Protozoa

Protozoa are classified according to locomotion organelles into:

1. Sub-phylum Sarcodina (Amoebae):

Uses pseudopodia for movement, sluggishly motile, e.g. Entamoeba histolytica.

Pseudopodium: Is a cytoplasmic extension of the amoeba which serve both for motility and engulfment of food particles.

2. Sub-Phylum Mastigophora (Flagellates):

Uses one or more flagella (whip-like structure) for locomotion, moderately motile and has undulating membrane.

e.g. Giardia lamblia.

3. Sub-phylum Ciliophora (Ciliates):

Uses cilia (eye lashes) for locomotion, activity motile and contains two types of nucleus (macronucleus and micronucleus).

e.g. Balantidium coli.

4. Sub-phylum Sporozoa:

No locomotion organelles, moves by means of body flexion.

e.g. Plasmodium vivax.

Amoebae are structurally simple protozoa which have no fixed shape. They are classified under the Phylum-Sarcomastigophora, Subphylum-Sarcodina, SuperclassRhizopoda, Order-Amoebida. The cytoplasm is bounded by a unit membrane and can be differentiated into an outer ectoplasm and an inner endoplasm. Pseudopodia are formed by the ectoplasm thrusting out, being followed by the endoplasm flowing in, to produce blunt projections. Pseudopodial processes appear and disappear, producing quick changes in the shape of the cell. These are employed for locomotion and engulfment of food by phagocytosis. Amoebae may be free-Iiving or parasitic.
A few of the free-living amoebae can, on occasion act as human pathogens,

producing meningoencephalitis and other infections. Some of them can act as carriers of pathogenic bacteria. The parasitic amoebae inhabit the alimentary canal.

PARASITIC AMOEBAE

Parasitic amoebae belong to the following genera:

Genus Species

1. *Entamoeba E.histolytica, E.hartmanni, E.coli, E.polecki*
2. *Endolimax E.nana*
3. *Iodamoeba I.butschlii*
4. *Dientamoeba D.fragilis* (now classified as Amoeboflagellate)

Entamoeba histolytica:

The most important type cause disease, amebiasis, amoebic dysentery, amoebic hepatitis, it destroy and lyses the host cell.

Geographical distribution:

- Wide spread on the world wide about 12% of people have been reported to be infected by the E. histolytica.
- Found in the cecal and sigmoido-rectal regions, and because of that it's location it is usually seen in the stool.

Epidemiology:

The incidence of infection throughout the world varies from 0.2%-50% being the highest in tropical and sub-tropical areas also found in cold climates. High incidence is found in lower socioeconomic classes, because of over-crowding, malnutrition and lack of sanitation. All races, ages, sexes are equally suspected and infants are not so commonly infected. Transmission occurs by ingestion of infective cysts through fecally contaminated food and drink, food contaminated by insects (flies, cockroaches). Hands of infected food handles. Infective remain viable in water, soil and vegetables for several days. Infective cysts resist routine chlorination and are killed by freezing and desiccation.

Morphology:

It is found in feces as:

- Trophozoite (vegetative form).
- Precystic (transitory stage).
- Cyst (Infective stage).

Trophozoite

The trophozoite or the vegetative form is the growing or feeding stage of the parasite. It is irregular in shape and varies in size from about 12 to 60 μm. It is large and actively motile in freshly passed dysenteric stools, while in convalescents and carriers, it is much smaller. The parasite as it occurs free in the lumen as a commensal is generally smaller in size, about 15 to 20 μm and has been called the minuta form.

The protoplasm is differentiated into a thin outer layer of clear, transparent, refractive ectoplasm and an inner finely granular endoplasm having a ground glass appearance. Pseudopodia are formed by a sudden thrusting movement of the ectoplasm in one direction, followed by the streaming in of the whole endoplasm. The direction of movement may be changed suddenly, with another pseudopodium being formed at a different site, when the whole cytoplasm flows in the direction of the new pseudopodium. Typical amoeboid motility is a crawling or gliding movement and not a free-swimming one. The cell has to be attached to some surface or particle for it to move. In culture tubes, the trophozoites may be seen crawling up the side of the glass tube. Pseudopodium formation and motility are inhibited at low temperatures.

The endoplasm contains the nucleus, food vacuoles and granules. The nucleus is not clearly seen in the living trophozoite, but can be distinctly demonstrated in preparations stained with iron-haematoxylin or Gomorri's trichrome stains. The nucleus is spherical, 4 to 6 μm in size and contains a small central karyosome surrounded by a clear halo. The karyosome is anchored to the inner surface of the nuclear membrane by fine radiating fibrils called the linin network giving a 'cartwheel appearance.'

The delicate nuclear membrane is lined by a rim of chromatin distributed evenly as small granules.

The trophozoites from acute dysenteric stools often contain phagocytosed

erythrocytes. This feature is diagnostic as phagocytosed red cells are not found in any other commensal intestinal amoebae.

The trophozoite divides by binary fission once in about 8 hours. Trophozoites are delicate organisms and are killed by drying, heat and chemical disinfectants, They do not survive for any length of time in stools outside the body. Therefore, the infection is not transmitted by trophozoites. Even if live trophozoites from freshly passed stools are ingested, they are rapidly destroyed in the stomach and cannot initiate infection.

Precystic Stage

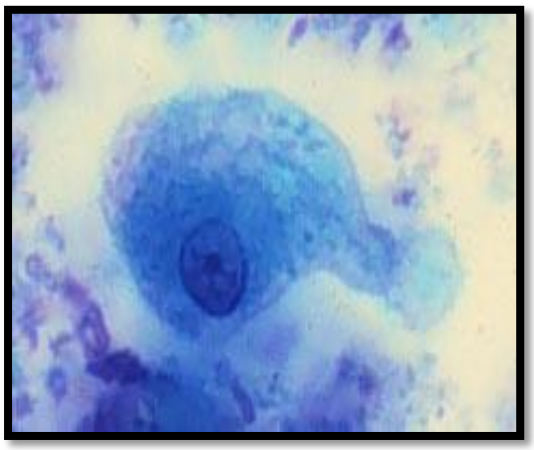
Some trophozoites undergo encystment in the intestinal lumen. Encystment does not occur in the tissues nor in feces outside the body. Before encystment the trophozoite extrudes its food vacuoles and becomes round or ovoid about 10 to 20 μm in size. This is the precystic stage of the parasite. It secretes a highly refractile cyst wall around it and becomes the cyst.

Cystic Stage

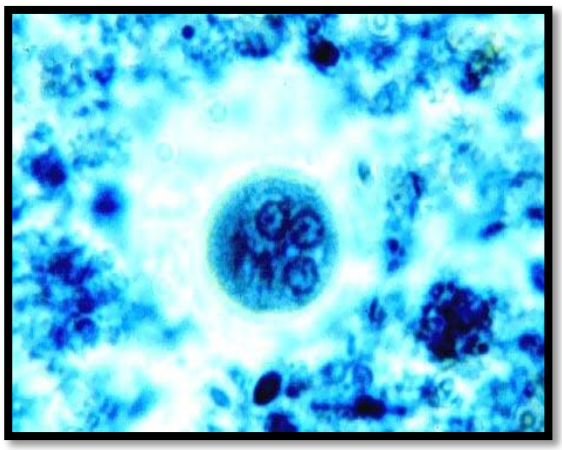
The cyst is spherical, about 10 to 20 μm in size. The early cyst contains a single nucleus and two other structures—a mass of glycogen and one to four chromatoid bodies or chromidial bars, which are cigar-shaped or oblong refractile rods with rounded

ends. The chromatoid bodies are so called because they stain with haematoxylin like chromatin. As the cyst matures, the glycogen mass and chromidial bars disappear.

The nucleus undergoes two successive mitotic divisions to form two and then four nuclei. The mature cyst is quadrinucleate. The nuclei and chromidial bodies can be made out in unstained films, but they appear more prominently in stained preparations. With iron-haematoxylin stain the nuclear chromatin and the chromatoid bodies appear deep blue-black, while the glycogen mass appears unstained. When stained with iodine the glycogen mass appears golden brown, the nuclear chromatin and karyosome bright yellow and the chromidial bars appear as clear spaces, being unstained.



Trophozoite Of E.histolytica



cyst Of E.histolytica

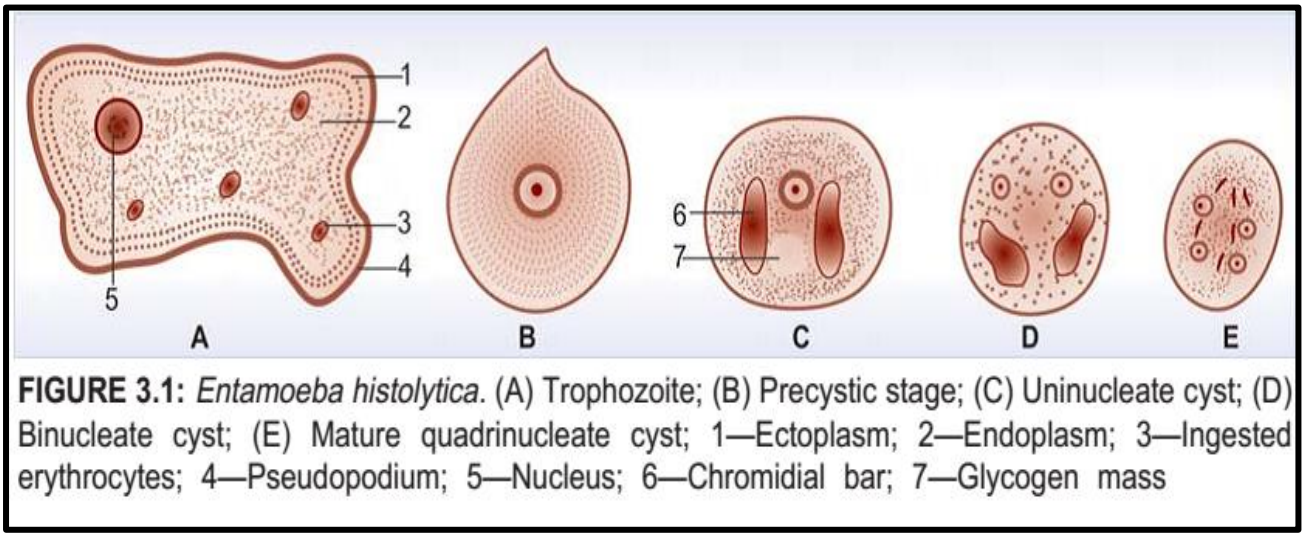


FIGURE 3.1: *Entamoeba histolytica*. (A) Trophozoite; (B) Precystic stage; (C) Uninucleate cyst; (D) Binucleate cyst; (E) Mature quadrinucleate cyst; 1—Ectoplasm; 2—Endoplasm; 3—Ingested erythrocytes; 4—Pseudopodium; 5—Nucleus; 6—Chromidial bar; 7—Glycogen mass

Life cycle of Entamoeba histolytica:

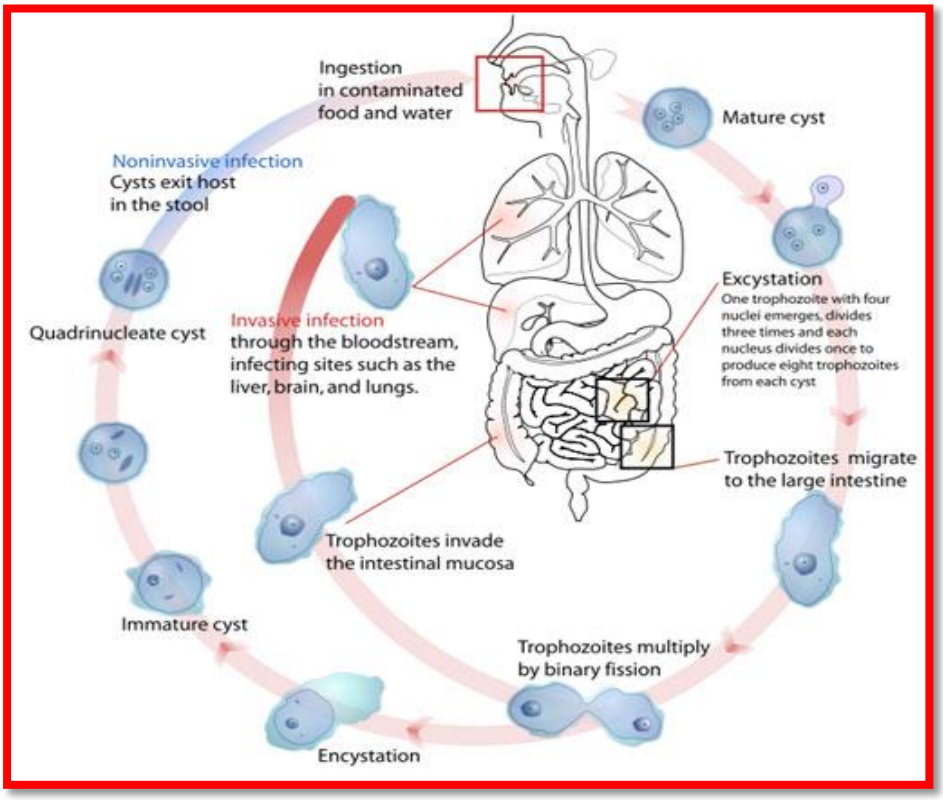
It is usually simple, direct (without other host) or (need other host).The man is the principle or reservoir host of this parasite. The trophozoite inhabit large intestine of man and it multiply and reproduce there. Under unfavorable conditions (change in pH; amount of oxygen; food in large intestine; and amoeba over population), the Trophozoite stops feeding and become sluggish, spherical in shape and it develops into precyst stage then the cystic wall will be formed to develop into cystic stage. Cystic stage is formed by a process called encystations, which is transformation of the trophozoite into cystic stage, this process occur in large intestine.

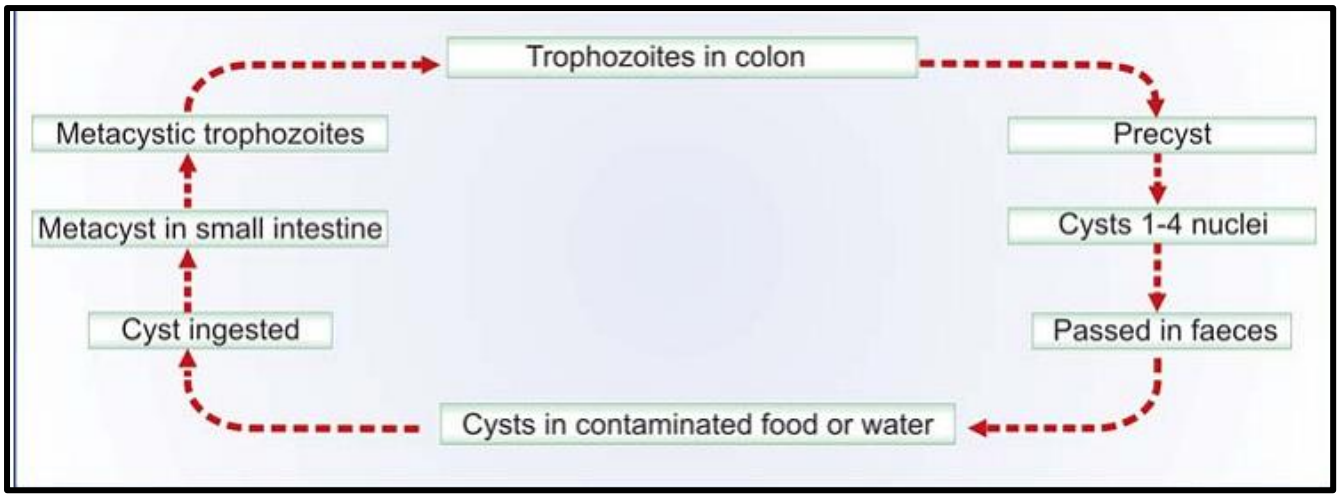
All the above conditions stimulate the encystations process, in which cause the trophozoite to transform into precyst, which is smaller than trophozoite and larger

than cyst. This precyst stop feeding and shrinked and start to secrete thick cystic wall forming "cyst".The cyst will be evacuated in feces outside the environment and it can stay living for about 10 days in moist areas. It can resist the external environment conditions and can survive several weeks. Usually about 40-50 thousands of cysts evacuated in the feces per day. Other man get infection by ingestion of infective cyst with contaminated food and water this is the method of infection . Only mature cysts can survive the acidity of digestive. Juice of stomach, while the immature ones will be dissolved by trypsin. In the lower portion of small intestine under influence of neutral or alkaline phosphates and digestive juice and the activity of parasite , the cystic wall will be disintegrated and the organism will be called 4-nucleated metacystic amoeba then they will have binary nuclear division and production of 8-nucleated metacystic stage then will have destruction of the cell wall then there will be release of 8 small trophozoites and each one is called amoebulae, which will migrate to large intestine and multiply they producing colonies.

So 4 stages of development is seen in the life cycle of E. histolytica :

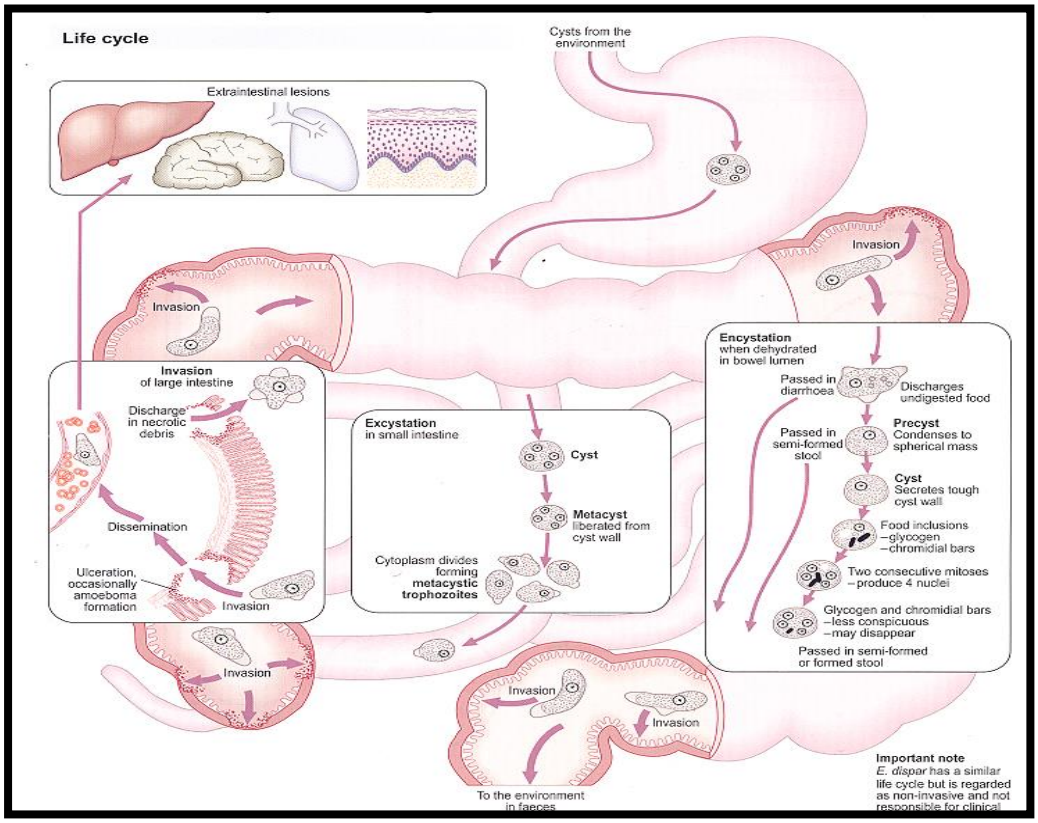
1. Cysts "infective stage"
2. Metacystic stage.
3. Trophozoites stage.
4. Short transmitary precysttic stage.





Types of infection:

1. Primary or intestinal infection leads to amoebic colitis or amoebic dysentery.
2. Secondary or extra-intestinal infection. When the trophozoite enter the venules and reach the liver then the other organs, Amoebic liver abscess, amoebic lung abscess, amoebic brain abscess, cutaneous amoebiasis may results from direct extension of trophozoite from rectum to perianal area or to the abdomen by fistula from colon or hepatic abscess. In tissue only trophozoites seen while in intestine cyst is seen.



Amoebiasis:

is human infection with E. histolytica with or without clinical; symptoms.

Sites of intestinal infection:

Is usually in the colon and the most common area of colon which is infected by trophozoite is the cecal and sigmoido-rectal regions because of the reduced colonic perisatlsis or the flow in these area are reduced.

Extra-intestinal infection usually occurs through blood stream by dissemination of trophozoite. The organ affected is liver lead to amoebic abscess; other method of infection is by direct extension from intestinal side by fistula.

Pathogenic activity of E. histolytica:

It depends on the following factors:

A. Virulence of strain:

There are several strains vary in their virulence; the more virulence cause more sever symptoms.

B. Environmental conditions: it includes:

1. Bacterial flora:

Normal flora plays an important role in the pathogenecity of E. histolytica. The bacteria will provide physiochemical conditions like: oxidation-reduction potential and other metabolic processes which enhance the invasiveness of the wall of intestine.

2. Serial passages:

The serial passage from host to host enhances the virulence and invasiveness of E. histolytica.

3. Nutritional status of the host:

The well-nourished man is less susceptible to infection than mal-nourished man.

4. Climate: virulence is restricted in tropical and sub-tropical regions of the world.

5. Immunological status:

The resistance depends on host. The more resistance, less chance of the parasite to invade the large intestine.

The immunosuppressed patients are more susceptible to be infected.

6. Drugs:

Some drugs may irritate the intestine that is more susceptible to infection.

e.x : Aspirin enhance invasiveness of amoeba .

7. Presence of other infections:

Persons who are not infected by other parasitic infection are less susceptible to be infected by E. histolytica; other persons who have parasitic infection in the wall of intestine may enhance the invasiveness and increase pathogenic activity of E. histolytica.

Invasion of the wall of the large intestine by the trophozoite and causing amoebiasis needs several steps:

1. Trophozoite multiplication.

2. Adhesion of trophozoite to intestinal mucosa that leads to colonization of trophozoites.

3. Destruction and invasion the wall of the large intestine.

Colonization:

- **The colonization of the parasite in large intestine depends on the following factors:**

1. Intestinal status:

Peristaltic movement plays an important role in colonization. Hyper motility will reduce the establishment of trophozoite. The most common areas of intestine which are infected by trophozoites are the caecal and segmoidorectal regions of colon because of reduced peristaltic movement in these regions.

2. No. of amoeba:

Which means the number of active trophozoites found on intestinal mucosa and this number depends on the number of viable quadrinucleated cysts, and the

chance of establishment in the intestinal mucosa is reduced when the number of amoeba is few.

3. The amount of food:

Large amount of food reduces the chance of colonization. (i. e. bulky food materials found in intestine make the parasite mixed with digested and undigested food, this cause less chance for colonization.

❖ **Invasiveness and destruction of intestine: It depends on:**

1. Motility of trophozoites (or its activity):

E. histolytica moves by the means of pseudopodia so the more active formation of pseudopodia gives more chance of destruction of the tissue.

2. No. of bacteria:

Which are either pathogenic or non-pathogenic bacteria (normal flora) which constitute 1/3 dry weight of the feaces.

The bacteria is a cofactor for the parasite to cause destruction and invasion of tissue.

3. Cytolytic enzymes: which are secreted by trophozoite to destruct host tissue (e.x : ptotease , hyaloronidase) .

4. Condition of intestinal tract:

e.g. infection with shigella and ascars , this will enhance the entrance of the parasite .

5. Resistance of the host: (immunity).

6. Type of diet:

Carbohydrates, enhance the multiplication of parasite and increase colonization as a result, it invades and destructs the wall of intestine.

Pathogenic anatomy of intestinal amoebiasis :

The amoebiasis of intestinal tract is commonly referred to as (Amoebic colitis) .

The first step in the pathogenesis of amoebic colitis is multiplication and colonization of trophozoite in the mucous membrane of large intestine, then

adhesion of troph. On the mucosa and epithelial cells and glands of large intestine leading to formation of (Early lesion).

Early lesion:

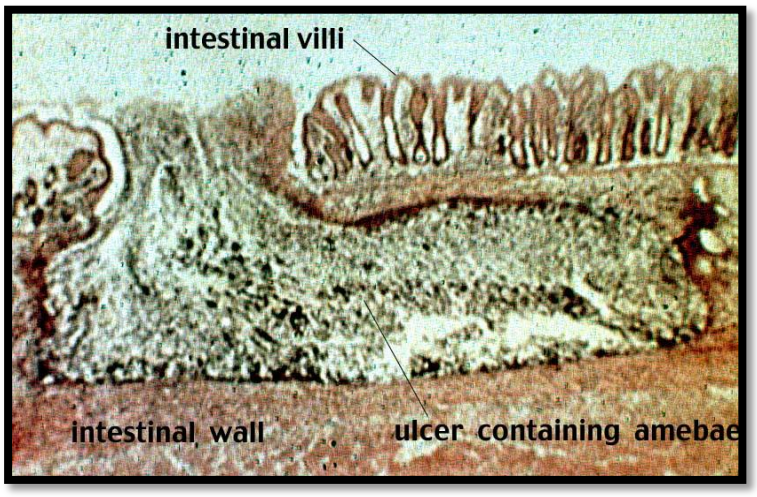
This lesion is produced when troph. Of E. histolytica invades crypts of (leiberkuhn) of large intestine by penetrating the columnar epithelial cells of intestinal mucosa, due to adherence of trophozoites on the mucosa; necrosis of epithelial cells will take place. So, early lesion is a necrotic process and no tissue reaction will take place unless it is complicated by secondary bacterial infections.

There will be a tiny necrotic areas on the superficial mucosa each one separated from the other by intact epithelial, and it will appear as nodular elevations with tiny or very small opening. So, the early lesion is a necrotic process and signs of inflammation other than hyperemia are usually absent.

Older lesion:

In older lesion, the trophozoites assisted by bacteria will break through muscularis mucosa then infilterated in the sub mucosa. The invasion or destruction of tissue by troph. will extend laterally in the sub mucosa along the axis of the intestine, leading to the formation of flask-shaped ulcer with wide base and narrow opening. The flask-shaped ulcer is diagnostic for E. histolytica infection, while in E. dispar infection the lesion remains superficial.

If the dissolution of tissue is so extensive and more than one lesion (ulcer) have been taken place, there will be a tunnel-like connection between two or more ulcers, and these tunnels are separated from each other by intact epithelial and it will cut-off, so, these areas will be sloughed-off exposing a large necrotic area covered by cytolysed tissue and this is called (Dyak's hair slough).



The process of sloughing off will enhance tissue reactions, and then fibrous thickening will be formed on the wall of the intestine.

The flask-shaped cavity of ulcer is usually filled with yellowish-brown necrotic material composed of :

1. Cytolysed cells (dead cells)

2. Mucous

3. Dead amoeba.

Dead amoeba are usually seen in the cavity of the ulcer, while living amoeba are found at the periphery (margin) of the ulcer and near its opening. As the lesion becomes chronic lesion and due to due to secondary bacterial invasion , tissue reaction will take place and the area will be filtrated by fibroblasts and granulation tissues that lead to the formation of (amoeboma) and (amoebic granuloma) which is confused with the neoplastic growth . So, slight fibrous thickening occur on the surface of the intestine and it is called (anemone ulcer).

Sometimes, destruction of tissue continues and reaches serosa, leading to intestinal perforations and this is a dangerous condition and may lead to death of the victim.

Entamoeba histolytica infection in the colon can initiate an intense post inflammatory response, both acute and chronic. In acute amoebiasis, an increase in the Th2 response is indicated by an elevation of IL-4. In chronic amoebiasis, patients exhibit little or no change in their CD4+/CD8+ ratio. The Th1 and Th2 responses in these patients remain unchanged as well. In the few asymptomatic patients investigated, high levels of IFN-g were observed, indicating a bias toward a Th1 response.

There are reports that some of E. histolytica's excretory and secretory products (e.g., proteins) may alter macrophage metabolism and thus reduce the efficacy of the patient's immune response, especially in the case of invasive amoebiasis.

For instance, it has been shown that E. histolytica trophozoites produce a small peptide, monocyte locomotion inhibitory factor (MLIF), which inhibits the motility of host monocytes and macrophages and also suppresses monocyte and neutrophil nitric oxide production.

It is probable that these factors contribute to the ability of trophozoites to survive within the host and even establish prolonged infections.

A degree of naturally acquired immunity to E. histolytica has been reported in humans. This immunity has been linked to a mucosal anti-adherence lectin IgA response.