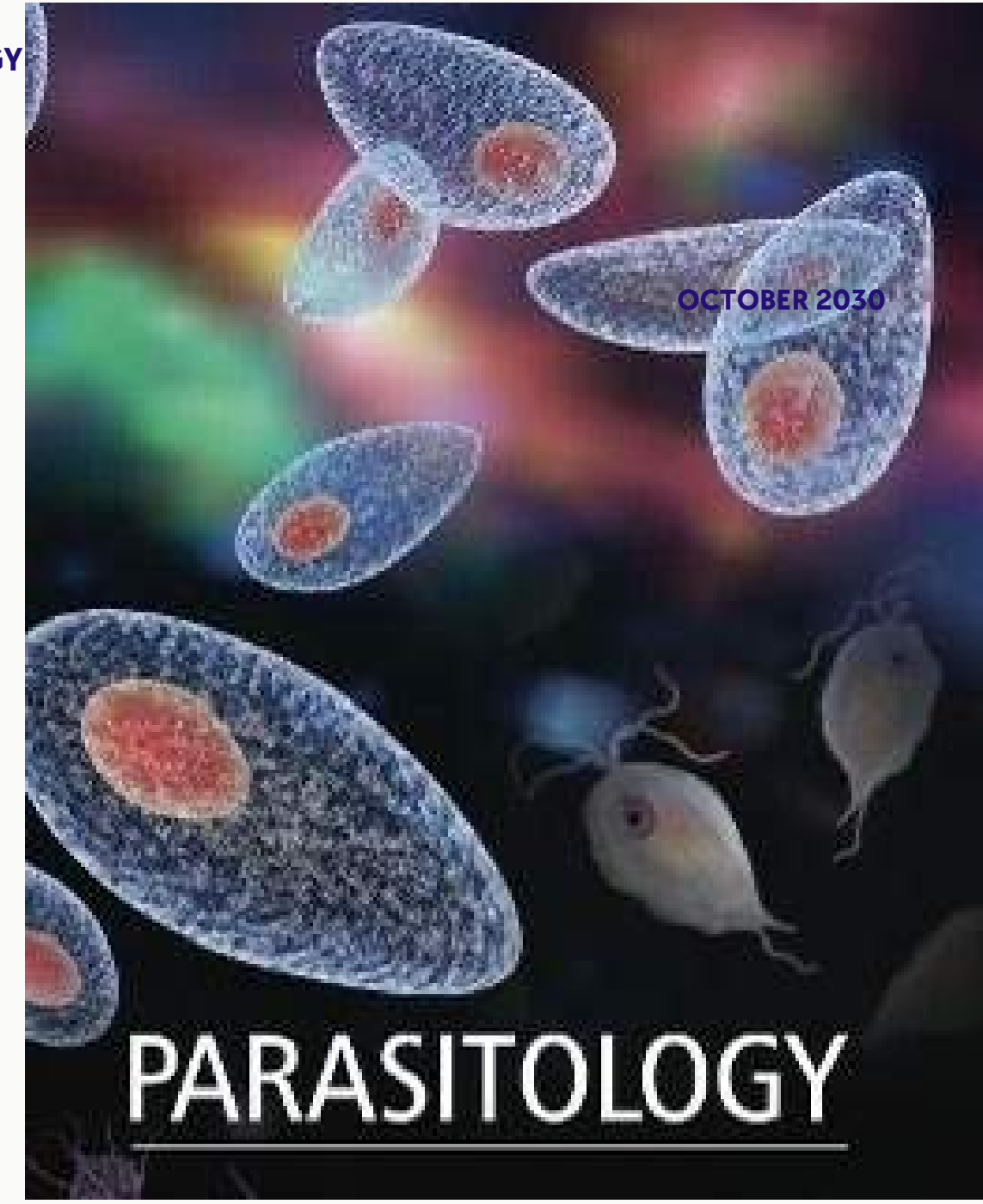
Lecture 2



Nonpathogenic Amoebae

A number of species of the genus ***Entamoeba*** are of worldwide distribution but do not appear to cause disease. The knowledge of these species is of value in differentiating the harmless commensals from potentially pathogenic ***E. histolytica***.

1. Entamoeba dispar

Formerly, it was believed that there is a pathogenic invasive strain and a nonpathogenic strain of *E. histolytica*, and *Entamoeba dispar* representing the nonpathogenic strain, but using the **isoenzyme-electrophoretic techniques**, and based on the antigenic differences, genomic DNA and ribosomal RNA, it is recognized now as a separate species.

The two species are morphologically identical but the trophozoites of *E. histolytica* contain ingested red blood cells. The cysts of *E. histolytica* and *E. dispar* cannot be differentiated microscopically and should therefore be reponedas ***E. histolytica / E. dispar.***

1.Entamaeba hartmanni

It's cosmopolitan in distribution, morphologically similar to *E. histolytica* but both its trophozoites and cysts are smaller and the cyst never contains ingested red blood cells. Therefore, it was earlier regarded as a small race of *E. histolytica*. The trophozoites and the cysts of *E. hartmanni* range from 4-12 µmand 5-10µm in diameter respectively. It is a nonpathogenic amoeba acquired by the ingestion of food or water contaminated with cysts. Its life cycle is similar to that of *E. histolytica*. The diagnosis can be established by the measurement of the size of the trophozoites and cysts and the absence of red blood cells in the endoplasm of the trophozoite.

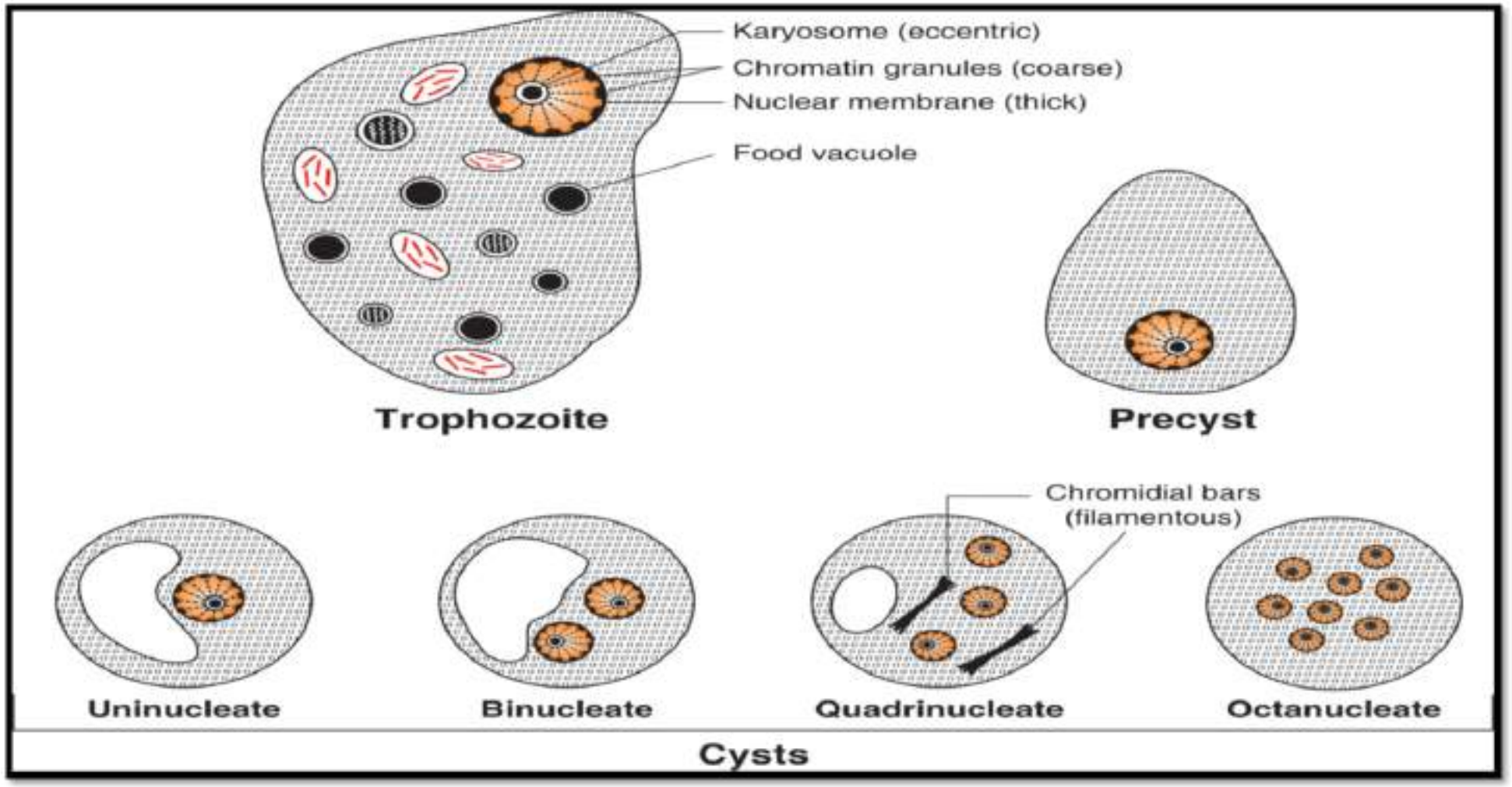
3. Entamoeba coli

Generally considered nonpathogenic in humans (commensal). The trophozoitedoes not ingest or invade the host tissues. It has a cosmopolitan distribution; its presence is evidence that the host has ingested fecal material. It lives in the lumen of the caecum and the lower level of the large intestine. The parasite has two stages trophozoite and cyst.

The trophozoite has a spherical shape with a diameter of 15-50µ, and the ectoplasm couldn’t recognize from the endoplasm. The food vacuoles contain bacteria, yeast and other enteric microbes and fragments of intestinal debris. The nucleus with eccentric and large karyosome.

The trophozoite has a sluggish movement, shortly extended pseudopodia. The mature cyst has a diameter of 10-35µ, 8 nuclei, and the chromatoid bodies have an irregular sharp ended (splinter-like).

The life cycle is similar to that of ***E. histolytica***, except that the trophozoite in this example doesn’t attack the mucosa of the intestine.

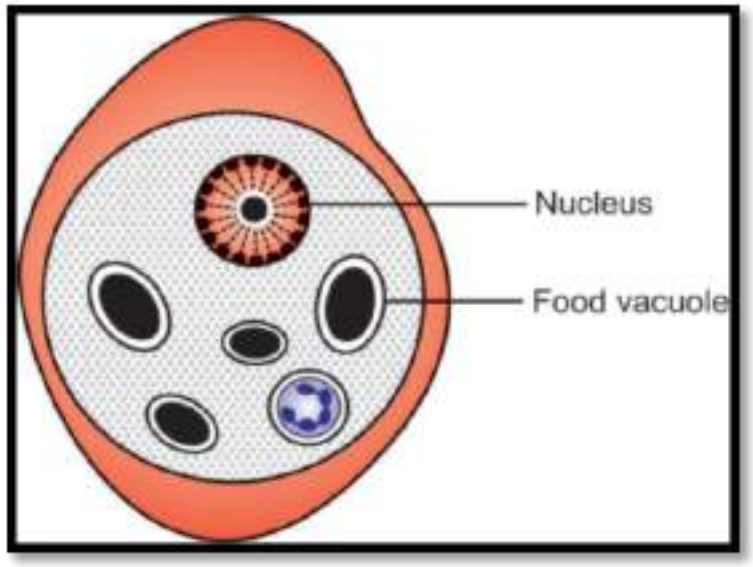


Morphological forms of Entamoeba coil

4. Entamoeba gingivalis

It is a parasite of the mouth of man and other mammals, including several species of monkeys, dogs and cats. It has a cosmopolitan distribution, commensal, commonly found in the tartar and debris associated with the gingival tissues of the mouth. It lives in/on the teeth, gum and sometimes tonsils, particularly if there is suppuration (purulence), as in pyorrhoeaalveolaris, but it also occurs in apparently hygienic mouths and on dental plates if they are not kept clean. There is little indication that it is pathogenic, and, while it abounds in people with unhealthy oral conditions (i.e., gingivitis or periodontitis), a cause and effect relationship has not been established.

Only trophozoite stage has been described in this parasite, which is measure 5-35µ in diameter. In most respects, it closely resembles E. histolytica, with a few to several fingerlike pseudopodia, finely granular endoplasm and clear ectoplasm.



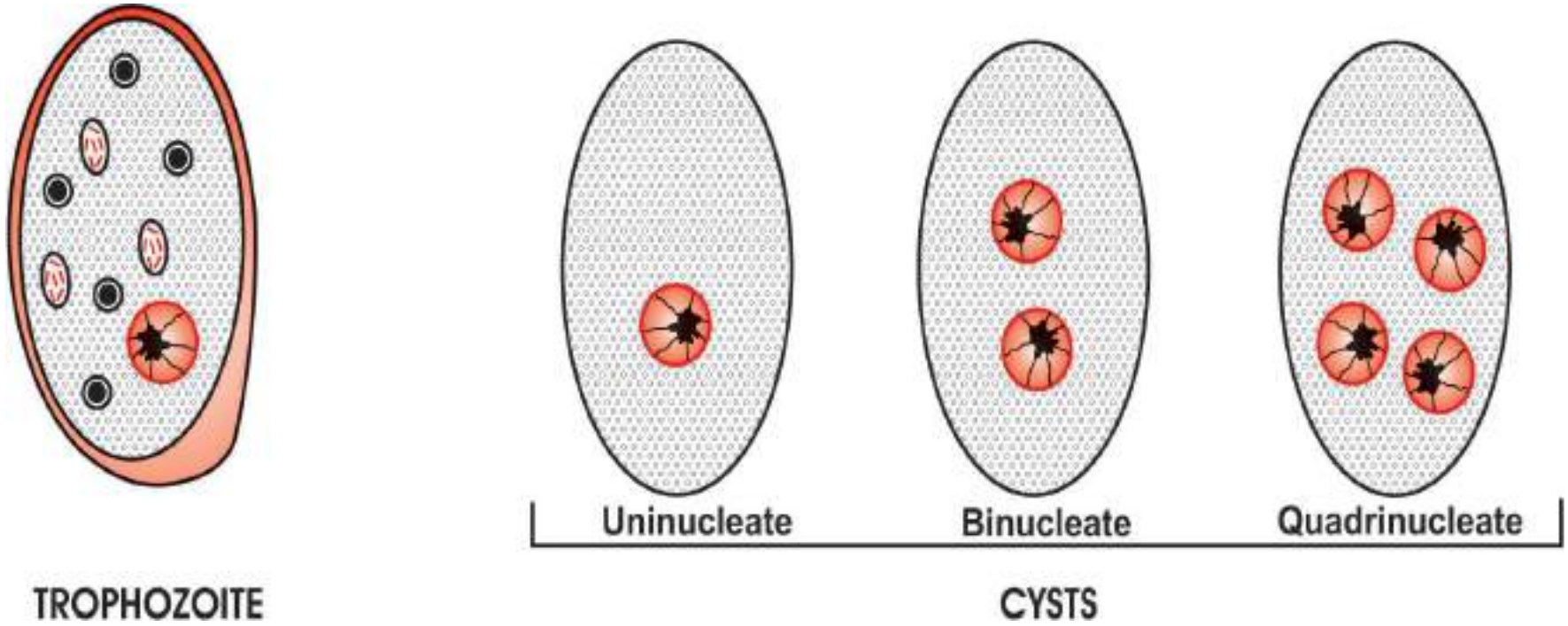
Trophozoite of Entamoeba gingivalis.

The nucleus contains a small karyosome that is central or slightly eccentric in position. Endocytotic vacuoles are often numerous and may contain oral epithelial cells, leukocytes, occasionally erythrocytes and various microbial organisms although it is not invasive.

No cysts are formed and transmission is either directly by oral to oral contact (kissing) or indirectly via trophozoite-contaminated food, chewing gum, toothpicks, contaminated drinking utensils, etc.

5. *Endolimax nana*

It is the smallest intestinal amoeba infecting humans where its trophozoite range from 6-15µ. The trophozoite is commensal in the lumen of the colon, caecum and the lower level of the large intestine, and is generally considered to be nonpathogenic, feeding on the bacteria. Its presence indicates that contaminated material has been ingested. It has a worldwide distribution and appears in two stages, trophozoite and cyst. The endoplasm is finely granular with numerous minute vacuoles (so it has a foggy appearance). The ectoplasm is hyaline and almost transparent. The food vacuoles contain bacteria, vegetable cells and some crystals. The nucleus is ovoid or subspherical with a relatively large karyosome, commonly eccentric. The trophozoites multiply rapidly by binary fission.

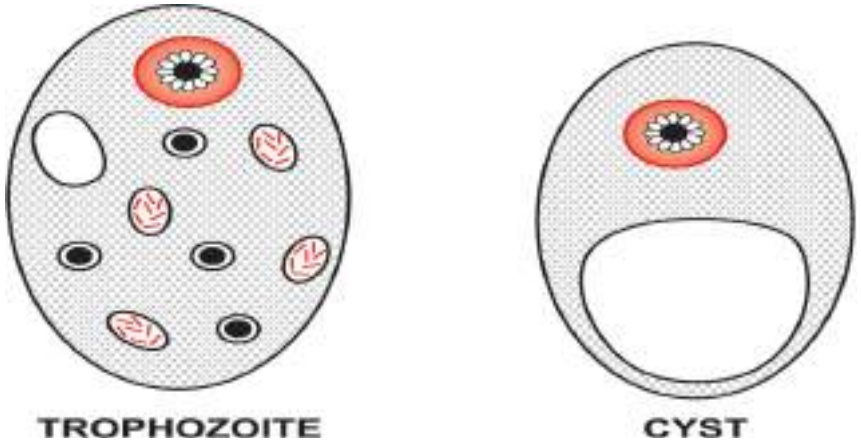


Morphological forms of Endolimax nana.

The life cycle is identical to that of other cyst-forming amoebae, with the cyst being the infective stage. The cysts of *E. nana* can be identified and distinguished from other cysts by their smaller size 5-14µ, ovoid shape and one to four vesicular nuclei, each usually containing a large, eccentric karyosome. The nuclear envelope is very thin and is difficult to see even in stained preparations.

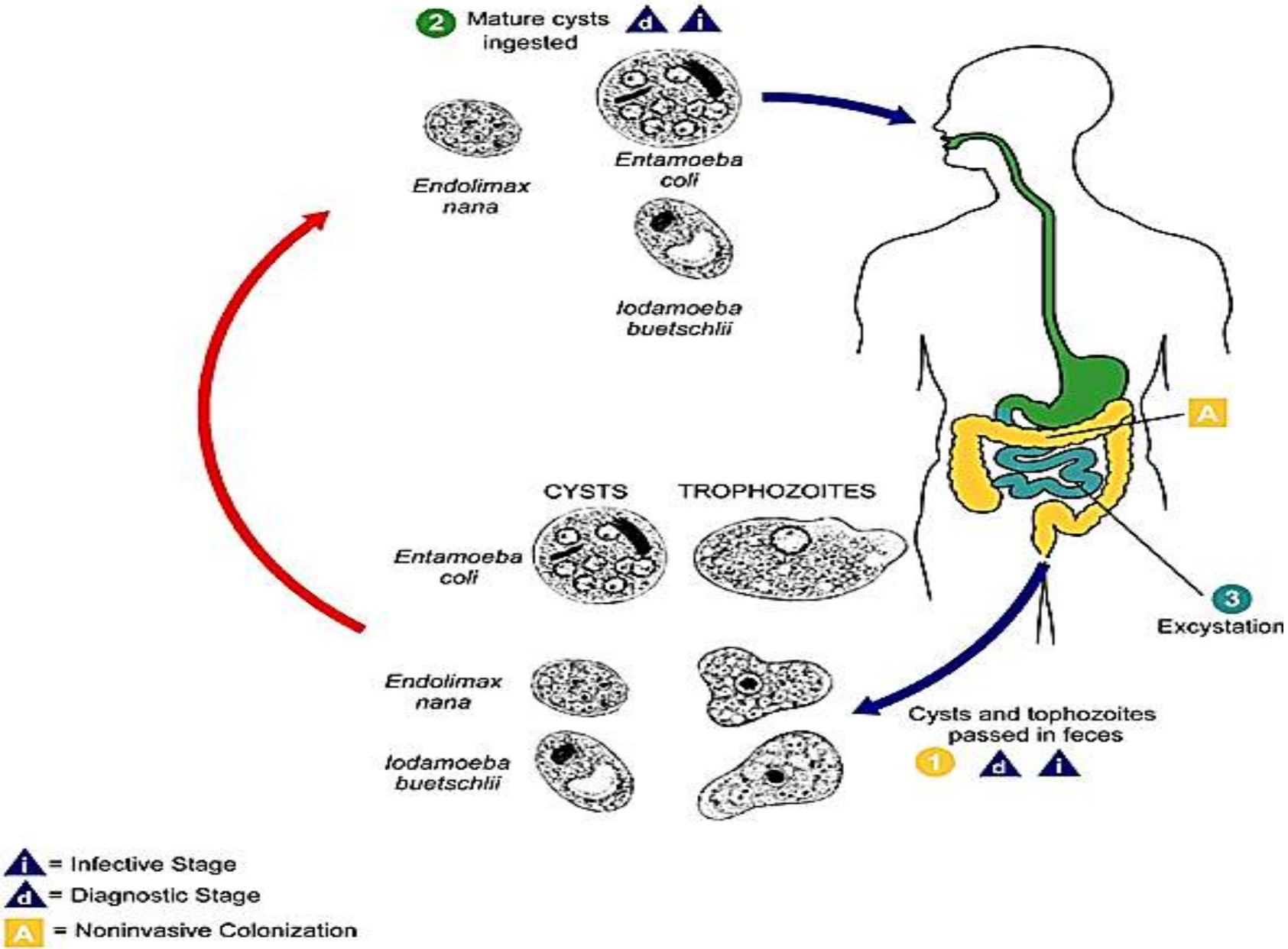
6. Iodamoeba butschlii

The parasite has a cosmopolitan distribution, but it is seldom as common as *E. coli* and *E. nana*. It is commensal, measures 8-20µ in diameter, lives in the lumen of the large intestine, especially the caecum. It has two stages, trophozoite and cyst. The ectoplasm of the trophozoite is not easily distinguished from the endoplasm. As it is evident from the contents of their food vacuoles, it is feeding on the bacteria and yeast. The nucleus is spherical, vesicular and has rather a thick membrane and large karyosome (centric or somewhat eccentric in position).



It is transmitted by the cyst which is very distinctive, facilitating its identification. The cyst is variable in shape, usually irregularly rounded (ovoid), and 5-18µ in diameter, and contains one nucleus.

There is a relatively big mass of glycogen that stains deep golden brown with iodine (the cause of the name ***Ioda.***), and also helps in the differentiation of this parasite from other intestinal amebae.

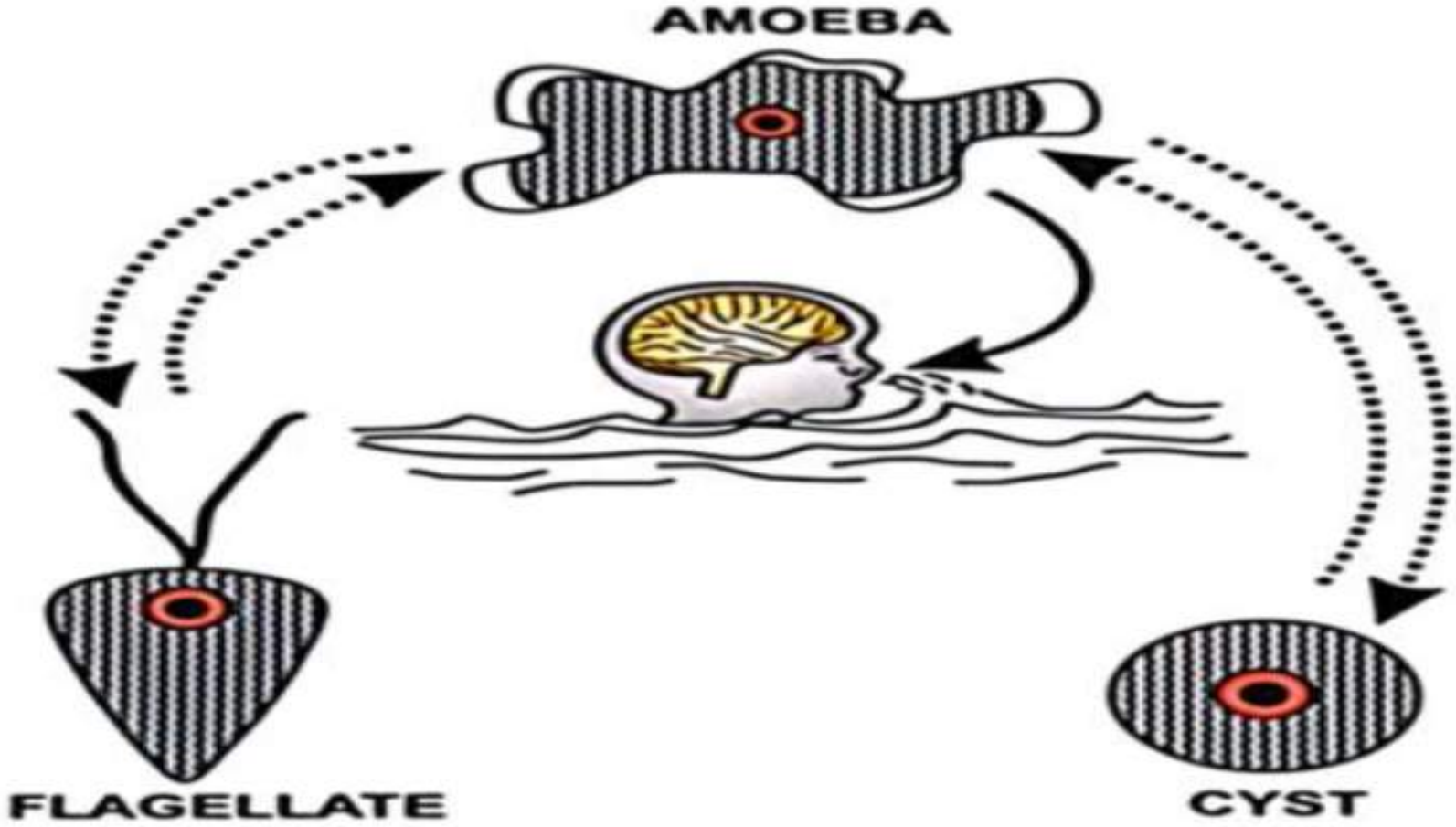

2 Mature cysts ingested
d i
Entamoeba coli
Endolimax nana
Iodamoeba buetschlii
A
CYSTS
TROPHOZOITES
Entamoeba coli
3 Excystation
Endolimax nana
Iodamoeba buetschlii
Cysts and tophozoites passed in feces
1
d i
i = Infective Stage
d = Diagnostic Stage
A = Noninvasive Colonization

Opportunistic free-living amoebae

Free-living amoebae of the genera *Naegleria*, *Acanthamoeba* and *Balamuthia* are facultative parasites of man. They are ubiquitous in nature, found commonly in soil and water (swimming pools, tap water, and heating and air-conditioning units) where they feed on bacteria, but as opportunists, they may produce serious infection of the central nervous system and the eye and thus, they are known as opportunistic amoebae.

Naegleria fowleri

It has two stages, motile trophozoites and nonmotile cysts. The trophozoiteoccurs in two forms amoeboid and flagellate. The amoeboid form is actively motile by means of eruptive, blunt pseudopodia called lobopodia. It measures 15-30 µm (average 22 µm) in length. It has distinctive phagocytic structures known as amoebostomes used for engulfment and vary in number depending on the strain. The nucleus is small, 3µm in diameter and has a large central karyosome. Reproduction is by simple binary fission of the amoeboid form.


AMOEBA
FLAGELLATE
CYST

The cysts are uninucleated, spherical, 7-15µm in diameter and are surrounded by a smooth double wall. Cysts and flagellate forms of *N. fowleri* have never been found in tissues or CSF. The amoeboid form of *N. fowleri* is the invasive stage of the parasite. Man acquires the infection by nasal contamination during swimming in freshwater lakes, ponds or swimming pools containing the infective form.

Infection may also be acquired by inhalation of dust containing infective forms. It is likely that flagellate forms or cysts of *N.fowleri* could enter the nose. However, since the amoeboid form is the invasive stage of the parasite, it appears that flagellate forms revert to amoeboid forms and the amoeboid form escape from the cysts in the nose.

The amoeboid forms invade the nasal mucosa, cribriform plate and travel along the olfactory nerves to the brain. They first invade olfactory bulbs and then spread to the more posterior regions of the brain leading to a rapidly fatal infection known as **primary amoebic meningoencephalitis (PAM)**. It occurs in healthy children and young adults with a recent history of swimming in freshwater

Symptoms

The patient develops a severe frontal headache, fever (39°-40°C), anorexia, nausea, vomiting and signs of meningeal irritation. Involvement of olfactory lobes may lead to disturbances in smell or taste. The patient may also develop visual disturbances, confusion, irritability, seizures and coma. The disease usually results in death within 72 hours of the onset of symptoms.

The period between contact with the organism and the onset of clinical symptoms vary from 2-3 days to as long as 7-15 days. PAM may resemble acute purulent bacterial meningitis, and these conditions may be difficult to differentiate particularly in the early stage.

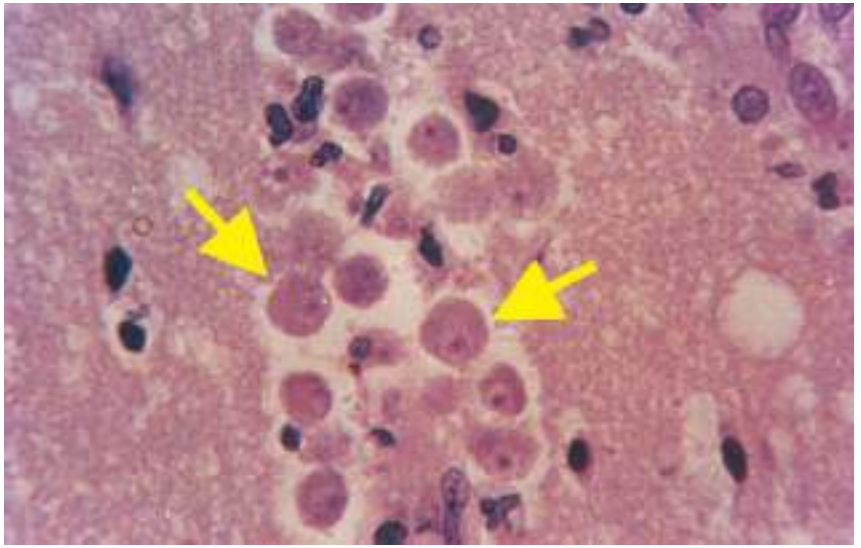
Diagnosis

The diagnosis of PAM can be made by microscopic identification of living or stained amoebae in CSF. Motile amoebae with characteristic morphology can be readily demonstrated in simple wet-mount preparation of fresh CSF specimens. Refrigeration of CSF is not recommended because this may kill the amoebae. When centrifuging the CSF, low speed (150 × g for 5 minutes) should be used so that the trophozoites are not damaged.

CSF smear may be stained with Wright or Giemsa stains. With these stains, amoebae have a considerable amount of sky-blue cytoplasm and relatively small, delicate pink nuclei.

These can be differentiated from mononuclear leucocytes which have a small amount of sky-blue cytoplasm and large purplish nuclei. Amoebae can also be demonstrated by fluorescent antibody staining of the CSF and in the histologic sections of the brain biopsied tissue by immunofluorescence and immunoperoxidase methods. The bacterial stain like Gram staining is of little value because heat fixing destroys the amoebae and causes them to stain poorly and appear as degenerating cells.

As in the case of fulminating bacterial meningitis, the leucocyte counts (predominantly neutrophils) vary from a few hundreds to more than 20,000 cells/ µL. CSF protein content is generally increased and glucose level is low. *N. fowleri* may be cultivated by placing some of the CSF on non-nutrient agar (1.5%) spread with a lawn of washed *Escherichia coli* or *Enterobacteraerogenes* and incubated at 37°C. The amoebae will grow on the moist agar surface and will use the bacteria as a food.



Treatment

At present, there is no satisfactory treatment for PAM. Antibacterial antibiotics and antiamoebic drugs are ineffective. Amphotericin B, a drug of considerable toxicity, is the antinaeglerial agent for which there is evidence of clinical effectiveness.