In *Helminthic* infections, the adult parasites are found inside the human body and no multiplication occurs except in cases of strongyloidiasis and hymenolepiasis. The number of invading organisms during primary infection or re-infection determine the clinical manifestations in helminthiasis. The effects caused therefore depend upon their habitat, i.e. the locations where the parasites attack the tissues and also on the pattern of laying eggs or larvae. In certain helminthic infections, the normal secretions and excretions of the growing larvae and the products liberated after the death of the parasites behave as foreign proteins and elicit various allergic responses. Various skin tests, therfore, are employed in diagnosis of these infections.

Miscellaneous Pathogenic Effects

Certain parasitic infections induce an immunosuppressive state, thereby making the patient vulnerable to bacteria (both pathogenic and otherwise), e.g. trypanosomiasis, kala-azar and malaria. Compromised immunological state (owing to infections or drugs) may help parasitic multiplication, resulting in fulminant parasitemia, as in falciparum malaria or may favour extensive invasion of tissue, as in strongyloidiasis or may help 'opportunistic infections', as in toxoplasmosis.

Parasites may cause chronic irritation and be responsible for development of a neoplastic lesion:

(i) Fascioliases and Clonorchiasis may lead to development of adenocarcinoma of bile duct and hepatocellular carcinoma.
(ii) Schistosomiasis may lead to carcinoma of colon, rectum, liver or urinary bladder.
(iii) Malarial infection may be responsible for Burkitt's lymphoma.

The migrating larvae may carry with them bacteria or viruses from intestine to blood or other tissues—as may occur in Strongyloidisis, Trichinosis and Ascariasis.

Immunological responses of the host may also be responsible for the pathological effects, e.g.

(i) Tropical splenomegaly syndrome (TSS) and nephrotic syndrome in malaria.
(ii) Autoimmune haemolytic anaemia observed in kala-azar and malaria.
(iii) Granulomatous reaction with subsequent fibrosis in schistosomiasis—consequent to cell-mediated immune responses.
(iv) Manifestations seen in occult filariasis.

Immunological Responses and Parasites

Akin to other infectious agents, parasites also elicit immune responses in the host, both humoral as well as cellular. But immunological protection against parasites is far less effective when one compares it with that of bacterial or viral infections. These are two main types of immunity: Innate and acquired. *Innate immunity* does not depend upon previous exposure to the infective agent and does not arise out of specific responses of immunocompetent cells. Genetic constitution of the host determines the innate immunity, e.g. Central/West Africans are more resistant than the white westerners to hookworm infection and vivax malaria. It is also well known that African children carrying the sickle-cell anaemia trait (HbS heterozygotes) are relatively resistant to *P. falciparum* infection. Acquired immunity may be gradually developed after a natural infection (following clinically apparent or a subclinical infection) or may be artificially induced, as in cutaneous leishmaniasis (Oriental sore). This is known as active immunity. Immunoglobulins may be passively transferred to a neonate via placenta

Method of Reproduction

A. **Excystation:** On reaching an appropriate climate (alimentary canal of man)—the trophozoites are transformed to cysts. Here a quadrinucleate cyst gives rise to eight amoebae and each one can develop into a trophozoite.
B. **Encystation** is the process of transformation of trophozoites to cysts and occurs in the intestinal lumen of an infected individual. Conversion occurs within hours and the mature cyst lives inside the intestinal lumen for about 2 days. Conversion to cyst *does not* take place in the human tissues, e.g. intestinal wall or in metastatic sites, but occurs in the intestinal lumen (this is basically a protective mechanism). Excystation and encystation may occur in the same host but once cysts are formed they need to enter another host to restart the cycle.
C. **Multiplication:** Occurs only in trophozoite phase. This occurs by simple binary fission, first of the nucleus which divides by a modified type of mitosis and then, of the cytoplasmic body of the organism.

Cultivation: Initially cultured by Boeck and Drbohlav in 1925 by using solidified blood agar or solidified egg slopes covered with Locke's solution. Growh of *E. histolytica* in cultures needs the presence of starch/ rice flour and some other metabolic associates, such as enteric bacteria or the parasitic flagellate such as *T. cruzi* (living or dead) or *organism t* (a nonpathogenic bacterium). Microcultures are prepared from a single washed amoeba in microtubes (measuring 4 × 50 mm) containing a medium of thioglycollate preparation, horse serum and an overlay of rich culture of *T. cruzi* (Philip's medium). *E. histolytica* has also been grown in a serum medium containing thioglycollate and penicillin inhibited streptobacilli which are living but not multiplying (Shaffer and Frye's medium).

Susceptible Animals: Experimentally, amoebic lesions may be reproduced in dogs, cats and monkeys. In kittens the infection is usually fatal while pups survive much longer.

Life cycle: *E. histolytica* completes its life cycle in only one host—man. There are mainly two stages (Fig. 2.4):
(a) Trophozoite
(b) Cyst, with a transitory stage of a precystic form.

Mature quadrinucleate cysts are the infective forms of the parasite. On gaining entry into the alimentary canal of a susceptible person—they pass unaltered in the stomach (not destroyed by the acidic environment), but the cyst wall is digested by trypsin of the intestine. The excystation occurs when the cyst reaches the caecum or the distal part of ileum (neutral or mildly alkaline medium). Here, the cytoplasmic body retracts from the cyst wall, vigorous amoeboid movements rupture the cyst wall through which a mass of cytoplasm and later the whole body comes out. Each cyst gives rise to a single amoeba with four nuclei (a tetranucleate amoeba) which forms eight amoebulae (metacystic trophozoites) by the division of nuclei with successive fission of cytoplasm. These actively mobile young amoebulae invade the tissues and ultimately lodge in the submucous tissue of the large gut. Hence, they grow and multiply by binary fission. The trophozoites are responsible for the production of the characteristic lesions of amoebiasis.

E. histolytica secretes histolysin (proteolytic enzyme) that destroys and brings about necrosis of surrounding tissue on which the parasite then survives. These invading amoebae gradually recede from the dead tissues towards the margin of fresh ones and wander in the gut wall. On entering into the deeper layers they may reach the radicles of

GROSS FEATURES

Amoebic liver abscesses vary in size and are usually confined to the posterosuperior surface of the right lobe. Usually a single large abscess is found. The abscess region appears to be reddish brown in colour with a semifluid consistency. Shreds of connective tissue may be seen in the abscess cavity. The abscess cavity wall is ragged and shaggy in appearance and is formed by the necrotic liver tissues merging into the healthy zones with intervening zone of hyperaemia. In an old abscess, however, the wall is smooth and composed of dense collagenous tissue. (Cases of multiple small abscesses or a single huge abscess are also seen sometimes.)

MICROSCOPIC FEATURES

From the centre towards the periphery—three zones can be identified.

A. A central zone of cytolysed granular material with no amoebae.
B. An intermediate zone of degenerated liver cells, a few leucocytes, connective tissue cells, RBCs and an occasional *E. histolytica* trophozoite.
C. A peripheral zone of congested capillaries, varying degrees of necrosis of hepatocytes. Amoebic trophozoites can be seen to be multiplying in the area and invading the adjoining healthy liver tissue.

In an old abscess, the third zone may show walling by actively proliferating connective tissue cells, lymphocytes and monocytes.

Liver Abscess Pus (Fig. 2.6)

The pus produced is not of suppurative origin but is an admixture of sloughed liver tissue and blood. It is thick and chocolate brown in colour—called 'anchovy sauce pus.' Microscopically it reveals degenerated hepatocytes, few RBCs and occasional leucocytes. The trophozoites of *E. histolytica* are not seen in the freshly aspirated liver-pus but may appear in the escaping 'pus' four or five days after the initial aspiration.

AMOEBIC LIVER ABSCESS—CLINICAL FEATURES

Onset is insidious. *Pain and tenderness* in the right hypochondrium initially due to stretching of the liver capsule (pain is sometimes referred to the right shoulder due to irritation of the phrenic nerve which supplies the undersurface of the diaphragm). A dry cough may be present initially. Occasionally, the pain may be referred to the lower abdomen or the right iliac region. *Fever* with mild evening rise or low remittent temperature that later becomes quotidian and takes a hectic character. This is on account of pyrogenic effect of necrosed hepatocytes. *Jaundice* is unusual. The patient gradually gets emaciated. *On examination* liver is palpable and tender. Liver dullness extends upwards. Right-sided chest movement may be diminished or absent. There may be marked rigidity of the upper part of right rectus that interfere with liver palpation. The left liver lobe may undergo compensatory hypertrophy. *Lung signs* occur due to collapse of right lung caused by growing liver abscess, a right-sided pleural effusion may be present. *Apical pulse:* This may be displaced upwards and laterally by a large abscess. *Intestinal symptoms* such as diarrhoea or dysentery are absent. On abdominal palpation, colonic thickening areas may be felt.

Nucleus: Measures less than 1 μm, is round or oval and usually situated in the middle of the cells or along the side of the cell wall.

Kinetoplast: Lies tangentially or at right angle to the nucleus. It consists of a DNA containing body and a mitochondrial structure.

Axoneme (Rhizoplast) is a delicate filament extending from the kinetoplast to the margin of the body. It represents the root of the flagellum.

Vacuole is a clear unstained space lying alongside the axoneme.

Promastigote stage (Flagellar stage)

Seen only in cultures and insect vectors

Shape and size: Young forms—2–3 × 5–10 μm, short oval or pear-shaped. Maturer form—long, slender spindle-shaped, 1–2 × 15–20 μm.

Nucleus: Central

Kinetoplast: At the anterior end lies transversely.

Eosinophilic vacuole: Pale staining area lying in front of kinetoplast over which the root of the flagellum runs.

Flagellum: Equal to or longer than body length and projecting from the front. There is no undulating membrane as the flagellum does not curve round the body. Using Leishman's stain, the cytoplasm appears blue, nucleus pink or violet and kinetoplast bright red.

Cultivation: *L. donovani* can be cultured in a medium composed of two parts of salt agar and one part of defibrinated rabbit's blood. This medium was first introduced by Novy and MacNeal and later modified by Nicolle and is commonly referred to as NNN medium. The material for culture is inoculated into the water of condensation of the medium and incubated at 22–24° C. Bacterial contamination should not occur or else it would cause degeneration and death of *L. donovani.* The presence of haematin and ascorbic acid favour the growth of the parasite. In NNN medium the amastigote form changes into the promastigote form which then multiplies actively by longitudinal fission to produce numerous flagellates. By subculturing every 2–3 weeks, the strain can be preserved in the laboratory. The intracellular growth of *L. donovani* can be maintained in tissue culture at 37°C for up to 32 days.

Susceptible animals: In the Mediterranean region dogs are naturally infected with *L. donovani.* Common laboratory animals, such as mice, rats and guinea pigs are not suitable for transmission of infection. Some varieties of hamsters, however, can be infected.

Methods of infection: Reservoirs have been outlined earlier. However, the natural transmission of *L. donovani* from man to man is carried by a certain species of sandfly of the genera Phlebotomus and Lutzomiya. Undermentioned are the species involved.

(a) Indian vector—*Phlebotomus argentipes*
(b) Mediterranean vectors
Phlebotomus perniciosus (Italy and Sicily)
Phlebotomus major (Crete)
Phlebotomus pernicious var. langeroni (Sudan)