Protozoans are unicellular parasites. Several protozoans, e.g. *Giardia lamblia, Trichomonas vaginalis, Blastocystis hominis* and *Cryptosporidium* spp., are present in the lumens of hollow organs without invasion of tissues. Only a few of the numerous protozoan species are pathogenic for man.

HAEMOFLAGELLATE INFECTIONS

Trypanosomiasis and leishmaniasis infections are caused by organisms with flagella only in the blood.

The trypanosomiases as autochthonous infections occur in geographically limited regions where causative agents and vectors live side by side. In man, the trypanosomes produce two distinct diseases: the American trypanosomiasis (Chagas disease) and the African trypanosomiasis (sleeping disease). Only in Chagas disease are amastigotes found, i.e. parasites without flagella, also called leishmanial forms, in the tissues of man and lower animals. Leishmaniasis, exists in man in three forms: the cutaneous, the mucocutaneous and the visceral forms. The leishmanias in the three forms are morphologically identical in tissues and structurally resemble amastigotes of *Trypanosoma cruzi*.

1. AMERICAN TRYPANOSOMIASIS

Introduction

This is an important protozoan infection, also called Chagas disease, which affects millions of people in Central and South America[1]. It is geographically limited. Chagas disease is endemic in Venezuela and new infections are coming to light because the vectors have not been totally eradicated[2–7]. During the sixties, new infections were not seen. Autochthonous infections are not found outside the Americas.

Dogs and rodents are reservoir hosts as well as man. More organs are involved in experimentally infected mice than in natural infections in man (Figs. 1.1 and 1.2).

The acute form occurs mainly in children. Often the infection is asymptomatic, or there are many clinical symptoms which depend in each case on the organ or organ system involved. Rarely is an early diagnosis made. In symptomatic cases, the prognosis is bad. The chronic form is observed mostly in older people and chronic myocarditis is one of the most frequent causes of sudden death. Chronic *T. cruzi* infections, however, do not appear to be a public health problem in Venezuela at the moment[8].

Clinical aetiologic diagnosis is not easy. The causative agent should be demonstrated. In the acute form, trypomastigotes should be looked for in the blood; this is done with good results at the University Hospital in Barinas. The search for amastigotes in muscle biopsies may be successful (Fig. 1.3). In the chronic form, xenodiagnosis* may be positive, or the infection may be proved only by serological methods. The indirect immunofluorescent procedure has been developed recently for detecting intact amastigotes and phagocytosed amorphous antigen, both intensely fluorescent in human and mouse myocardia[9].

The parasite

The genus *Trypanosoma* belongs to the family Trypanosomatidae. The species *Trypanosoma cruzi* seems to be the only causative agent of Chagas disease. Lately, *Trypanosoma rangeli* has been discussed as a causative agent too. Numerous people in Venezuela, Colombia and Panama are infected with this other species. However, the parasites have been found only in the blood of man and not in tissues.

The trypomastigote (flagellate form) of *T. cruzi* in the blood is 3 μm wide and 16–22 μm long including the flagellum. In fixed smears, it is seen as a (C) or (U) form (Fig. 1.4). Its nucleus is situated in the centre of the parasite, is oval-shaped and stains reddish pink with Giemsa. The kinetoplast, 1 μm in diameter, is prominent and located in the pointed rear part. It represents a particular form of mitochondria. Near the kinetoplast is the base of the flagellum, called the blepharoplast. This structure cannot be differentiated under a light microscope but it is possible with an electron microscope. The trypomastigotes do not multiply in blood. *T. rangeli* shows only minimal structural differences when compared with *T. cruzi* (Fig. 1.5). The flagellum in the latter is shorter. Furthermore, both species may be differentiated by their sialic acid content[10].

After penetrating the tissue cells, the flagellum cannot be discerned with the light microscope within the cell. The parasite transforms into an amastigote with a rudimentary flagellum visible only under an electron microscope. Under the light microscope, these amastigotes, without flagella, reveal a peripherally localized nucleus, measuring 3–5 μm in diameter and are arranged in nests or pseudocysts, mostly in muscle and glial cells (Figs. 1.6 and 1.7). These pseudocysts do not have an individual capsule. The amastigotes contain, in addition to the nucleus, a characteristic rod-like kinetoplast, generally darker than the nucleus in the H&E and Giemsa stain. The amastigotes of *T. cruzi* are called also 'leishmanial forms'. Parasites in the amastigote form are also found in tissue culture.

*Xenodiagnosis is the confirmation of a causative agent in a (human) patient through the development of the parasite in a non-vertebrate (vector) which has been exposed to the patient. For example, promastigotes of *T. cruzi* are observed in a previously healthy triatomid after it has sucked blood from a patient with Chagas disease.

K. Salfelder et al., *Atlas of Parasitic Pathology*

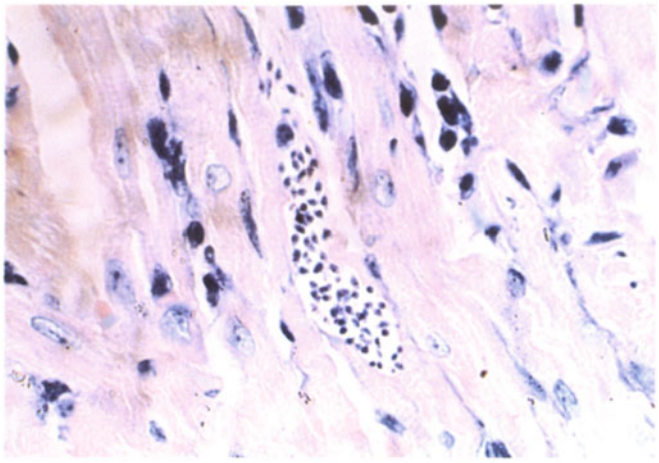

Fig. 1.1 Nest of amastigotes of *Trypanosoma cruzi* in the mycoardium of a mouse inoculated intraperitoneally with the excrement of parasitized vector bugs. H&E

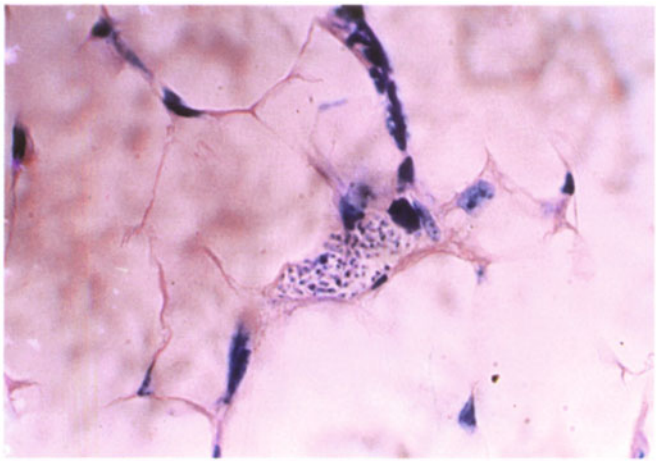

Fig. 1.2 Nest of amastigotes of *Trypanosoma cruzi* in the mediastinal fat tissue of the same mouse as in Fig. 1.1. H&E

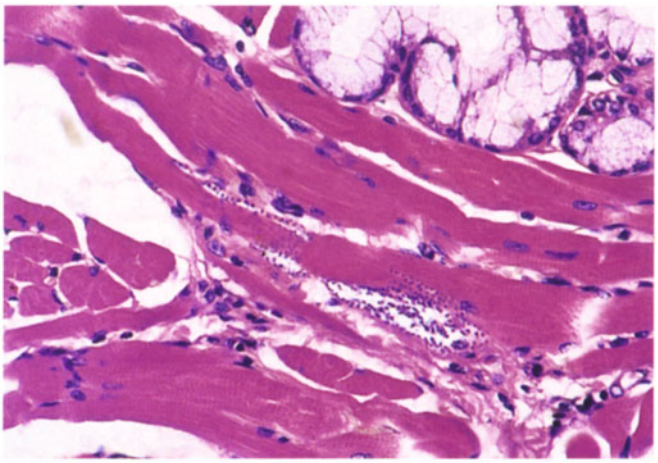

Fig. 1.3 Amastigote nest of *Trypanosoma cruzi* in the muscular fibres of a human tongue. H&E

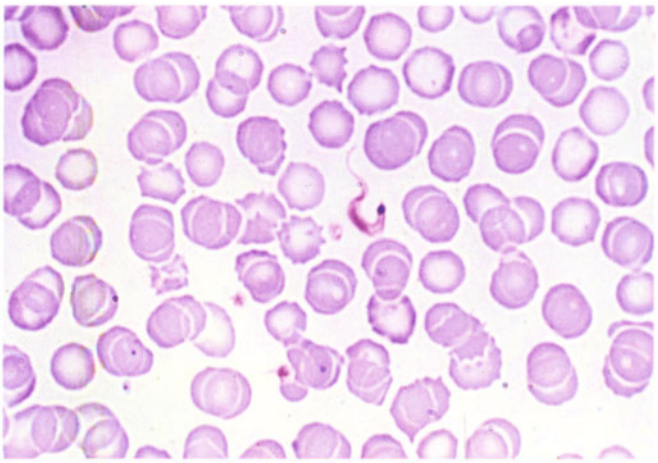

Fig. 1.4 Trypomastigote of *Trypanosoma cruzi* in a blood smear. Giemsa

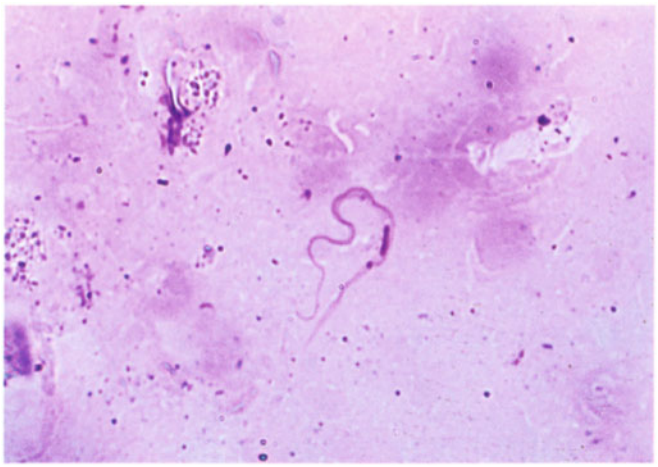

Fig. 1.5 Trypomastigote of *Trypanosoma rangeli* in a blood smear. Giemsa

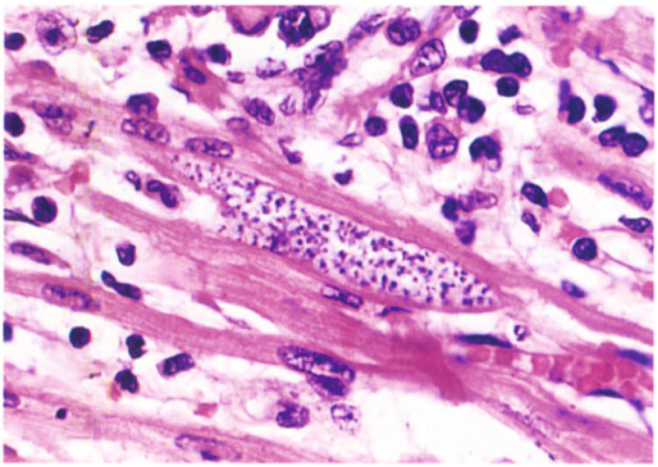

Fig. 1.6 Nest of amastigotes (parasites without flagellum) of *Trypanosoma cruzi* in a muscle fibre of myocardium. H&E

Trypanosoma cruzi may be isolated from human and animal blood relatively easily, as well as from the intestinal tract of the vectors in the culture medium known as NNN (for composition, see the section on Visceral Leishmaniasis). In the culture media, the trypomastigotes of *T. cruzi* are called epimastigotes and are often arranged in a rosette pattern. If preservation of live strains of trypanosomes for a longer period is planned, it is necessary to include intermediate steps in animals and subcultures.

Numerous species of mammals are reservoirs of *T. cruzi*, including, among others, marsupials, bats, rodents, dogs, cats, pigs and monkeys.

Regarding the vectors, 66 species of the genus *Triatoma*, bugs of the family Reduviidae, have been found to be naturally infected with *T. cruzi* (Fig. 1.8). Their habitat extends from sea level to an altitude of 800–1200 m. They are found predominantly in the thatched roofs of rural houses.

Pathogenesis

In the **acute form** of the infection, pathogenesis is easy to understand. Transmission of *T. cruzi* occurs from man to man or animal to man through defecation after the insect bite, more rarely through blood transfusions or, occasionally, diaplacentally. By contrast, transmission of *T. rangeli* takes place directly through an insect bite and not via evacuation.

The metacyclic trypomastigotes, the infectious forms of *T. cruzi* which develop from the epimastigotes in the insect gut contents, enter the host through damaged mucosa or skin. At the conjunctiva, the so-called 'Romaña sign' may be observed (Fig. 1.9). It consists of a unilateral palpebral oedema with conjunctivitis and swelling of the regional lymph nodes. In other cases, a skin nodule may appear at the site of penetration by the parasite, appearing 1–2 weeks after the bite, and is called the 'chagoma of inoculation'. It must be emphasized, however, that, in the majority of cases, the point of entrance cannot be determined. We do not know of any reports of the histological patterns of the portal of entry by biopsy.

Once in the vertebrate host, at the point of entrance, the trypomastigotes enter tissue cells where they lose the flagellum and replicate as rounded amastigotes. Later, they reach the blood stream as trypomastigotes and may thus reach other organs. Here, again, they become amastigotes and produce a tissue reaction; in the heart this takes the form of an acute diffuse myocarditis. The amastigotes reproduce in the intracellular nests until they rupture 2–4 weeks after formation (Fig. 1.10), and the parasites 'disappear'. In reality, they do not disappear; they may return later to the blood stream in the flagellate form (trypomastigotes) and so an endogenous re-infection may take place. In addition, they may be withdrawn from the blood stream by another insect bite. Experimentally, it has been shown recently that, in lower animals, amastigotes are circulating in the blood stream and that they are as infective as trypomastigotes[11].

The pathogenesis of **chronic Chagas myocarditis** remains unknown. This is, in part, due to the difficulty of detecting parasites in the myocardium at this stage of the disease and to the similarities between this and the tissue reaction of so-called idiopathic myocarditis of Fiedler[12]. Several theories of pathogenesis have been proposed[13–15]. The two most favoured hypotheses are the antiheart immune or autoimmune reactions[16,17] and the neurogenic theory of the influence of damage to the vegetative nervous system[18,19]. There are several aspects of the neurogenic theory which deserve comment: the morphological and functional bases of this theory were initially obtained from study of Chagas disease patients at very advanced stages of the disease[20]. More recent clinical[21–23] and experimental[24,25] studies indicate that myocardial damage may precede the cardiac parasympathetic abnormalities. The reduction in the number of cardiac vagal neurons[26] and the functional disorders of cardiac parasympathetic innervation[27] are also seen in patients with ventricular dilatation who do not have Chagas disease. It seems, then, that the cardiac parasympathetic abnormalities are secondary to the progressive dilatation of the cardiac chambers[28].

We believe that chronic Chagas myocarditis may be due to repeated exogenous and/or endogenous re-infections.

Pathology

The organs which may have intracellular amastigotes of *T. cruzi* in man are those with various types of the musculature cells: smooth muscle cells of the digestive tract as well as skeletal muscle fibres and myocardial muscle fibres. Damage of the latter is of most importance. Furthermore, brain tissue may be affected with amastigote nests in the glial cells; also, the placenta with subsequent congenital trypanosomiasis and, rarely, the testicles. In numerous autopsies performed by the authors, parasites were looked for in the organs of the PMS (lymph nodes, liver, spleen, bone marrow), in other parenchymal organs and in the vegetative nervous system, but were not found[29]. This is in contrast to the findings reported in numerous books. However, in experimentally infected lower animals, amastigotes of *T. cruzi* may be located in cells of numerous viscera.

Gross lesions in the **acute form** of Chagas disease are observed in the heart (Figs. 1.11–1.13), brain and the musculature of the digestive tract, including the tongue. The heart shows hypertrophy and dilatation, the latter developing in the final days with diminished consistency. The myocardium may reveal a fish-flesh or cooked-meat-like aspect or foci of various sizes of a yellowish-white colour are found. In the brain, oedema and multiple petechiae are observed.

In the **chronic form**, hypertrophy and dilatation, mostly of the left ventricle are present. Coronary arteries, even in older patients, are not usually affected. On the surface of the heart, a subepicardial rosary, i.e. a chain of small fibrotic nodules, is seen (Fig. 1.14). Quite often, parietal aneurysms can be observed, in addition to various numbers of whitish scars in the myocardium of variable sizes and shapes. However, none of these lesions is pathognomonic. This means that all these alterations may also appear in cardiopathies with other aetiologies (Fig. 1.15). Aneurysms are mainly located at the apex of the left ventricle; they are thin-walled showing fibrotic tissue and contain thrombi (Fig. 1.16). From these thrombi, arterial embolism often takes place with subsequent infarction in numerous organs.

Regarding **histology**, the parasites appear in tissue sections as amastigotes in intracellular nests, i.e. as parasites without flagella. It must be emphasized that characteristic kinetoplasts in the amastigotes are seen mostly in preparations after recent penetration into tissue cells and when the tissue is removed early and well preserved. This means that these structures are not found in each and every case. In tissue sections of routine autopsies, we only rarely saw the kinetoplasts. We therefore stress that amastigotes are almost never recognized as such, with all the structural details, in tissue sections. They are mostly indistinguishable from other small microorganisms with the H&E stain.

Morphologically, amastigotes are also indistinguishable from species of *Leishmania*. In order to differentiate between these two, we must consider that each of these parasites has particular and specific locations in tissues and the corresponding organs. Small yeast cells are

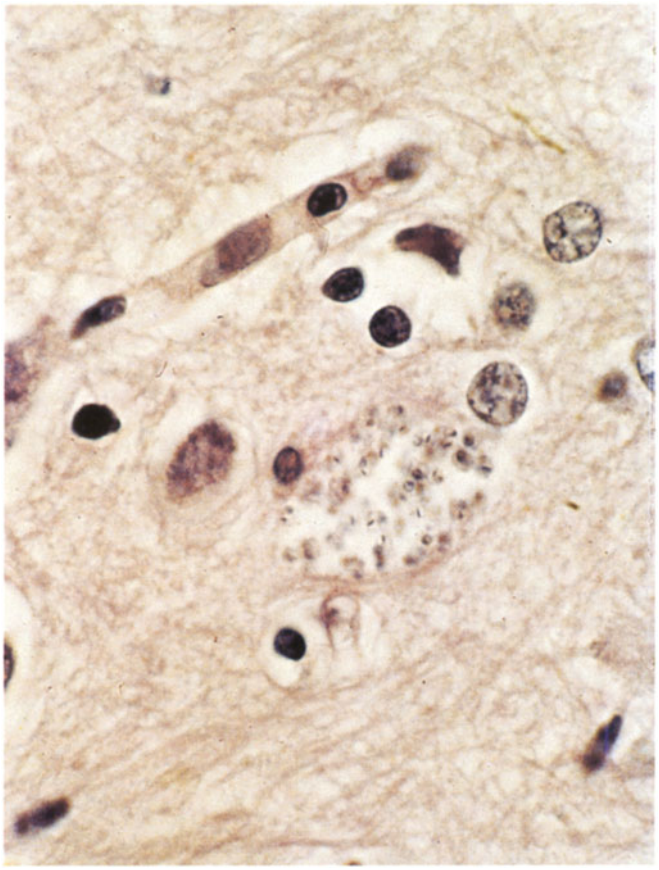

Fig. 1.7 Nest of amastigotes of *Trypanosoma cruzi* in a glial cell (pseudocyst). Rod-like kinetoplasts are visible. H&E

Fig. 1.8 Triatomines (*Rhodnius prolixus*), vectors of Chagas disease

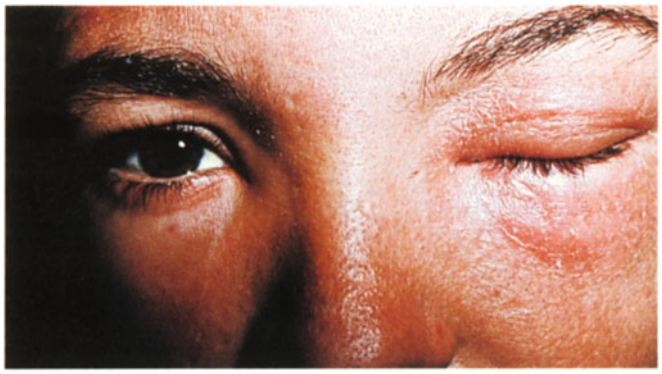

Fig. 1.9 'Romaña's sign' in a *Trypanosoma cruzi* infected patient from Venezuela. This periorbital and unilateral oedema marks the site of entry of the parasite

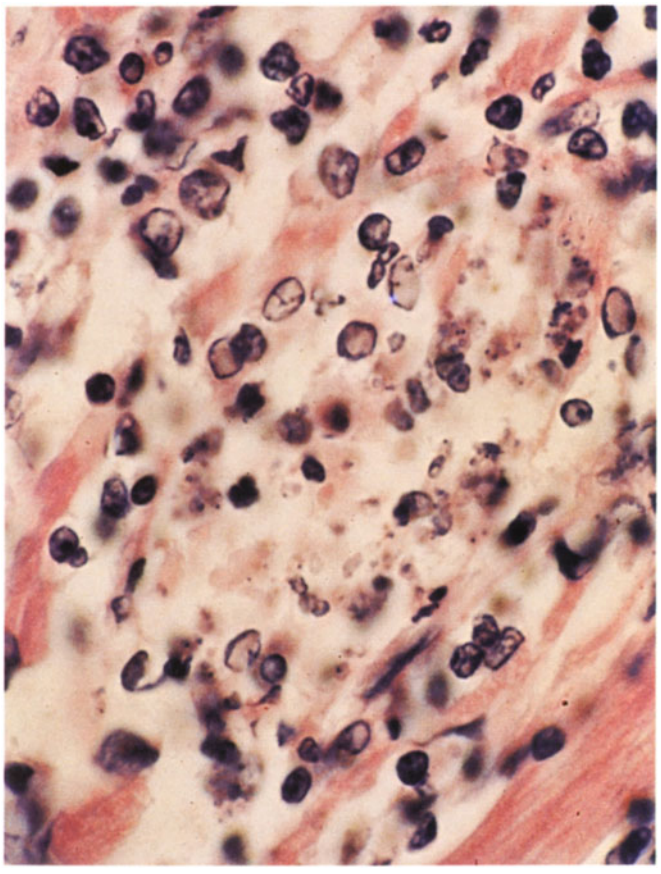

Fig. 1.10 Ruptured nest of *Trypanosoma cruzi* in the acute myocarditis of an infant, one month after becoming ill with fever. H&E

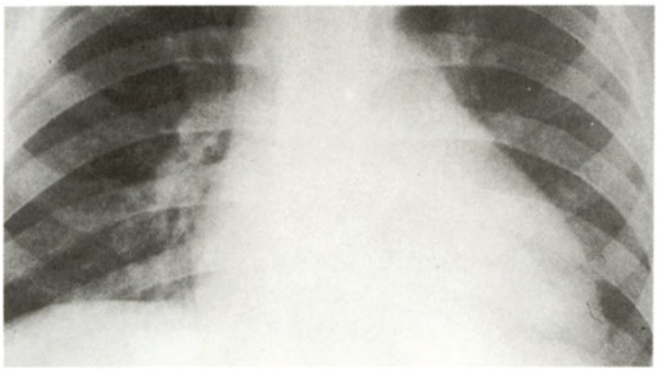

Fig. 1.11 Acute fatal form of Chagas disease with cardiomegaly

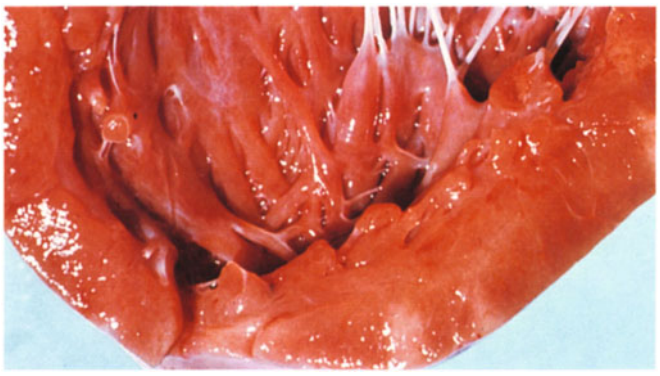

Fig. 1.12 Fish-flesh appearance of the myocardium in acute Chagas myocarditis

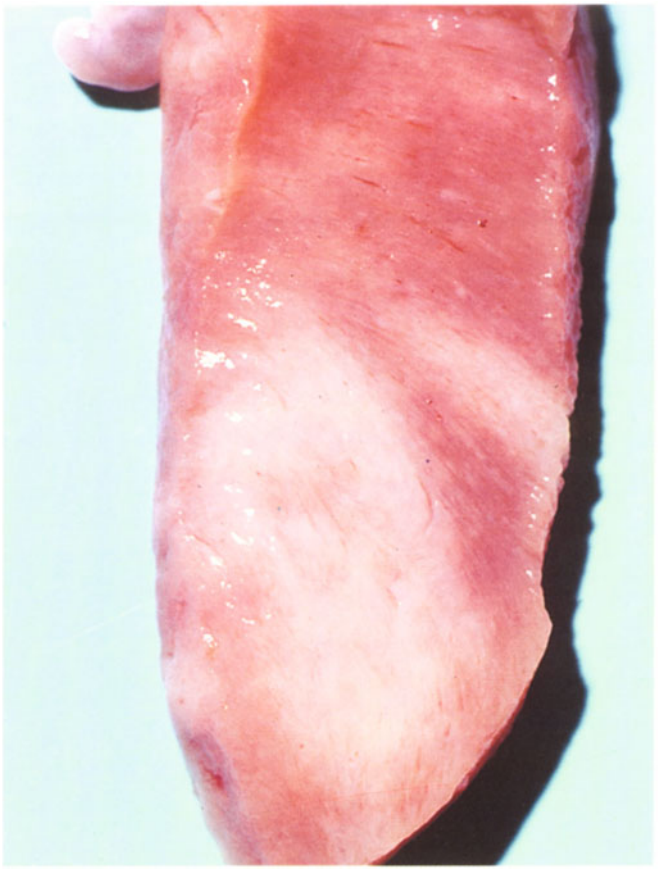

Fig. 1.13 Whitish spots on the cut surface of myocardium in a case of acute Chagas myocarditis

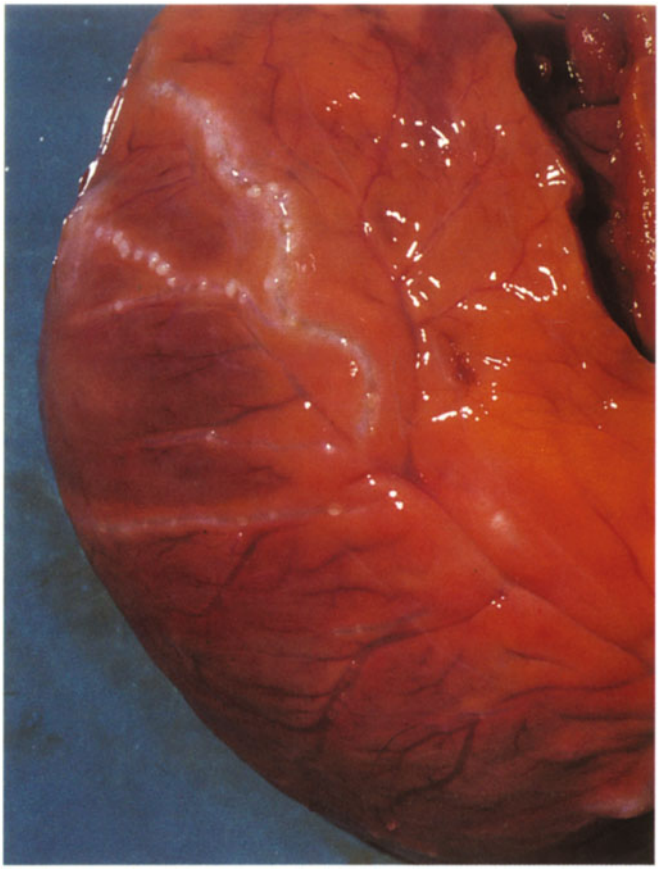

Fig. 1.14 Subepicardial 'rosary' considered typical in cases of chronic Chagas myocarditis

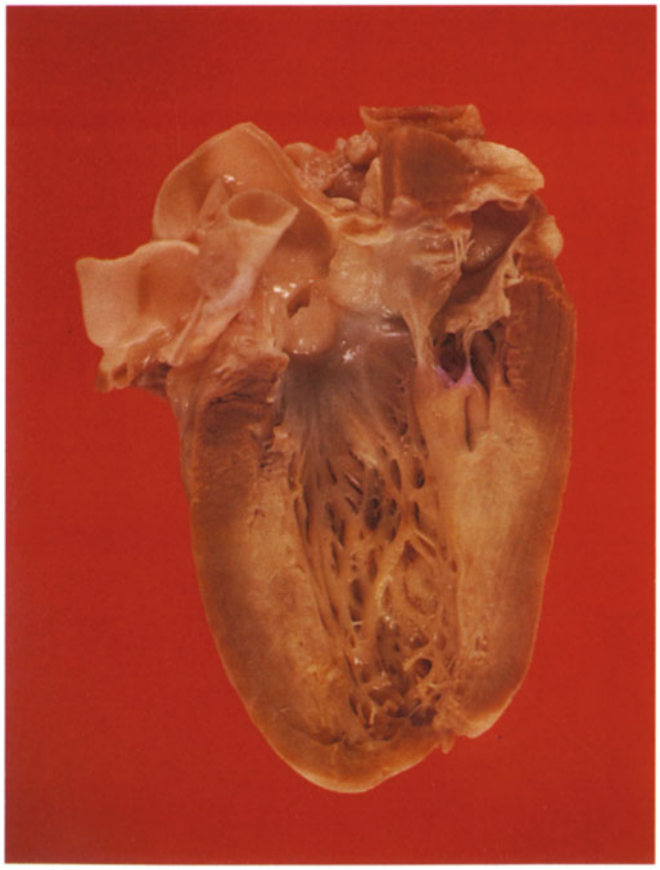

Fig. 1.15 Acute myocarditis in an infant from Mérida. Parasites could not be found in tissues in this case

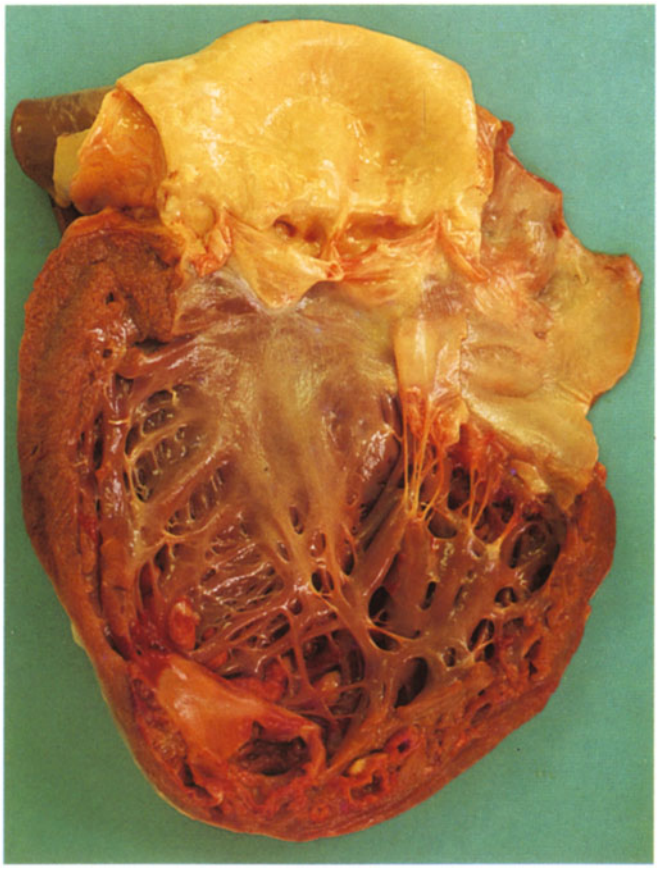

Fig. 1.16 Apical aneurysm of the left ventricle with thrombus in a case of chronic myocarditis

Grocott-positive while small protozoan organisms generally are Grocott-negative. Outside muscle fibres and glial cells, we have never seen parasites in other cells (macrophages or endothelial cells).

The tissue reaction found at the level of myocardium in the **acute form** is a marked cellular infiltration, more or less diffuse (Figs. 1.17 and 1.18), where lymphocytes, histiocytes and plasma cells can be seen while the number of leukocytes is reduced. In wide areas, the muscle fibres disappear because they have been totally replaced by the infiltrates. This is a true myocarditis, apparently as a result of the action of parasites present in variable numbers of intracellular (myocardial fibres) nests. We are, therefore, at a loss to understand why nowadays numerous researchers call this typical inflammatory process of the disease myocardiopathy, instead of myocarditis.

As far as the central nervous system is concerned, the inflammatory process is disseminated and localized in the white and grey matter. In the white matter, there is a diffuse gliosis present. The reaction of the neurocytes is mild and non-specific. The parasitic cells or nests of them are hardly ever surrounded by cellular infiltrates. Notably, the intracellular localizations of amastigotes are seldom recognized as such with a light microscope. There are three main types of lesions:

1. Small granuloma-like cell infiltrates (100–500 μm), often situated near arterioles but also 'free' in the parenchyma, composed of lymphocytes and histiocytes as well as microglial rod-like cells.

2. Cuff-like cellular infiltrates in the perivascular Virchow–Robins' spaces formed by lymphocytes, plasma cells and monocytes.

3. Small glial nodules consisting of astrocytes with rod-like microglial cells. A non-specific meningitis occurs occasionally, in addition to acute Chagas encephalitis. Primary Chagas meningitis has not, to our knowledge, been reported (Figs. 1.19–1.23).

In other organs, the parasites cause a non-specific, often scarce, inflammation, usually of the interstitial type (Figs 1.24–1.28). In Chagas orchitis, parasites are located inside seminiferous tubules, and cellular infiltrates are present in the interstitium (Fig. 1.29).

In the **chronic form**, the heart is the main organ involved. Lesions in the brain are not observed. The chronic myocarditis shows areas of fibrosis of variable size, with and without cellular infiltrates, either totally or partially replacing the muscle tissue (Figs. 1.30–1.34). If exclusive fibrosis is observed, without cellular infiltrates or with only scarce ones, a diagnosis of myocarditis may be questionable.

The cellular infiltrates are made up primarily of lymphocytes, histiocytes, plasma cells and, very rarely, neutrophilic leukocytes. Sometimes, a variable number of eosinophilic leukocytes is observed and, occasionally, myogenous multinuclear giant cells are found. The origin of the myogenous giant cells, i.e. that they grow from muscle fibres, could be corroborated in one case, when lipofuscin pigment was observed in a giant cell (Fig. 1.35). The presence of giant cells does not indicate a granulomatous reaction because of the lack of epithelioid cells. Micronecroses are found occasionally in cases of chronic myocarditis as well as typical acute cardiac infarcts.

In the great majority of all these cases, parasites cannot be found in the tissues in spite of intensive and prolonged search. In practice, therefore the question arises of whether one is faced with myocarditis due to infection with trypanosomes or with the so-called 'chronic idiopathic myocarditis' which occurs all over the world and, obviously, also in regions of endemic Chagas disease.

Regarding the so-called endomyocardial fibrosis, a well-known entity in Africa and in some way related to African trypanosomiasis, it cannot be ruled out that this cardiopathy in South America has links with the *Trypanosoma cruzi* infection. However, only few cases of this sort have been observed in South America.

So-called mega-organs, mostly reported in Brazil, Argentina and Chile, have not been found in Venezuela.

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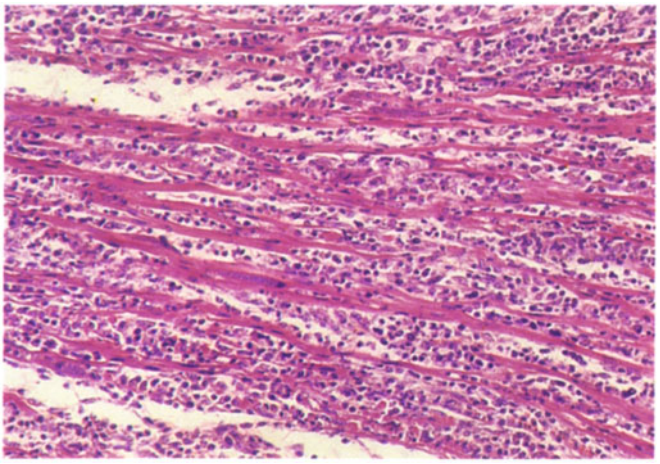

Fig. 1.17 Acute diffuse chagasic myocarditis. Nests of parasites are difficult to discern at this magnification. H&E

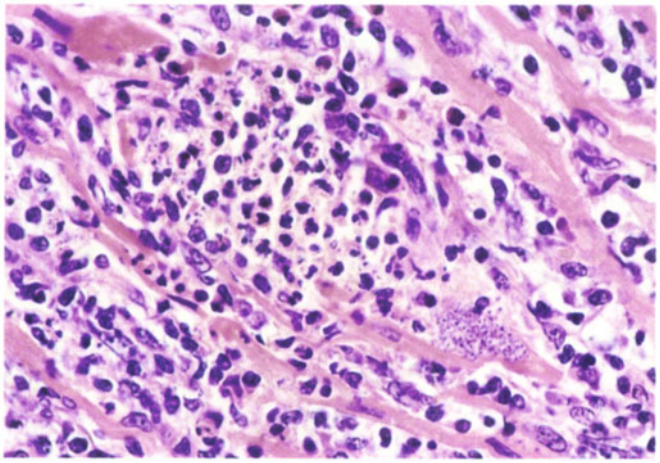

Fig. 1.18 Acute chagasic myocarditis at higher power with muscle fibres replaced by cellular infiltrates and a nest of parasites faintly visible. H&E

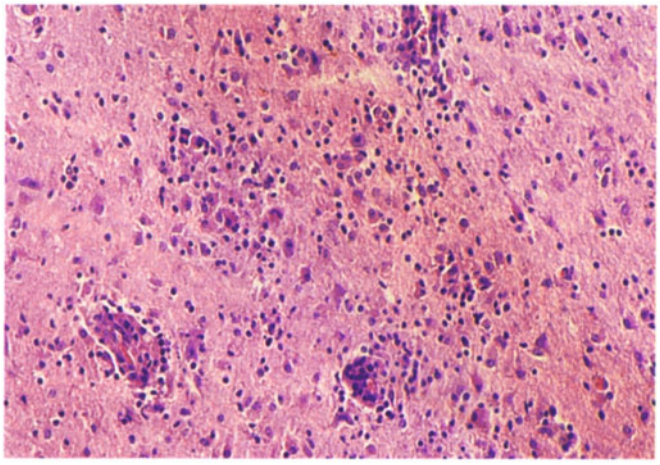

Fig. 1.19 Acute chagasic encephalitis at low power. H&E

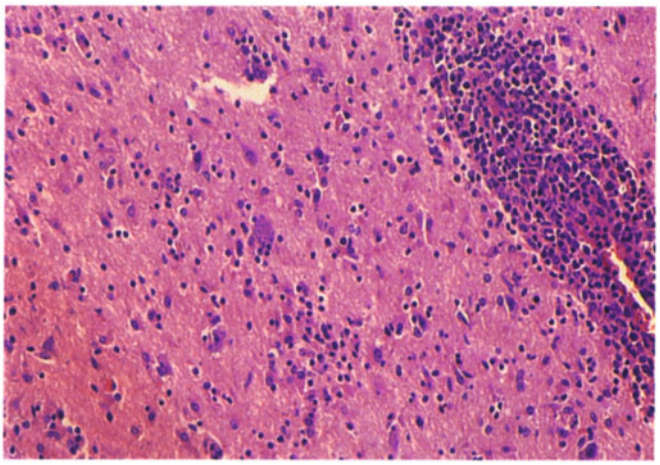

Fig. 1.20 Acute chagasic encephalitis with a marked perivascular cell infiltrate. H&E

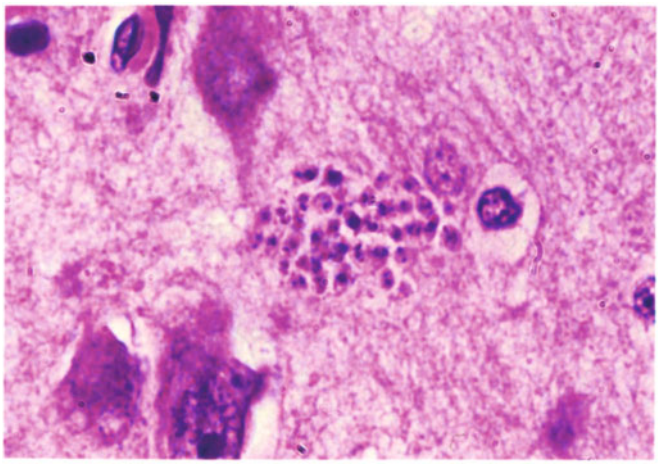

Fig. 1.21 Nest of *Trypanosoma cruzi* amastigotes in the brain without inflammatory reaction. Outlines of a glial cell not visible. H&E

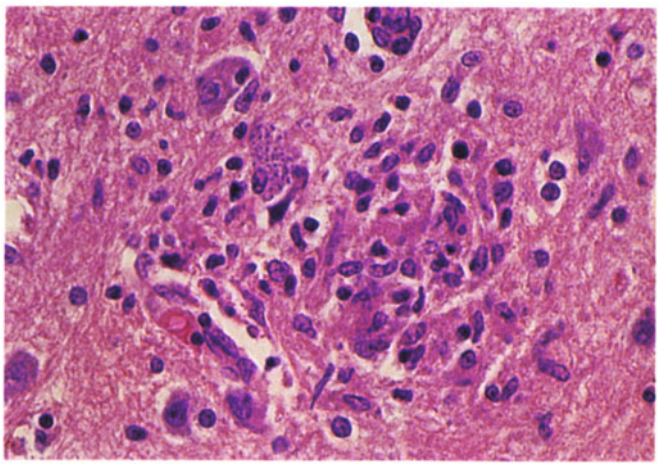

Fig. 1.22 Parasitic nest in the cellular infiltrate of an acute chagasic encephalitis. H&E

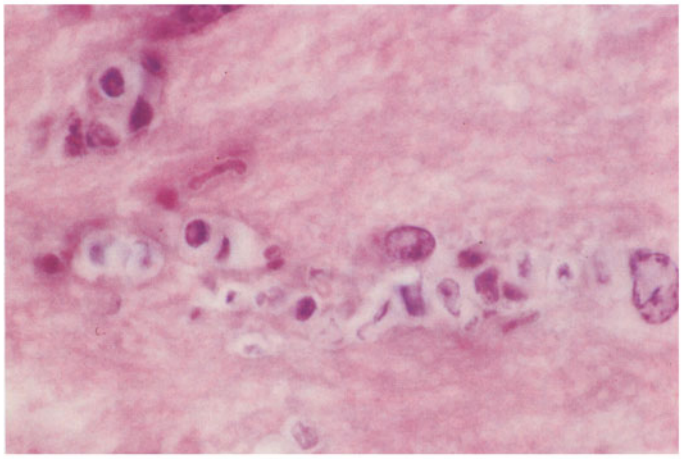

Fig. 1.23 Necrobiotic cells in an acute chagasic encephalitis mimicking parasitic structures. H&E

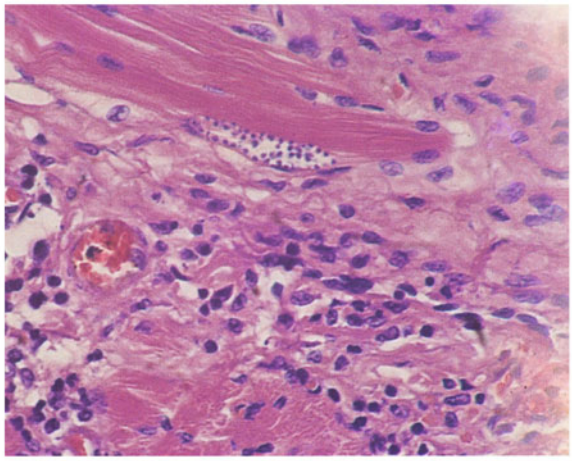

Fig. 1.24 Marked inflammation in acute chagasic oesophagitis. H&E

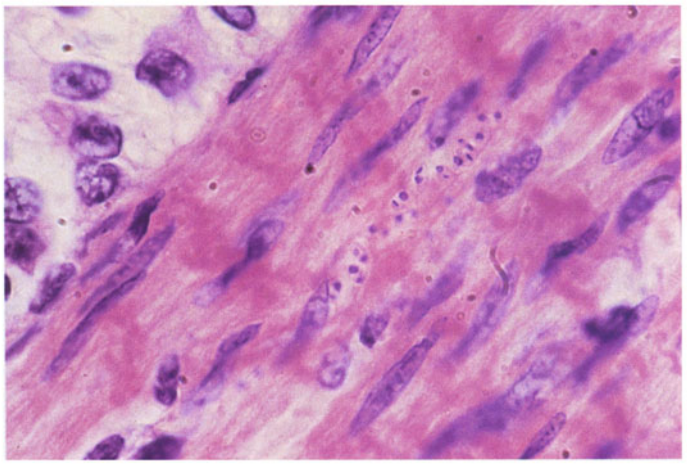

Fig. 1.25 Acute chagasic enteritis. H&E

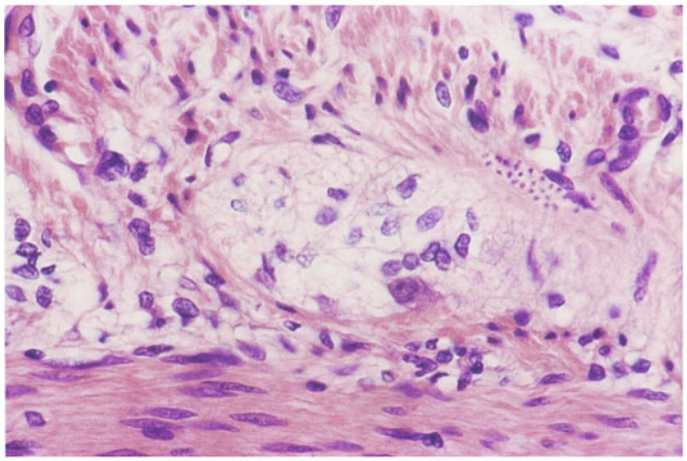

Fig. 1.26 Acute chagasic colitis. Note parasites localized in the digestive tract in muscle cells. H&E

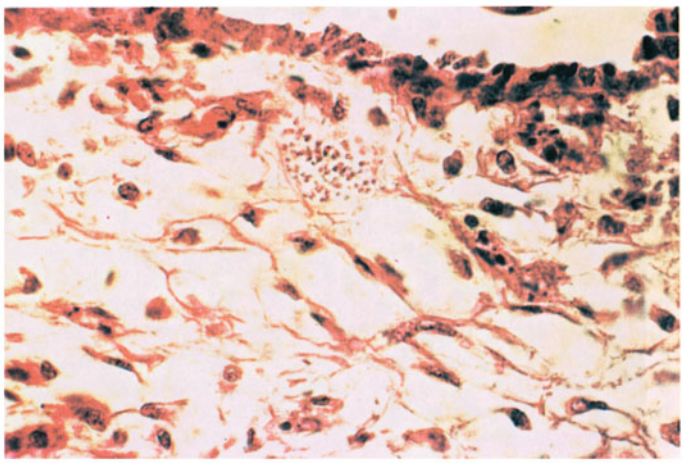

Fig. 1.27 Nest of *Trypanosoma cruzi* amastigotes in placental villus. A case of congenital Chagas infection. H&E

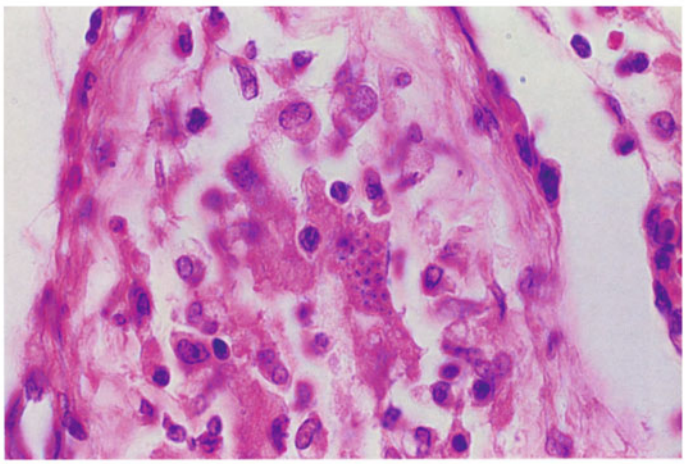

Fig. 1.28 Same case as Fig. 1.27 at higher power. H&E

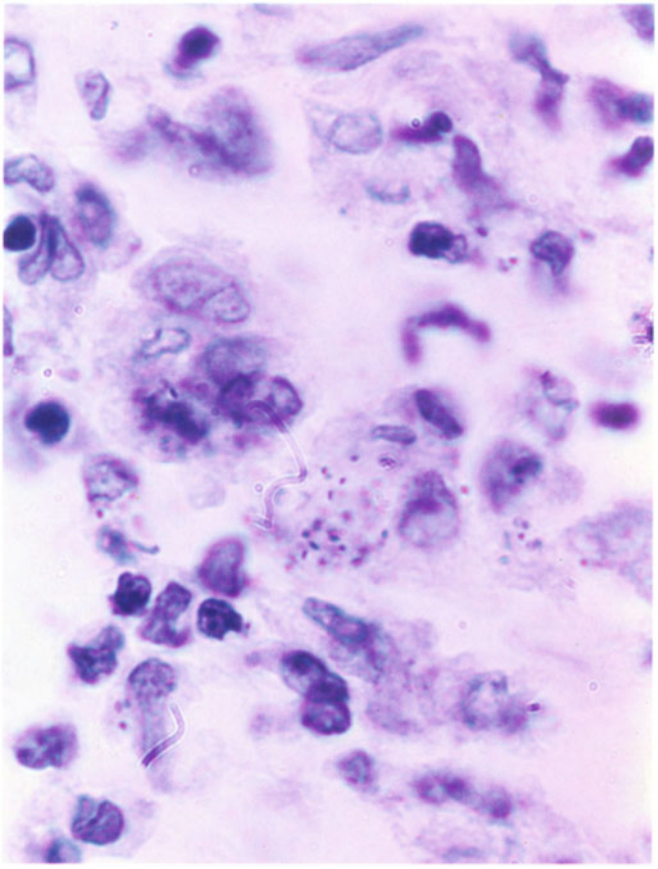

Fig. 1.29 Chagas orchitis with nests of parasites in a seminiferous tubule. Giemsa

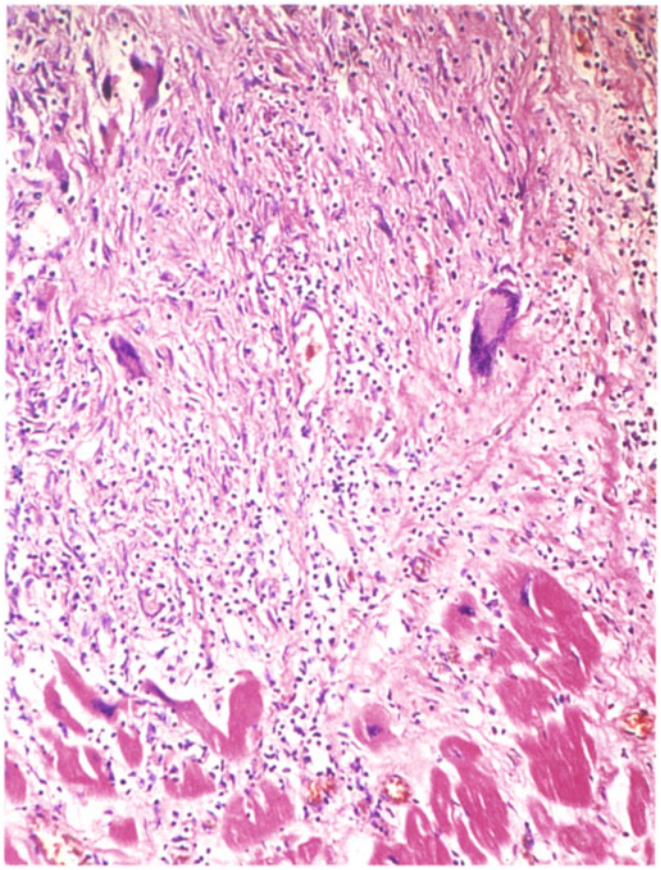

Fig. 1.30 Chronic myocarditis with several myogenous giant cells. H&E

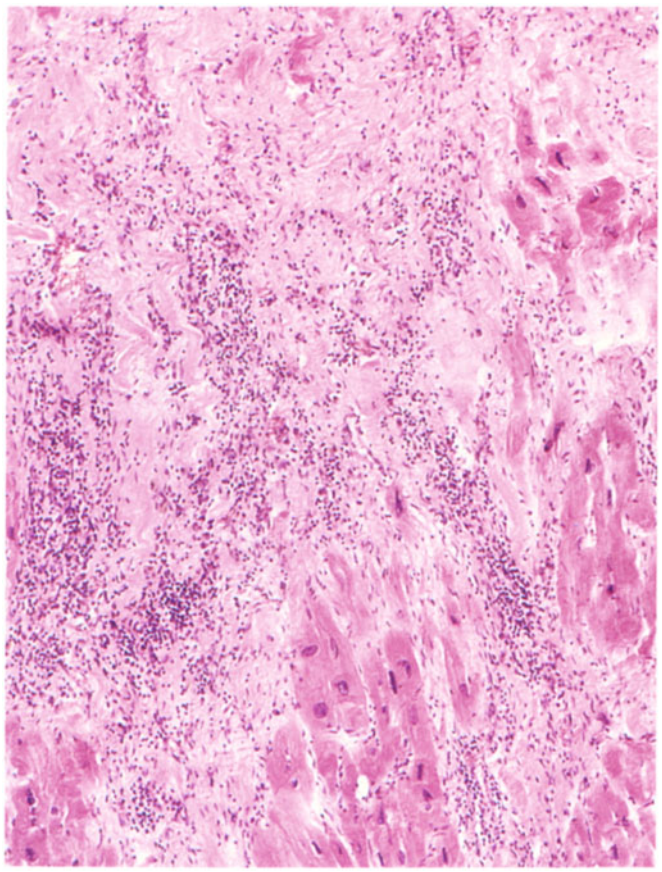

Fig. 1.31 Chronic myocarditis with fibrosis and focal cellular infiltrates. H&E

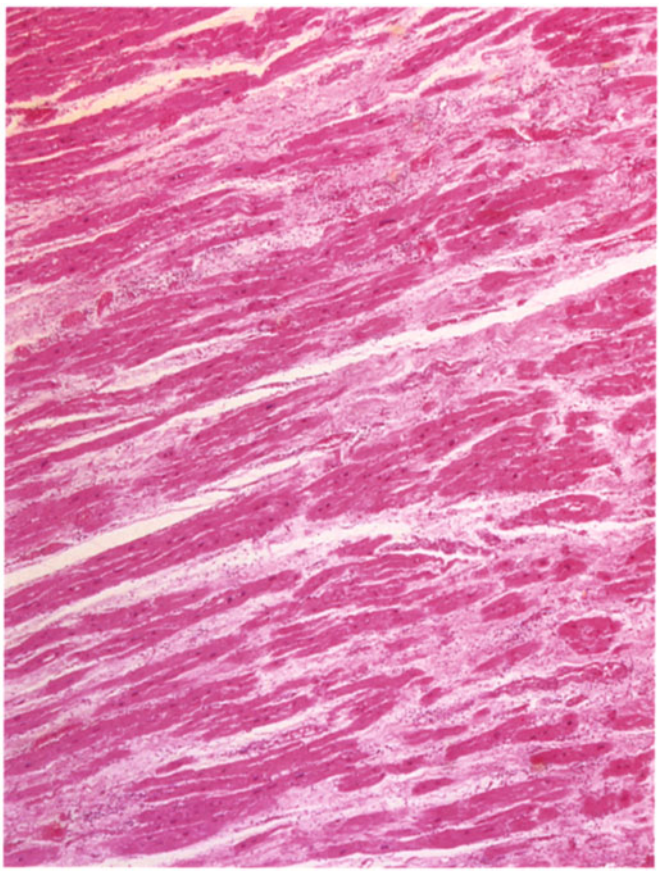

Fig. 1.32 Chronic myocarditis with marked fibrosis and scarce cellular infiltrates. H&E

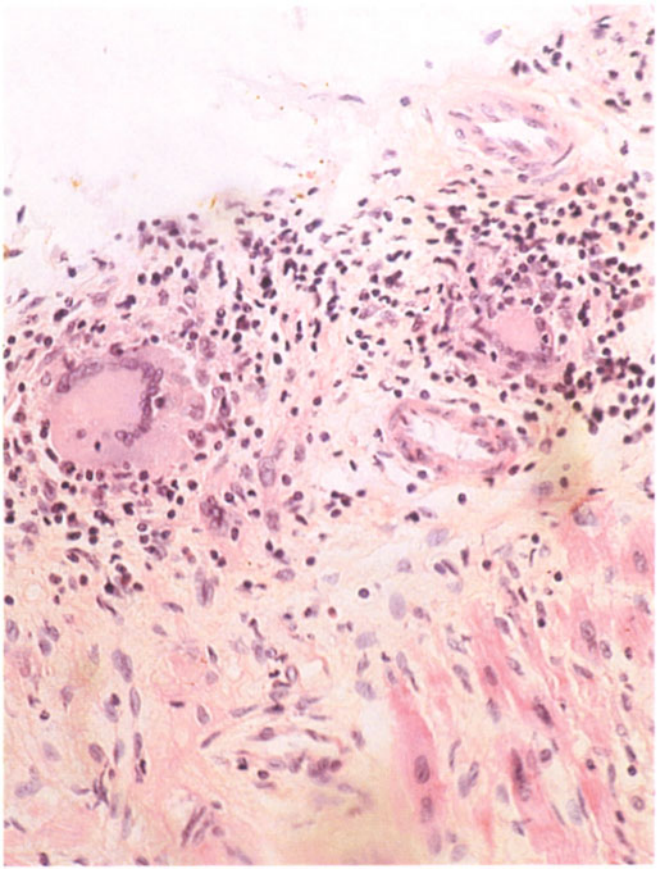

Fig. 1.33 Chronic granulomatous myocarditis of unknown aetiology. H&E

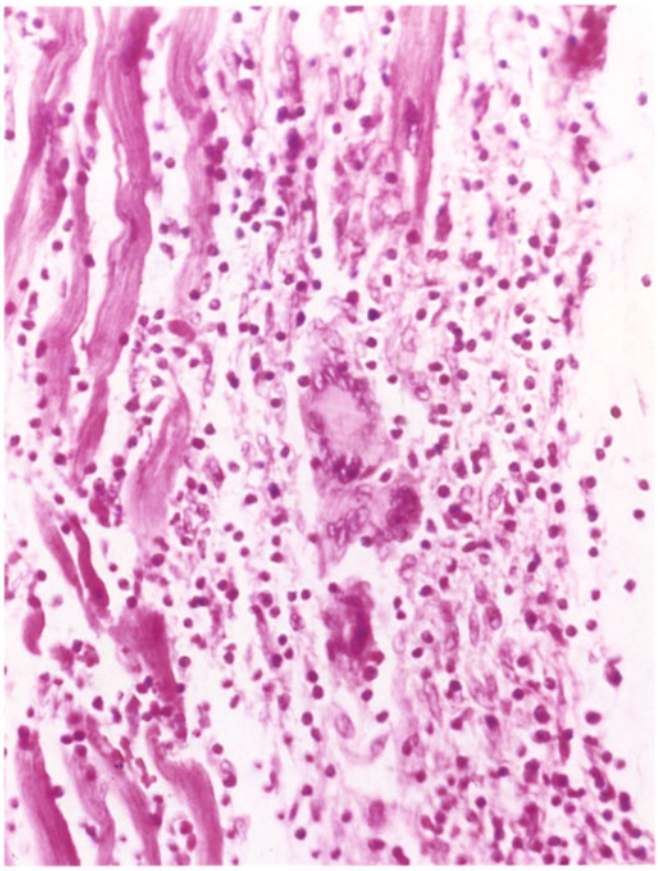

Fig. 1.34 Another case of chronic myocarditis with giant cells and undetermined aetiology. H&E

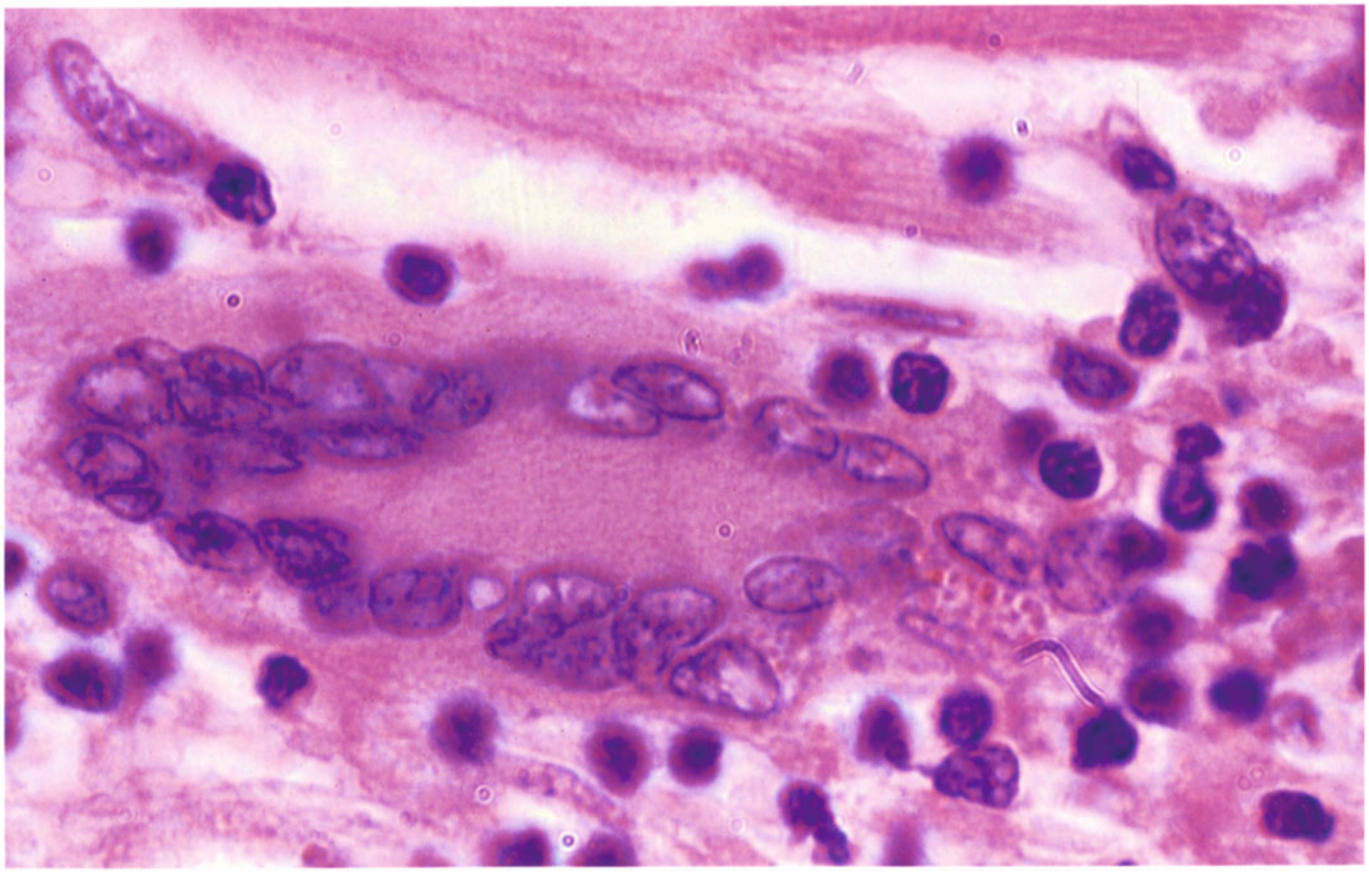

Fig. 1.35 Myogenous giant cell in a case of chronic myocarditis showing granules of lipofuscin pigment, thus revealing the myogenous origin of the giant cell. H&E

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2. AFRICAN TRYPANOSOMIASIS

Introduction

This infection is also known as sleeping sickness. Epidemics with high mortality rates had depopulated vast regions of Africa. The preventive campaigns of the WHO to control the vectors have had the result of limiting considerably this disease. Also, it has been possible to treat cases with effective drugs. However, eradication of vectors has not been total; still more or less important epidemics occur. WHO experts believe that, in spite of all efforts, complete extermination of this disease will not take place in the near future.

Natural infections occur in domestic and wild animals[1]. The disease may be produced by inoculation into laboratory animals[2–5] (Fig. 2.1).

In African trypanosomiasis, histological preparations do not show the parasites as amastigotes, in contrast to American trypanosomiasis. This must be emphasized as the most important morphological difference between these two parasitoses.

The infection is limited to equatorial Africa. Two forms of the disease may be distinguished: in the western and central parts of this continent, a chronic and relatively benign disease is observed[6]. In the later stages, symptoms of the CNS appear and the outcome may be fatal. In the eastern parts of the continent, on the other hand, the disease takes a more severe course. In untreated patients, death occurs, frequently 1–3 months after infection[7]. Clinical diagnosis in early stages of the disease may be made by observing trypomastigotes in blood smears and smears of puncture fluids taken from the chancre, lymph nodes, body cavities and also of the spinal fluid. Cultures can be made on artificial media[8] or by inoculation of rodents and monkeys.

The parasite

The three species of African trypanosomes belong, like *Trypanosoma cruzi*, to the genus *Trypanosoma* and the family Trypanosomatidae. *Trypanosoma brucei gambiense* is responsible for human infection in the western and central parts of the continent, *Trypanosoma brucei rhodesiense* in eastern parts and *Trypanosoma brucei brucei* is only a pathogen in domestic animals. All three are structurally identical. They differ in their pathogenicity in man and lower animals.

Both *T. gambiense* and *T. rhodesiense* each have a specific group of vectors, live in different geographical regions and have different reservoir hosts. They do infect superior vertebrates, but, in general, do not cause disease; that is, these animals are reservoir hosts and a source of infection for man but do not become ill. On the other hand, domestic animals, such as horses, donkeys, camels, dogs and cats, fall ill when infected by trypanosomes belonging to the species *T. brucei brucei*. The disease is called 'Ngana', which means 'useless' or 'weak' and has a fairly high mortality rate. Some wild animal species, certain domestic animals and man, on the other hand, do not become ill when infected by *T. brucei brucei*.

The three species appear only in the flagellate form (trypomastigotes) and have variable sizes (Fig. 2.2). Trypomastigotes succumb fast: a good fixation is required to preserve them. In stained smears, the trypomastigotes measure 1.5–3.5 μm in width and 15–30 μm in length, including the flagellum. The nucleus stains dark with the Giemsa, Wright and other similar stains and is situated towards the centre.

The trypomastigotes are difficult to recognize in unstained smears because they are colourless, thin and transparent. However, it is possible to detect them when they are alive because of their motility. This type of trypanosome has a small kinetoplast situated near the rear end. The flagellum originates in the blepharoplast near the kinetoplast, which, however, is not identifiable with a light microscope. Trypanosomes of this type may multiply by longitudinal division of the trypomastigote. This is in contrast to *Trypanosoma cruzi* which reproduces only in the amastigote form.

Man is the principal reservoir host for *T. brucei gambiense*, but wild and domestic animals (pigs for example) may act as reservoir hosts. However, wild animals (above all antilopes) and also domestic animals are the principal hosts for *T. rhodesiense*, infection from man to man being less frequent.

The vector for *T. brucei gambiense* and *T. brucei rhodesiense* is the tsetse fly (Fig. 2.3), *Glossina palpalis* and *Glossina morsitans*, respectively. These species do not have important structural differences. Either male or female fly may act as vector.

Pathogenesis

When the non-infected tsetse fly (*Glossina* spp.) bites an infected man or lower animal, it sucks blood and, with it, the trypanosomes, which then reach the intestinal tract of the vector. The parasites multiply in the gut of the fly and three weeks later they reach the salivary glands, in the meantime having become metacyclic and infectious.

Therefore, when an infected fly bites a healthy man, it transmits the trypanosomes. This bite, by the way, is painful. At the site of the bite, which is almost always the cervical region, a skin nodule is produced and inflammation of the regional lymph nodes develops. Both lesions constitute the 'inoculation chancre' of the African trypanosomiasis, also named Winterbottom's symptom (Fig. 2.4). From this focal cutaneous lesion, haematogenous dissemination results, with clinical manifestations of fever, general malaise and splenomegaly. In experiments recently carried out on lower animals, it has been found that the parasites first reach the meninges; encephalitis develops later in addition to the meningitis[2].

Pathology

Both the gross and the microscopic lesions are similar in the two forms of African trypanosomiasis[9]. The CNS, lymph nodes, spleen and heart are all involved and sometimes there are effusions in all corporal cavities. In the central nervous system oedema and petechiae are found as gross lesions. The lymph nodes and the spleen may be enlarged. Apparently, no other gross manifestations have been observed and no specific gross features characteristic for this infection are known.

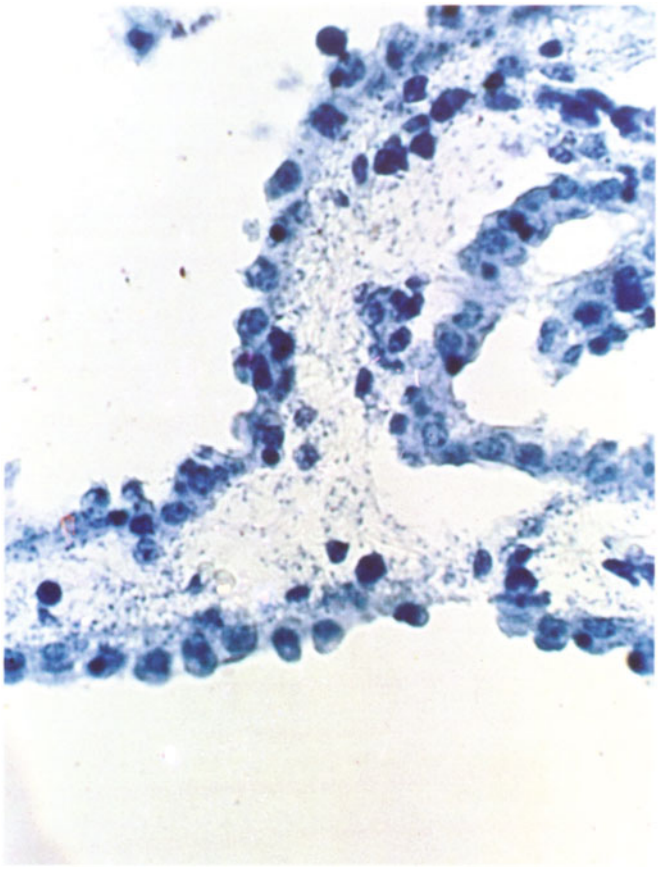

Fig. 2.1 Numerous amastigotes of *Trypanosoma rhodesiense* in a villus of the choroid plexus. Experimental infection of a mouse (H. Schmidt, 1983). Giemsa

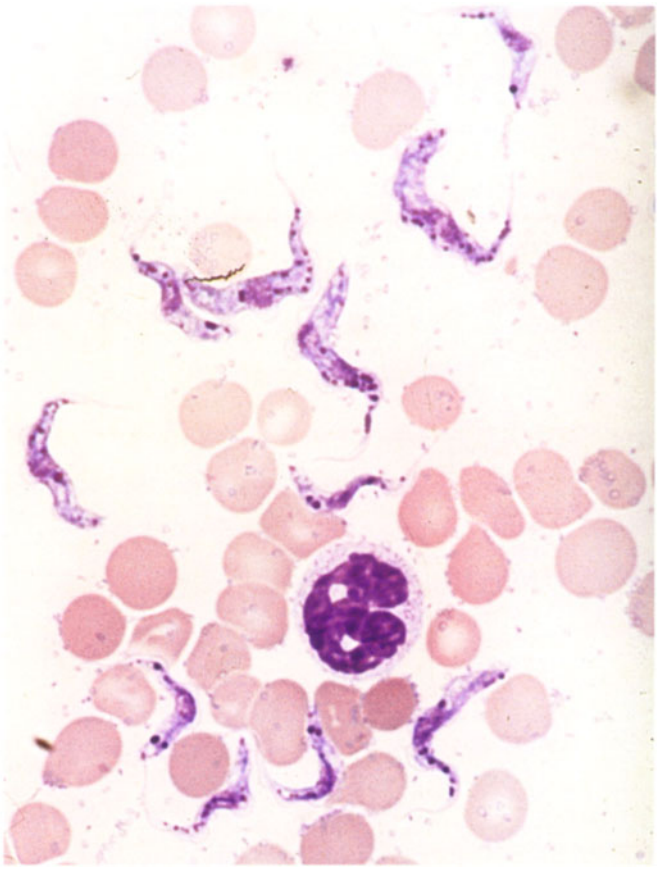

Fig. 2.2 Numerous trypanosomes of *Trypanosoma gambiense* in a blood smear. Giemsa

Fig. 2.3 Tsetse fly (*Glossina*), vector of African trypanosomiasis

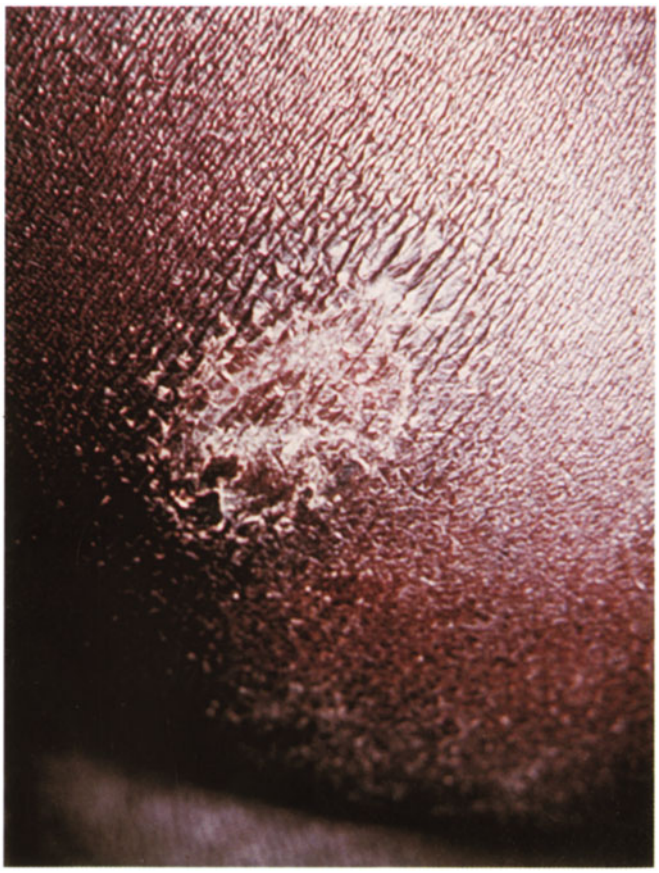

Fig. 2.4 Skin chancre. Residual scar of an infection in African trypanosomiasis

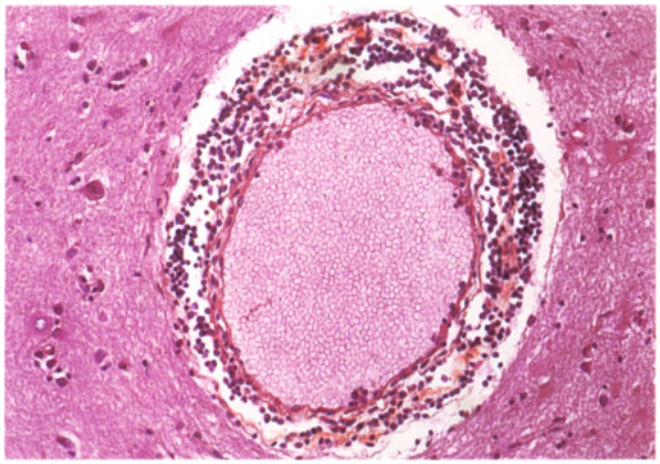

Fig. 2.5 Typical perivascular infiltrates in the encephalitis of sleeping sickness. H&E

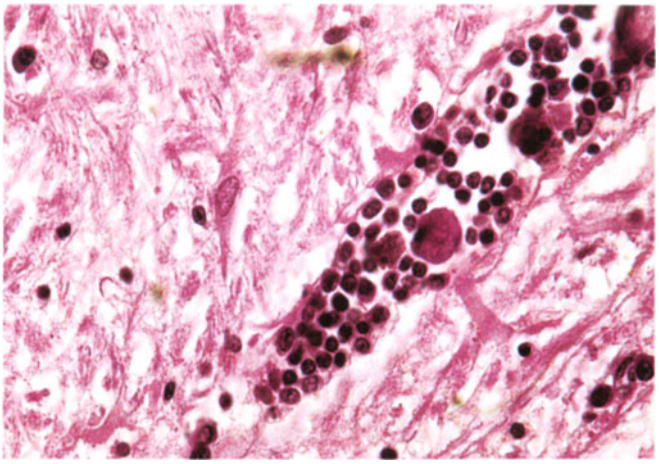

Fig. 2.6 Perivascular infiltrate with a 'morula cell'. H&E

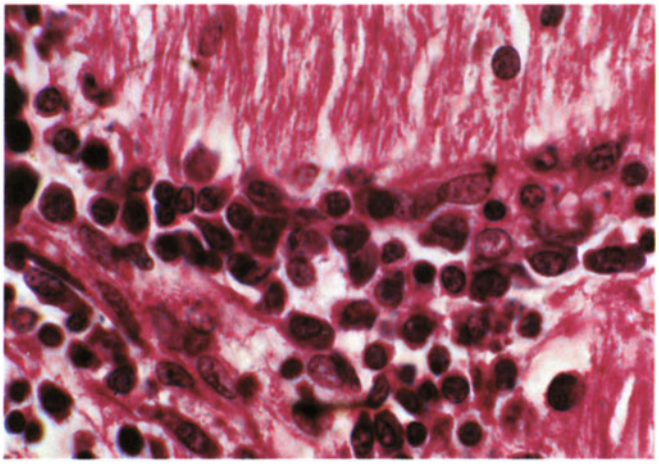

Fig. 2.7 Higher magnification of a perivascular infiltrate in African trypanosomiasis encephalitis. H&E

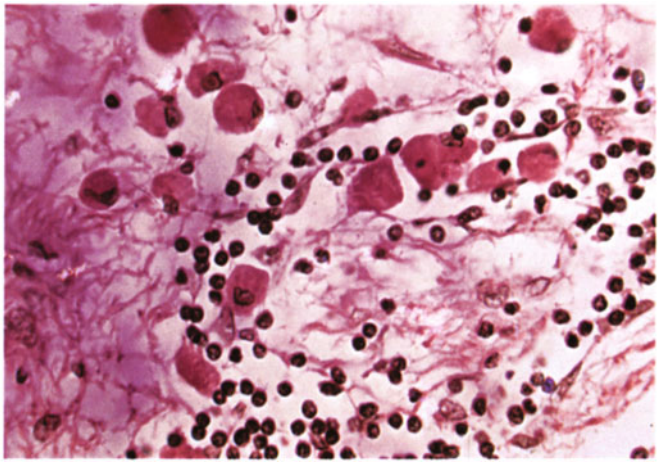

Fig. 2.8 Numerous 'morula cells' in a case of cryptococcosis with brain lesions. H&E

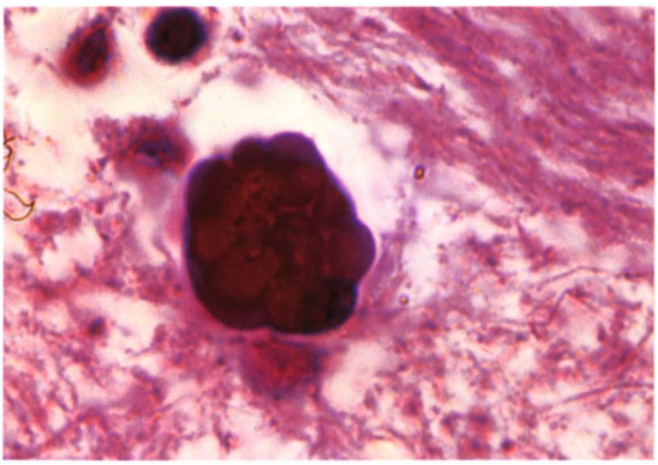

Fig. 2.9 Higher magnification of a 'morula cell' in African trypanosomiasis encephalitis. Note the confluence of the intraplasmatic corpuscles. H&E

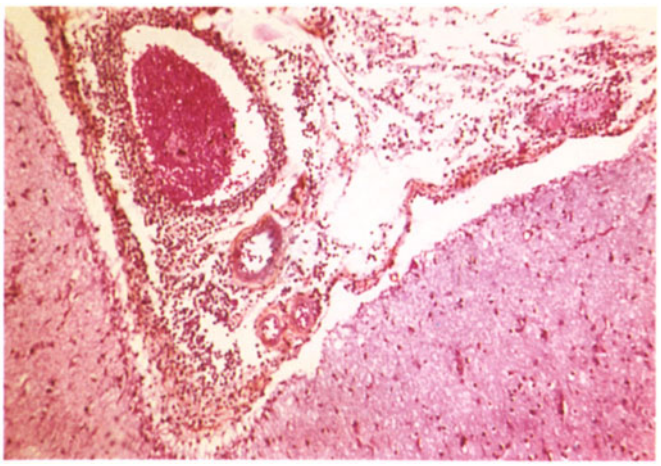

Fig. 2.10 Leptomeningitis in a case of African trypanosomiasis encephalitis

Under the microscope, the causative agent cannot be found in tissue sections as is the case in the acute form of Chagas disease. Only in experimental animals are parasitic elements seen in the tissues, and amastigotes and trypomastigote-like forms have been reported[2].

Regarding tissue reaction, the inflammation is present in the white and grey matter and there are regions with oedema. Here, also, progressive forms of astroglial elements are seen with an homogeneous eosinophilic cytoplasma which is irregularly shaped (gemistocytic transformation). Neurocytes do not show any specific lesions.

In the often-dilated perivascular spaces, cellular infiltrates, consisting of lymphocytes, plasma cells and monocytes are found. In addition, there are many 'corpuscular structures', measuring from 20–30 μm in diameter, which are strongly eosinophilic, spherical or ovoid in shape, and situated in the cellular infiltrates or in the oedematous areas. They consist of small eosinophilic bodies measuring 2–3 μm in diameter, either showing a morula aspect or appearing homogeneous. The latter is due to confluence of the small bodies. These structures represent Russell or Mott bodies and are characteristic of this disease but are also observed in cases of encephalitis due to other aetiologies. We have seen them in encephalitis due to *Cryptococcus neoformans* or *Histoplasma capsulatum* var. *capsulatum*. Mononuclear inflammation is seen also in the pia mater spreading along blood vessels into the cerebral cortex (Figs. 2.5–2.9).

In enlarged lymph nodes and the spleen, a reactive hyperplasia and infiltrates of histiocytes and plasma cells are seen histologically. In the infrequently encountered pancarditis, lymphohistiocytes and plasma cells are observed in infiltrates of the endocardium, myocardium[10] and the pericardium. Occasionally, fibrosis of variable degree is also present at these sites.

Endomyocardial fibrosis, frequently seen in Africa, is usually of undetermined aetiology. Nevertheless, it has been interpreted as a possible residual lesion of pancarditis caused by trypanosomes[11,12].

The authors of this atlas were able to review personally only a limited number of histological preparations of a few cases of sleeping sickness provided by friends. Together with other colleagues, we believe that the absence of causative agents in tissues, as reported in the literature, may be because histology has not been carried out systematically and thoroughly after postmortem examinations.

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LEISHMANIASES

Traditionally, three types of disease are produced by species of the genus *Leishmania*:

1. The cutaneous leishmaniasis or oriental sore, seen in the Old World and caused by *Leishmania tropica* (Wright).

2. The mucocutaneous leishmaniasis, seen in the New World and due to *Leishmania braziliensis* (Vianna).

3. The visceral leishmaniasis or Kala-azar, seen in both the Old and New World, and produced by *Leishmania donovani* (Laveran, Mesnil).

There are arguments against this classification; mainly that skin lesions may be seen in all three types. Nevertheless, it is useful from the geographical point of view and for other practical reasons.

Some features common to the three species of leishmanias must be emphasized:

1. The amastigotes of the three species of *Leishmania* (and of *Trypanosoma cruzi*) are structurally identical and therefore these species cannot be distinguished in tissues.

2. The leishmanias do not show flagellate forms (trypomastigoes) in mammalian blood.

3. The leishmanias (and trypanosomes) may be cultured in the artificial NNN medium. This medium (Nory, McNeal, Nicolle) contains bactoagar, sodium chloride, distilled water and, in addition, defibrinated blood, from diverse animal species, and penicillin.

Many details of the pathogenesis of the different types of leishmaniasis and of the virulence of their causative agents still need to be investigated. The gross and microscopic features of cutaneous and mucocutaneous leishmaniasis are similar. The only difference is that the mucosae may be involved in the latter.

We describe the mucocutaneous leishmaniasis in most detail; our personal experience has been gained studying leishmaniasis in the New World.

3. CUTANEOUS LEISHMANIASIS

Introduction

This infection is called also oriental sore or boil, tropical ulcer, sore of Jericho, Aleppo, Delhi or Biskra, or leishmaniasis of the Old World[1–3].

The disease occurs in the Mediterranean region, Asia Minor, India, China and Africa stretching from east to west between the 10° and 13° of latitude. Natural infections exist in several lower animal species[4]. Rodents may be used for experimental purposes[5,6].

Three types of the disease are considered: the urban type (*L. tropica minor*) which is mostly anthropozoonotic; the rural type (*L. tropica major*) which is zoonotic; and the diffuse cutaneous leishmanias (*L. tropica aethiopica*). The two types first mentioned usually show solitary lesions. The last is characterized by multiple lesions. This is apparently due to an anergic disposition of unknown origin; the Montenegro skin test in this type is negative.

The cutaneous leishmaniasis may heal spontaneously. Clinical diagnosis is made by confirmation of the causing agents in smears or biopsies; also, fine needle aspiration biopsy may be performed[7].

The parasite

Leishmania tropica is encountered in tissues in the amastigote form. The amastigotes are spherical, measure 2–4 μm in diameter and show a kinetoplast. Structurally, the amastigotes of all the *Leishmania* species are identical, not only the species causing the cutaneous, mucocutaneous and visceral leishmaniasis (*L. tropica, L. braziliensis* and *L. donovani*), but also *L. tropica minor, L. tropica major* and *L. tropica aethiopica*. It is therefore difficult to understand why different names were given to the leishmanias producing the different clinical forms of the disease.

The reservoir host for the urban form of *L. tropica* is predominantly man; for the rural form of cutaneous leishmaniasis, however, dogs, cats, rodents and monkeys.

Vectors of the disease are female sand flies of the genus *Phlebotomus*.

Pathogenesis

Infection takes place through the bite of a sand fly (Fig. 3.1), mostly on uncovered parts of the skin. The incubation period varies from 2–8 months. Usually, a solitary skin nodule develops which grows slowly and may later ulcerate and become a scar.

Multiple skin lesions appear to be due to an anergy of unknown origin. There is no satisfactory explanation at present for the differences in localization of the lesions in the two forms of leishmaniasis found in different geographical regions, i.e. why, in case of the cutaneous leishmaniasis, neither mucosae nor internal organs are affected.

Pathology

In the great majority of cases, a single skin lesion is found. The urban type of cutaneous leishmaniasis is characterized by the so-called 'dry' form of the disease, i.e. the skin nodules do not have a tendency to ulcerate. When it relapses, it is called 'lupoid leishmaniasis'. In the rural type, on the other hand, there is a tendency to ulcerate and then it is called the 'humid' form.

While only solitary skin lesions used to be seen in the *L. tropica* infection, recently multiple lesions, mainly in the face, have been described, mostly in certain regions of Africa. This form of the disease which was unknown in the Old World previously, is called diffuse tegumentary, lepromatoid, keloid or tuberculoid leishmaniasis. These cases of leishmaniasis with single or multiple lesions in the skin are practically identical to the leishmaniasis lesions in the New World; lesions are limited to the skin without involvement of the mucosae (Figs. 3.2 and 3.3).

In some reports recently, it was stated that, histologically, the tissue reaction in the American form of leishmaniasis shows special patterns which allow differentiation from the disease in the Old World. However, as yet, these data have not been confirmed.

As we do not have much personal experience of leishmaniasis of the Old World, we shall refrain from giving detailed descriptions of this infection. The few cases we could examine showed infiltrates of numerous plasma cells, a pattern not, perhaps, so pronounced in the mucocutaneous leishmaniasis. In a case of cutaneous leishmaniasis of the Old World, numerous parasites were seen (Fig. 3.4). All the following details of mucocutaneous leishmaniasis also apply to the cutaneous form. Only the data about epidemiology and, of course, all details about the involvement of the mucosae relate solely to the American leishmaniasis.

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4. MUCOCUTANEOUS LEISHMANIASIS

Introduction

This disease is also named American tegumentary leishmaniasis or leishmaniasis of the New World and has been confirmed as an autochthonous infection from Northern Argentina to Southern Mexico[1]. The name depends on the particular anatomical locations and the country or region (Mexico, Panama, Peru, the Guianas, Amazonas or Brazil). Some other local names are 'chicleros' ulcers', espundia, buba, uta and pianbois (forest yaws). The disease is well known in Venezuela[2–5]. It is usual to

Fig. 3.1 *Phlebotomus*, male. This genus of sand-flies is the vector for all the species of leishmanias. Only the females are vectors

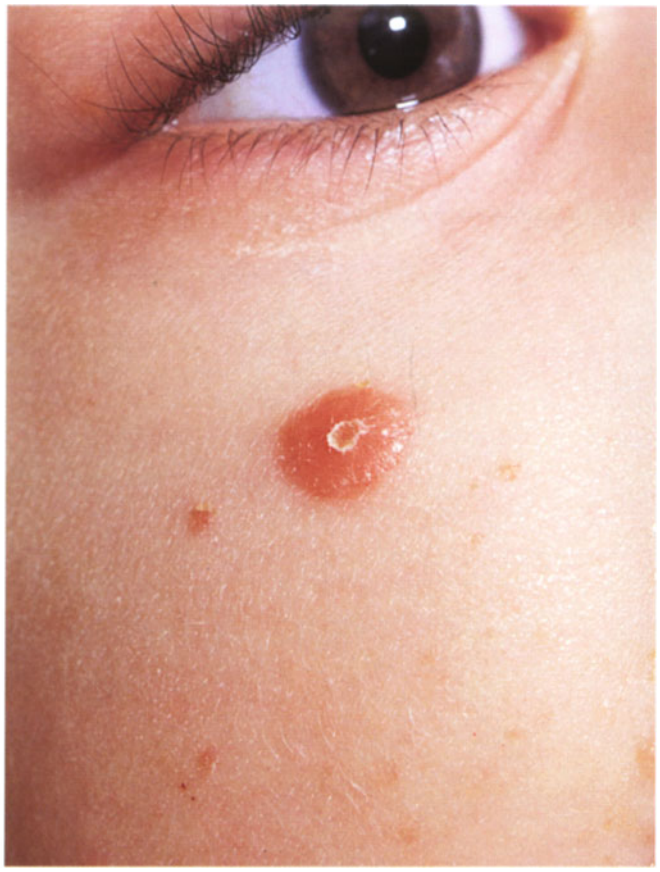

Fig. 3.2 Early stage of leishmanial infection in the Old World

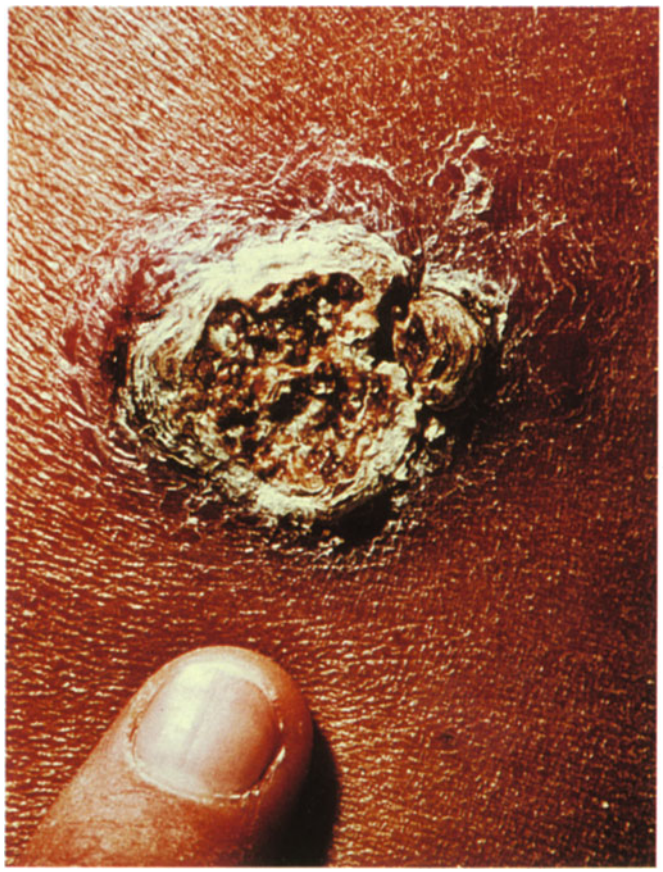

Fig. 3.3 Advanced or chronic stage of oriental sore with a single skin ulcer

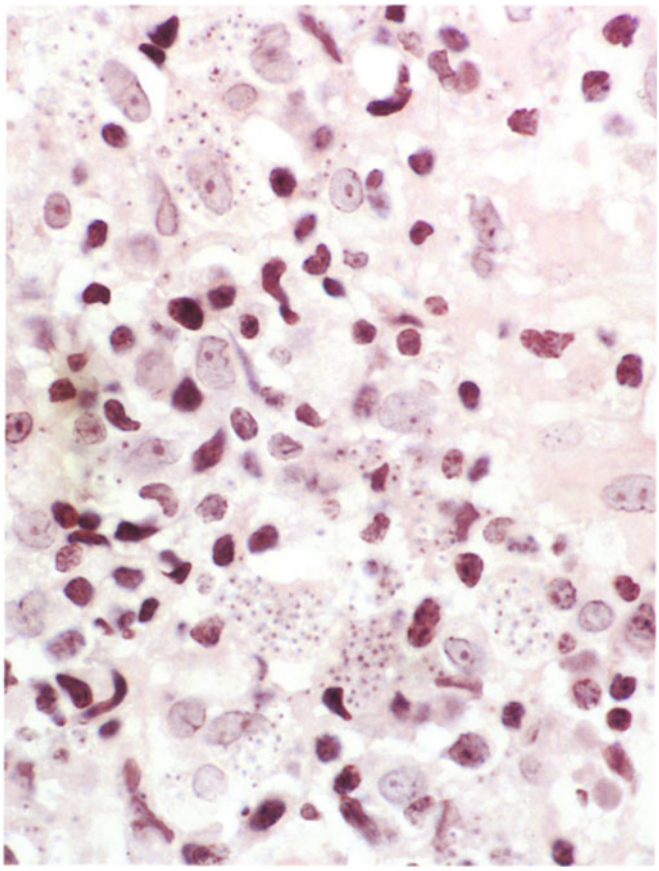

Fig. 3.4 Oriental boil. Case from Afghanistan with numerous parasites in this field. H&E

observe groups of patients with this infection, not single cases, in rural areas with abundant woods and vegetation.

Natural infections occur in dogs and certain wild lower animal species. Experimentally, visceral lesions may be produced in hamsters by inoculation with *L. braziliensis*[6].

Cutaneous lesions are similar to those of the Old World, but usually more extensive and not as easily cured as the latter. The mucosal lesions are located in the oral or nasal cavity and may lead to perforation of the nasal septum or the pharyngeal wall. Prognosis is less favourable than in the cutaneous leishmaniasis of the Old World.

Clinical diagnosis is made by confirmation of the causative agent in biopsies or smears from the surface of ulcerations. The cutaneous test of Montenegro[7] is positive in more than 75%. The immunoperoxide method facilitates diagnosis[8].

The parasite

Leishmania braziliensis is the causative agent of mucocutaneous leishmaniasis. Other species described as causative agents are apparently only subspecies or variants of *L. braziliensis*. This may be the case, for instance, in the 'new species' isolated in the Venezuelan Andes by Scorza *et al.* in 1978[9]. Also *Leishmania mexicana*[10] will not be considered here since it is of no importance in this context. This species may be differentiated from *L. braziliensis* by using DNA probes[11].

L. braziliensis has one form with (in the vector and in cultures) and one without flagella. The aflagellate forms, leishmanias or amastigotes, measuring 2–3 μm in diameter, are found in mammalian tissues (Fig. 4.1). They are slightly smaller than the amastigotes of *Trypanosoma cruzi* and the nucleus is situated on the edge attached to the cellular membrane, which is thin and often not discernible. The rod-like kinetoplast of the amastigotes may be easily seen in well-preserved recently infected tissue, which has been fixed immediately after being removed from the patient. In tissues of routine biopsy material, we have not seen kinetoplasts, but have seen them convincingly in selected cases only.

It must again be emphasized that *Leishmania braziliensis* is structurally identical to *Leishmania tropica* and *Leishmania donovani*, as well as to the amastigotes of *Trypanosoma cruzi*.

Reservoir hosts for *L. braziliensis* are rodents, certain wild animal species and, more rarely, dogs which show cutaneous lesions produced by *L. braziliensis*.

Vectors are females of sand fly species belonging to the genus *Phebotomus* or *Lutzomyia*, for instance *L. longipalpis*. In Venezuela, the most important vector is *Phlebotomus panamensis*.

Pathogenesis

When the females of infected phlebotomes (sand flies) bite healthy men or lower animals, they inject promastigotes into the superficial layers of the dermis. Here, they lose their flagella and penetrate into histiocytes, or are phagocytosed by macrophages. The inflammatory reaction leads to a nodular skin lesion which becomes visible between 2 weeks and 4 months after the bite of the insect.

If a patient has several leishmania lesions, three pathogenic possibilities exist:

1. They are the result of multiple bites,
2. They originated through autoinoculation from a single primary lesion as a result of a single bite, or
3. There was a haematogenous dissemination with the consecutive appearance of multiple skin nodules.

The diffuse tegumentary leishmaniasis starts, apparently, with a single nodule which probably spreads haematogenously, producing multiple skin lesions. This process may take years.

Why and how involvement of the facial mucosae occurs mostly is not clear. Probably, it is due to haematogenous dissemination. However, in the course of parasitaemia, involvement of the internal organs never takes place.

Pathology

Mostly, lesions are found in areas of the skin usually not covered by clothing. Weeks or months after the bite, one or several papules develops or skin nodules appear, reaching variable dimensions. Later, they ulcerate and become chronic lesions; when untreated, there is no tendency to spontaneous healing. On the whole they are difficult to differentiate from skin afflictions due to other causes, especially as they have a chronic evolution (Figs. 4.2–4.9).

The clinical form known as 'diffuse tegumentary leishmaniasis' with multiple skin lesions shows characteristic papulomatous nodules in contrast to the solitary or isolated lesions of the common leishmaniasis (Fig. 4.10). The nodules become confluent and are transformed into cauliflower-like lesions which present a papillomatous appearance with an irregular surface. Characteristically, epidermal ulceration does not occur regularly[12].

The lesions in the mucosae are localized in the nose, oral cavity (Fig. 4.11), pharynx, larynx and trachea, and may or may not be accompanied by cutaneous lesions. In cases without active cutaneous lesions, a scar in the skin may indicate a former primary cutaneous infection by leishmanias. The alterations of the mucosae vary considerably in shape and size and are often tumour-like with ulcerations. Sometimes, they produce stenosis and occlusions in the upper parts of the respiratory and digestive tract which occasionally require surgery.

Exceptionally, the amastigotes are observed histologically in epithelial cells of the epidermis or the mucosae; predominantly they are encountered inside the histiocytes where they reproduce by division and lead to clusters of parasites inside individual tissue cells. The cytoplasm of these tissue cells often shows a clear or vacuolized aspect and, consequently, these cells seem to be 'small cysts'. Numerous histiocytes with clear cytoplasm and many parasites are seen, mainly in acute infections and in diffuse tegumentary leishmaniasis. When the parasitized histiocytes rupture, the liberated amastigotes invade other cells or are phagocytosed by other macrophages. It must be emphasized that the liberated and single amastigotes outside tissue cells are difficult to recognize as such and, therefore, diagnosis cannot be made. The number of leishmanias in each inflammatory focus is very variable. If they are present in great numbers, the intracellular leishmanias are easily recognizable in the 'vacuoles' of histiocytes which, however, are frequently empty due to the loss of parasites during the cutting procedures. On the other hand, if they are scarce or situated extracellularly, or in smears, their visualization may be difficult and often it is impossible to see them. In addition to their intracellular localization in histiocytes with a clear cytoplasm, the borders of necrotic areas should be reviewed in order to detect leishmanias. When the parasites are clearly recognizable, the kinetoplast is not usually well preserved (Fig. 4.12). Some parasitologists insist upon making a diagnosis of leishmanias only when kinetoplasts are visible. We have not often seen kinetoplasts in our material and would like to stress that our experience leads us to make a diagnosis of leishmanias only when organisms are Grocott-negative.

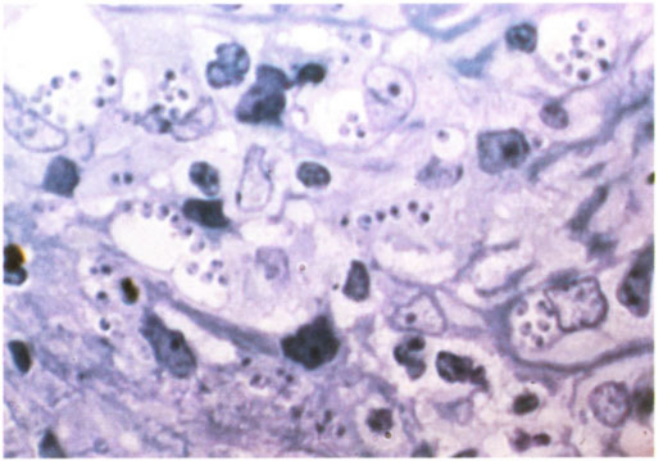

Fig. 4.1 Numerous amastigotes of *Leishmania braziliensis* arranged in clusters. Thick section, Giemsa

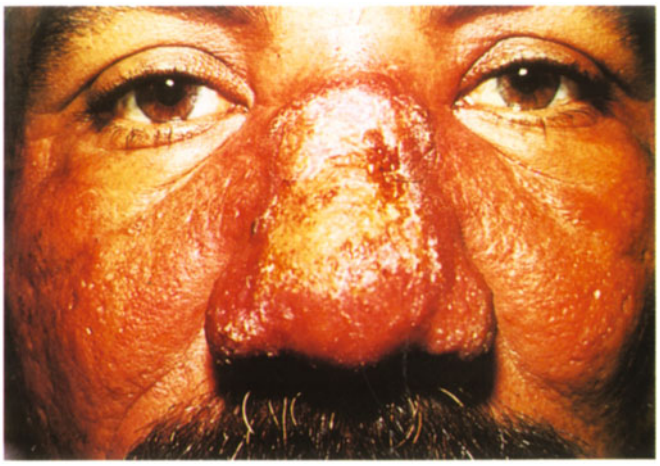

Fig. 4.2 Muco-cutaneous leishmaniasis. Large ulcerative lesion of the nose

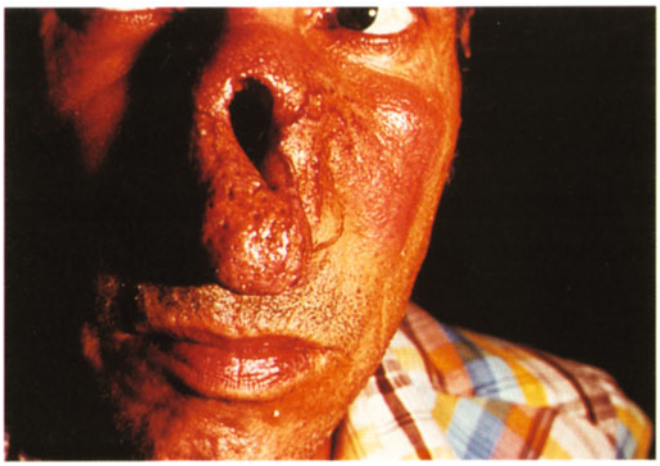

Fig. 4.3 Muco-cutaneous leishmaniasis. Profound ulcer with perforation of the ala nasi

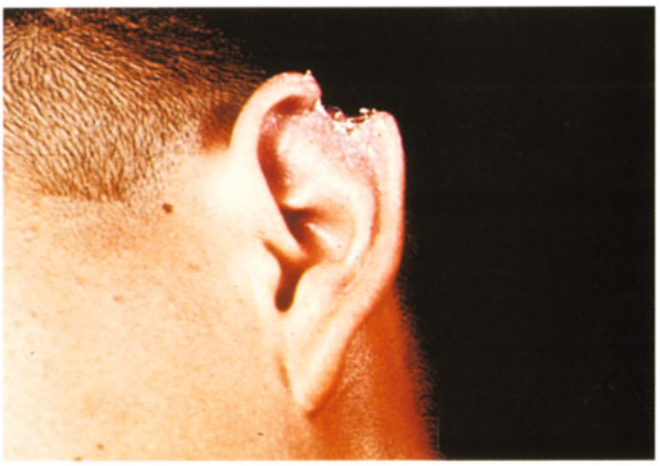

Fig. 4.4 Muco-cutaneous leishmaniasis. Deep skin lesion with defect of the ear lobe

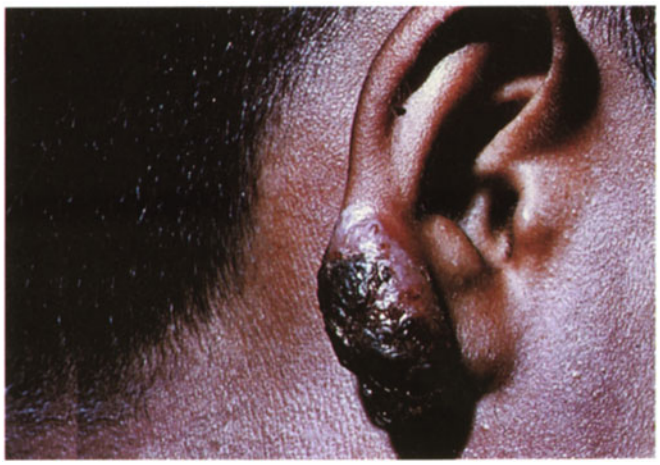

Fig. 4.5 Muco-cutaneous leishmaniasis. Papulomatous lesions with haemorrhages

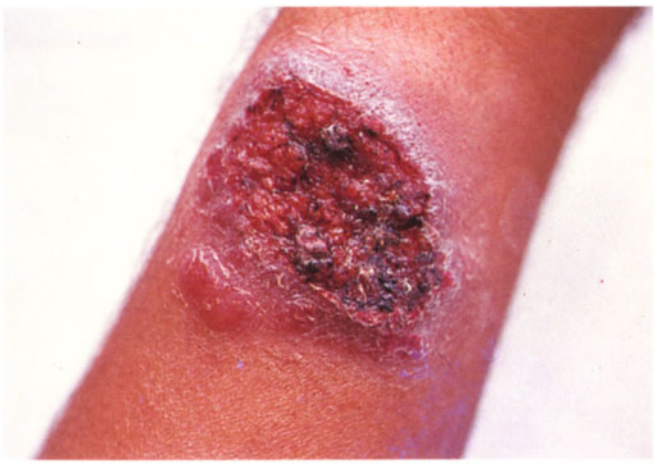

Fig. 4.6 Muco-cutaneous leishmaniasis. Large skin ulcer with a granular ground on an arm

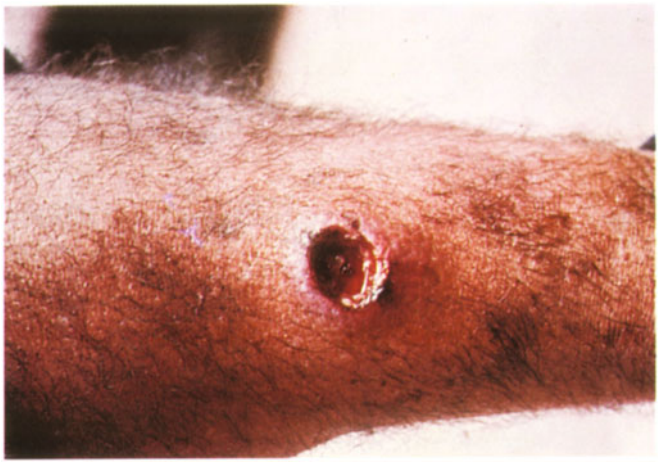

Fig. 4.7 Muco-cutaneous leishmaniasis. Deep skin ulcer with sharp borders on a thigh

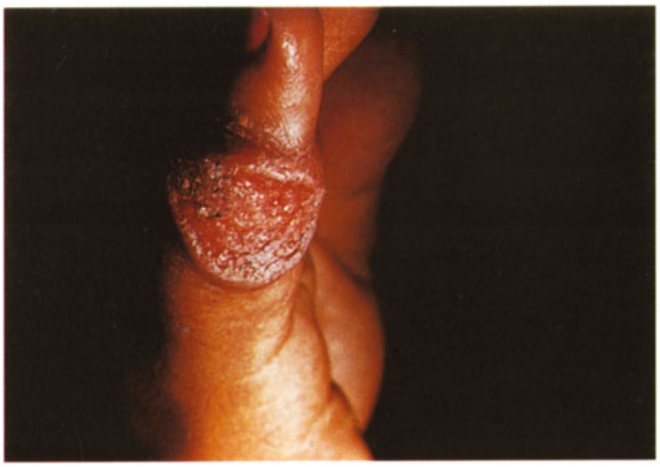

Fig. 4.8 Muco-cutaneous leishmaniasis. Large skin ulcer with thick borders on a finger

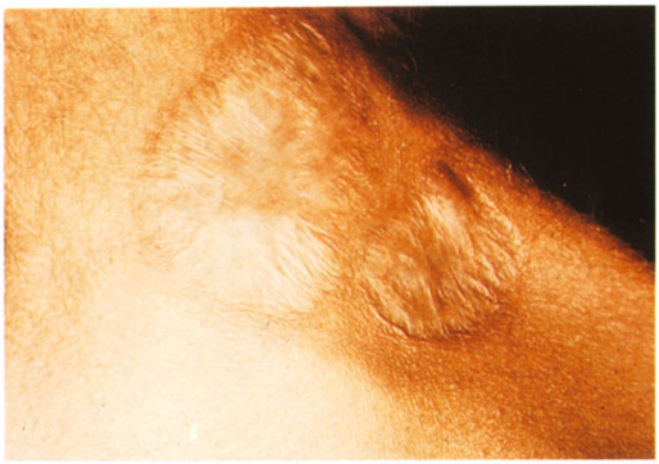

Fig. 4.9 Typical leishmaniatic skin scar

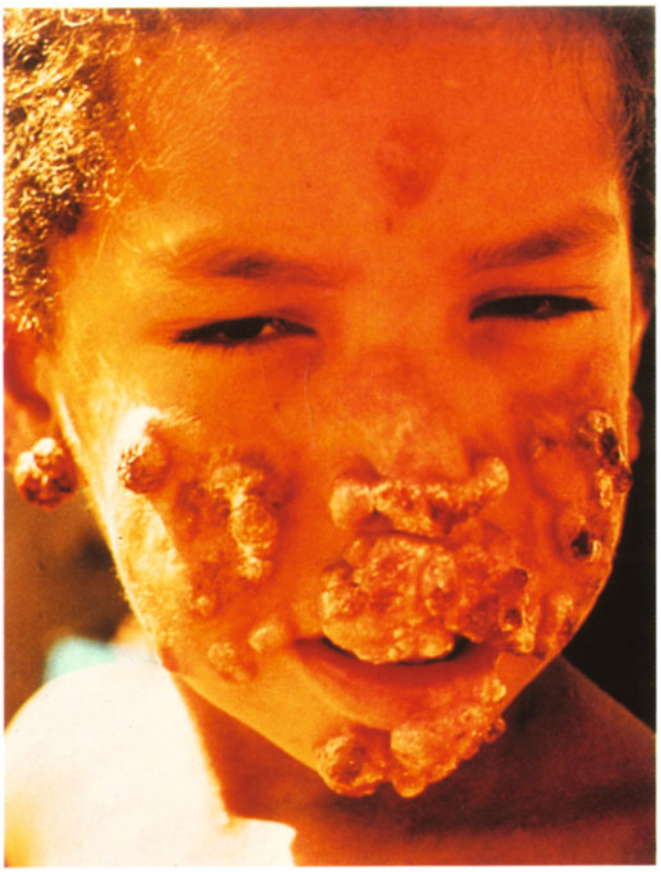

Fig. 4.10 Multiple nodular foci in the face, typical of 'diffuse tegumentary leishmaniasis', also called 'leproid'

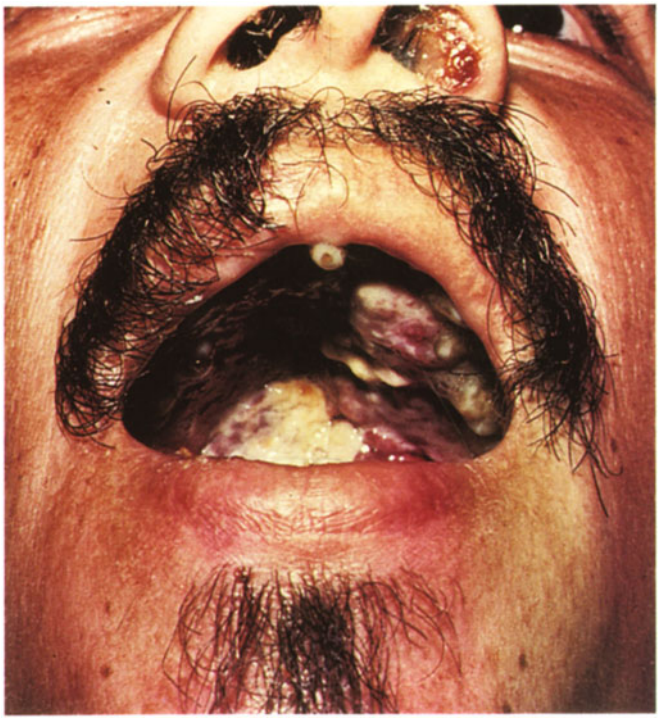

Fig. 4.11 Muco-cutaneous leishmaniasis. Extensive lesions in the mucosa of mouth, pharynx and nose

In our material, parasites have been confirmed in tissue sections in only 50% of all cases of mucocutaneous leishmaniasis[13,14]. Even scarcer were the parasites in other series of routine biopsies[15].

When making a differential diagnosis, histiocytes with clear or vacuolated cytoplasm are commonly observed in skin and mucosa in lesions of leprosy, rhinoscleroma and histoplasmosis capsulati.

In addition, amastigotes of *L. braziliensis* in tissues must be differentiated from amastigotes of leishmanias of other species and *Trypanosoma cruzi*. Furthermore, *Toxoplasma gondii* and small yeast cells, e.g. *Histoplasma capsulatum* var. *vapsulatum*, species of *Candida, Torulopsis glabrata* and *Penicillium marneffei* must be distinguished, as well as other small forms of yeast cells in mycoses which normally show large yeast cells in tissues, such as *Coccidioides immitis, Blastomyces dermatitidis* and *Paracoccidioides brasiliensis*. Fungus cells may be ruled out when the elements under discussion are Gram-, PAS- and Grocott-negative. Before making a histological diagnosis of leishmaniasis, we always perform the Grocott test[16]. Haematoxylin-positive chromatin particles may also look like leishmanias in tissues (see reference 17, Chapter 23).

Tissue reaction in skin and mucosa lesions is identical. The factors which determine the type of tissue reaction are:

1. The stage of evolution and the duration of the disease,
2. The virulence of the species or strain of the infectious leishmanias,
3. The human organism's immunological status,
4. The presence or lack of a secondary infection, and
5. The action of drugs in cases where the patient receives treatment.

What is usually seen is a diffuse tissue reaction with dense cellular infiltrates composed of a variable number of lympho- or histiocytes, plasma cells and, occasionally, mast cells and/or eosinophilic granulocytes. If there are numerous histiocytes with clear cytoplasm and intracellular parasites, some scientists call the tissue reaction 'histiocytoma'. In a recently observed case with marked infiltrates of histiocytes showing a clear cytoplasm containing numerous amastigotes (Figs. 4.13 and 4.14), a unique (for us) feature was detected: a single multinuclear giant cell contained several tissue cells which harboured amastigotes. This means that there was a double phagocytosis: first, amastigotes were engulfed by histiocytes and later the histiocytes were engulfed by a giant cell (Fig. 4.15).

In addition to diffuse cellular infiltrates, one can also observe granulomatous reactions with epithelioid and giant cells. Epithelioid granulomas sometimes show central necroses, features which are indistinguishable from granulomas caused by *Mycobacterium tuberculosis*. We have not seen as many cases with typical granulomas. Instead, there have been quite numerous instances when the exclusive presence of epithelioid cells leads to the diagnosis of leishmaniasis (Figs. 4.16–4.19).

Pseudoepitheliomatous hyperplasia of the epidermis is typical of chronically ulcerated leishmanial lesions, but this feature is also found in chronic cutaneous lesions of deep mycoses and in other chronic dermatopathies.

Exudation of granulocytes and microabscesses are not common in mucocutaneous leishmaniasis. When granulocytes are found, they are mostly located near the surface of a tissue defect at the base of an ulceration. Microabscesses are the typical lesions in infection due to *Sporothrix schenckii*.

We were never able to confirm the presence of parasites in lymphangitis or regional lymphadenitis which are common in patients with leishmania lesions. This is probably because the patients were not examined at the right time or because these lesions in the lymph nodes were due to secondary bacterial infections. However, experimentally, parasites have been confirmed in regional lymph nodes of lower animals[18]. The differences that exist between the leishmaniases of the Old and New World have not been elucidated. Further studies are needed on this subject.

In the rather rare 'diffuse tegumentary leishmaniasis', tissue reaction shows numerous histiocytes with clear cytoplasm containing many parasites in the inflammatory foci; a granulomatous reaction is never observed.

The lack of parasites in tissues in numerous apparently chronic cases makes it imperative, in practice, to give at least a presumptive histological diagnosis based only on a more or less characteristic tissue reaction.

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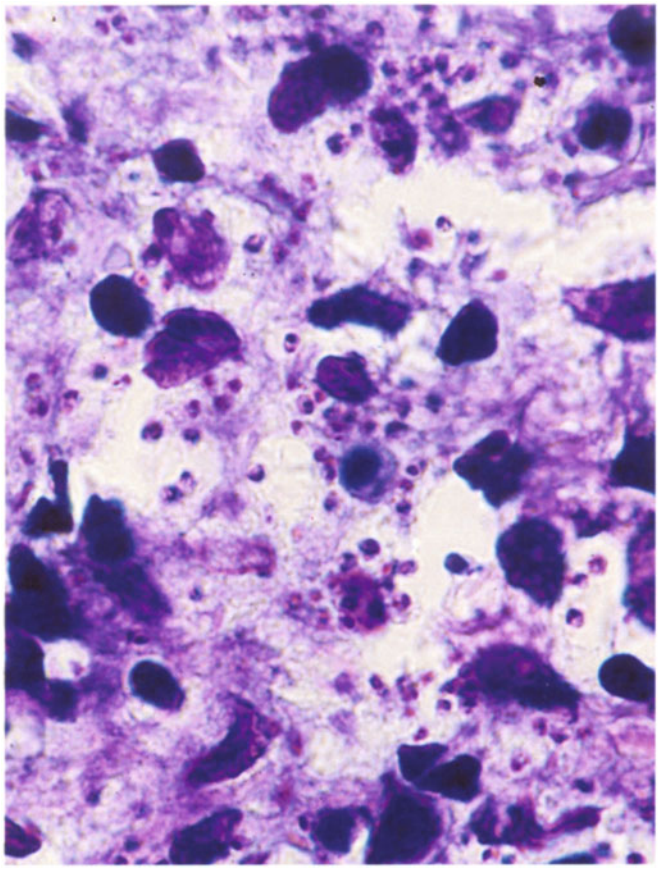

Fig. 4.12 Numerous amastigotes of *Leishmania braziliensis* arranged diffusely and in clusters. Giemsa and Wright

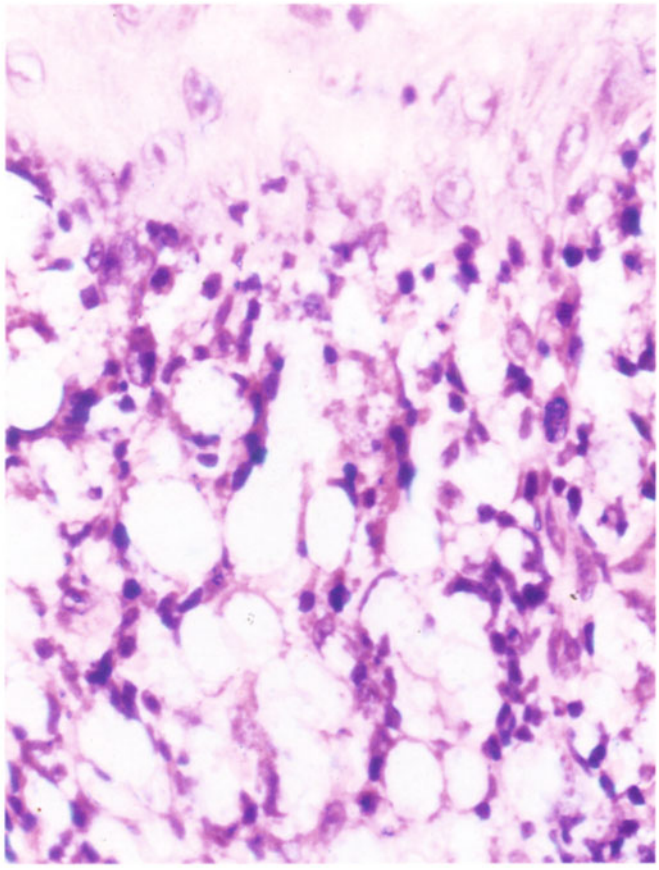

Fig. 4.13 Muco-cutaneous leishmaniasis. Numerous histiocytes with clear or vacuolic cytoplasm. The parasites have fallen out during the process of cutting and staining. H&E

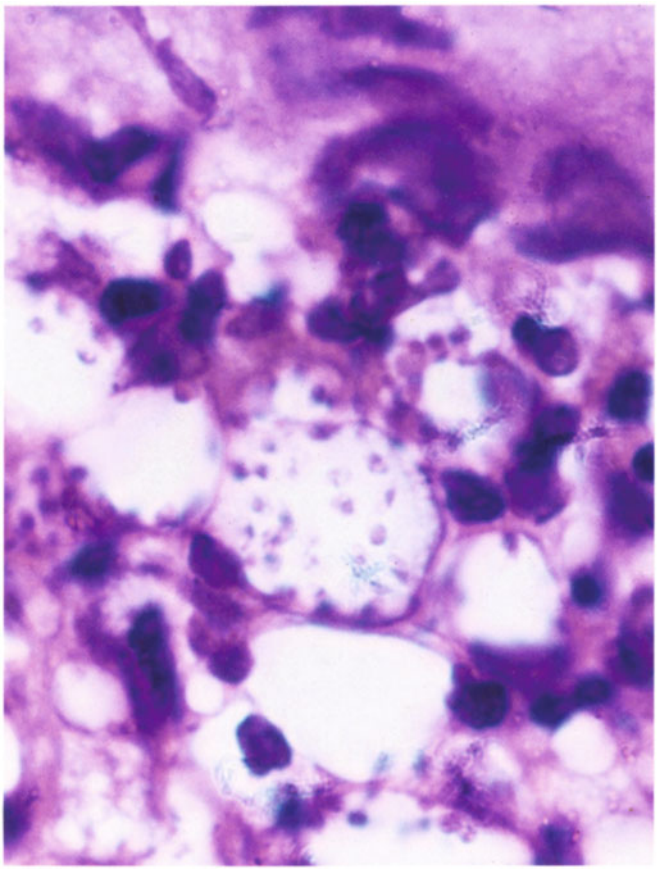

Fig. 4.14 Higher magnification of lesions in Fig. 4.13 with preserved leishmanias in a histiocyte. H&E

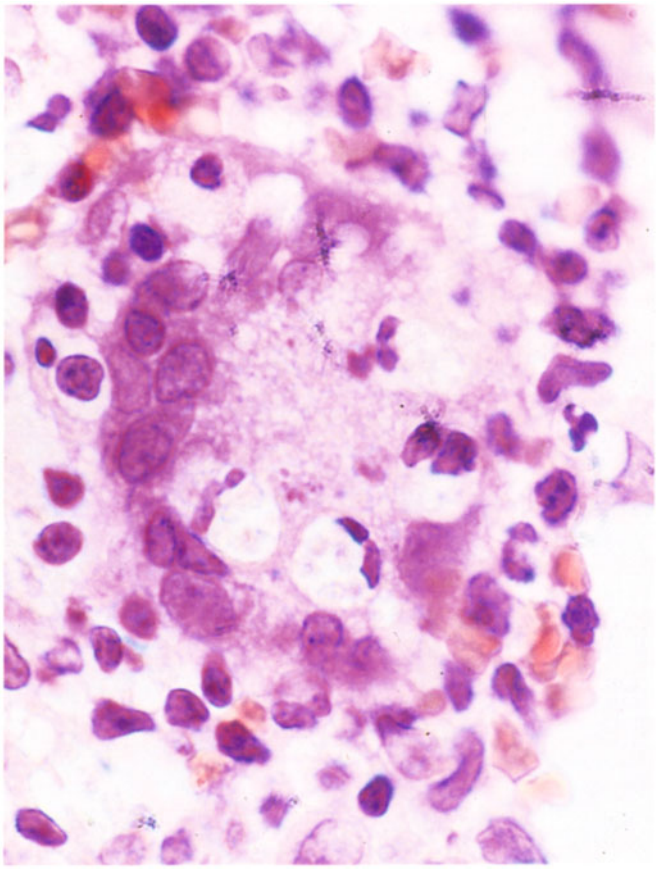

Fig. 4.15 Same case as Figs. 4.13 and 4.14. Giant cell with a 'double phagocytosis'. First, macrophages engulf leishmanias and then the giant cell phagocytoses the leishmania-containing macrophages. H&E

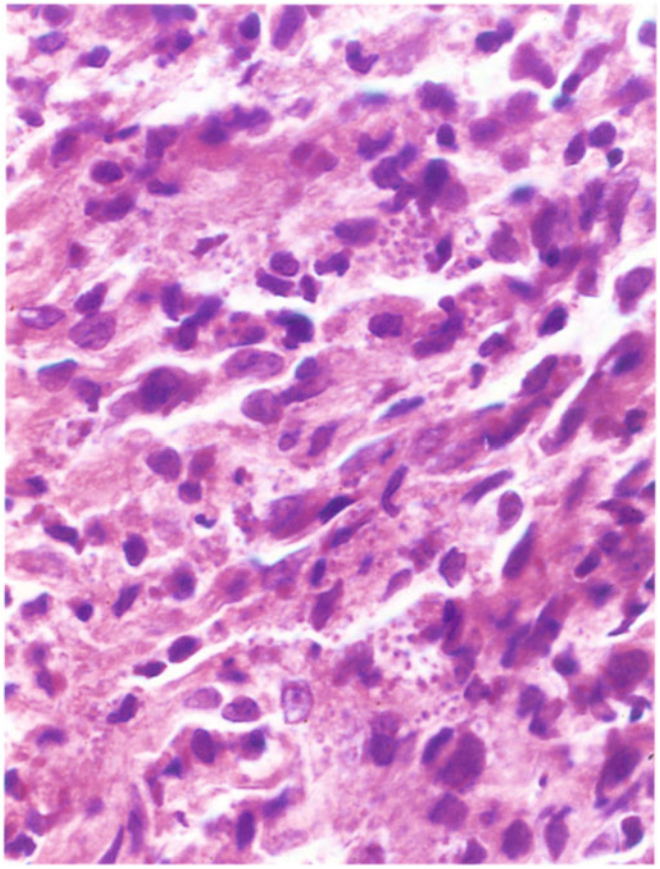

Fig. 4.16 Muco-cutaneous leishmaniasis. Typical epithelioid cell reaction. Furthermore, small nests of parasites (intracellularly located) are seen. H&E

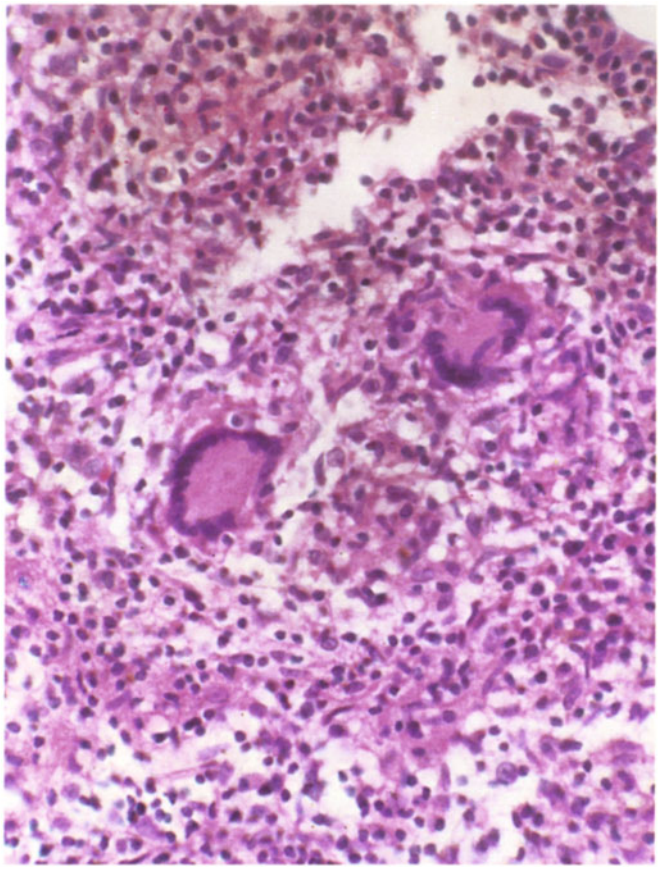

Fig. 4.17 Muco-cutaneous leishmaniasis. Dense cell infiltrates and granulomatous reaction with giant cells. H&E

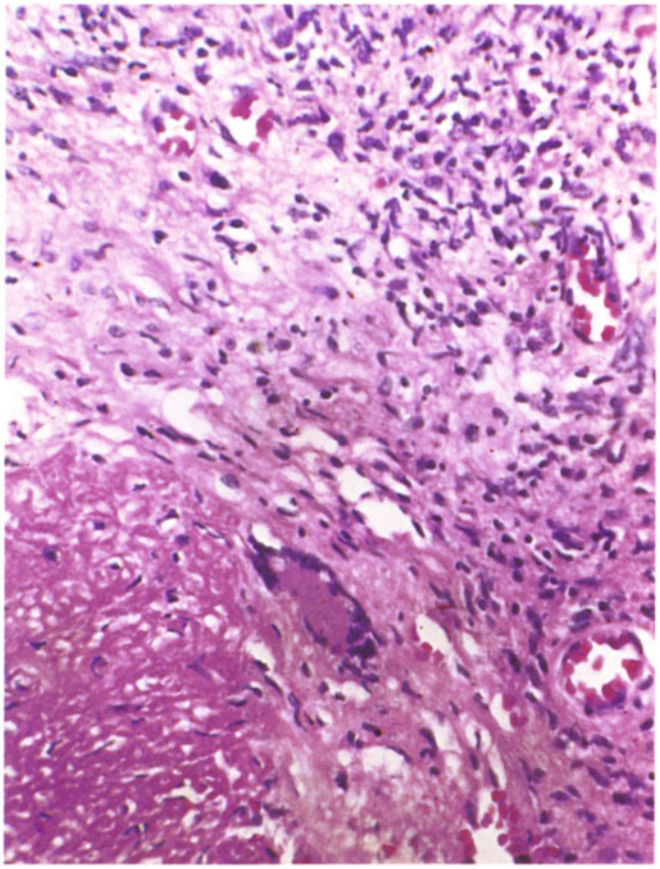

Fig. 4.18 Muco-cutaneous leishmaniasis. Granulomatous reaction with giant cell and necrosis. H&E

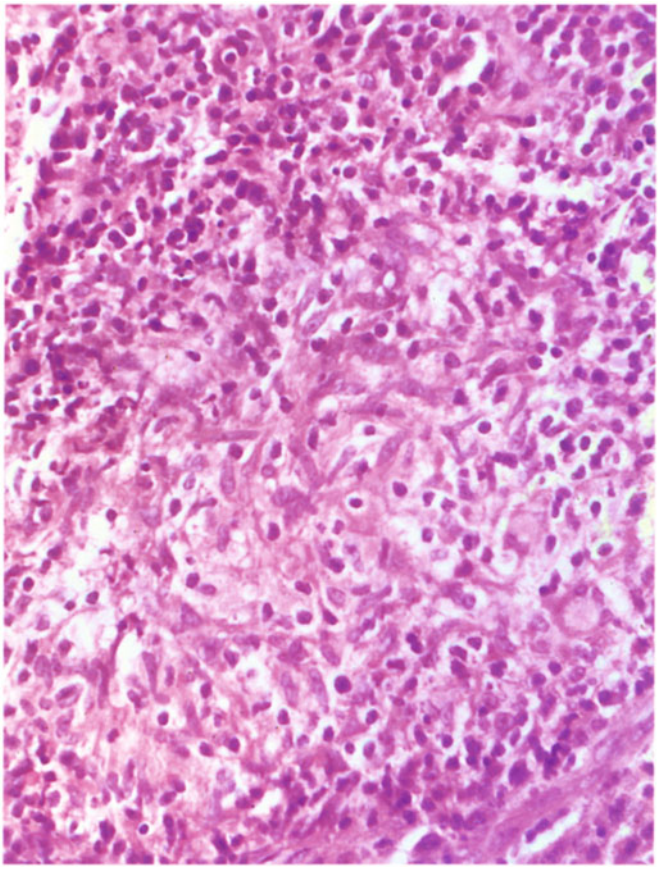

Fig. 4.19 Muco-cutaneous leishmaniasis. Dense cellular infiltrate and numerous 'clear' epithelioid cells. This is a characteristic histological feature of chronic leishmaniasis. Parasites are mostly not visible. H&E

5. VISCERAL LEISHMANIASIS

Introduction

This disease is called also kala-azar, which means black fever, or tropical splenomegaly, as well as 'infantile anaemia with tumour of the spleen'. The prevalence of this infection is low. The vector has been controlled by anti-anopheles campaigns, but has not been totally eradicated.

The infection is endemic in the countries surrounding the Mediterranean Sea, the Middle East, Asia, Africa and South America; only a few cases are reported in Central America and Mexico and human cases of Kala-azar are known in Venezuela[5,6].Mostly, children are affected[1–4].

Dogs, canine species and wild animals (foxes and several rodent species) can be naturally infected[7]. Experimentally, mice, dogs, monkeys, cats, hamsters and other animals may be inoculated[8–10].

Anaemia, increased IgG and marked splenomegaly are the clinical signs. Almost 90% of untreated patients die 1–2 years after the beginning of the disease. With current therapy, almost 90% can be cured. Visceral leishmaniasis has also been found in AIDS patients[11].

Clinical diagnosis is made by confirmation of the causative agent, for instance, in biopsies of the bone marrow, culture and inoculation into laboratory animals.

The parasite

Leishmania donovani is the causal agent of the disease in man and lower animals. The amastigotes of *L. donovani* have the same structure as *L. tropica* and *L. braziliensis* and are called Leishman and Donovan (L-D) bodies*. *L. donovani chagasi* is a new clinical variant of cutaneous leishmaniasis in Honduras[12].

Amastigotes of *L. donovani*, as well as the other species of *Leishmania* stain well in mammalian tissues with H&E and Giemsa and are negative when the methods of Gram, PAS and Grocott are used. They are situated in intracellular clusters inside the histiocytes and phagocytes of the PMS†. They have either a spherical or an oval shape, and measure 1.5–3 μm in diameter (Fig. 5.1). They are slightly smaller than the amastigotes of *Trypanosoma cruzi*, but otherwise cannot be distinguished from the latter. In smears, they appear to be larger than in tissue sections. The nucleus and kinetoplast may be seen in well-preserved material. When kinetoplasts are not detected in the tissue sections, it is due to the age, degeneration or necrobiosis of the amastigotes, or it may be that the tissue was not preserved in time or in the correct manner.

The flagellate forms of *L. donovani*, the promastigotes, are found in cultures at temperatures of 20–30°C. Characteristically, as in other species of leishmanias, flagella are not encountered in the blood of mammals.

Reservoir hosts of *L. donovani* vary according to geographic region: man almost exclusively in India; dogs and other canine species in Mediterranean countries; man and certain wild animals, but not dogs, in Africa. Wild and domestic animals are reservoir hosts in South America. For information about asymptomatic carriers of the disease, see under Pathogenesis below.

Vectors are species of the genus *Phlebotomus* (sand flies) and, in the New World, mosquitos of the genus *Lutzomyia*.

*The so-called Donovani bodies, however, are causal agents of inguinal granuloma, a quite different nosologic entity.

†PMS = phagocytic–mononuclear (cell) system and has replaced RES (reticulo-endothelial system from Aschoff) and RHS (reticulo-histiocytic system).

Pathogenesis

The bite of an infected female sand fly almost always produces the so-called 'leishmanioma', a circumscribed skin nodule which soon disappears. In some cases, this nodule does not appear or is not noted by the patient. General malaise, fever and weakness, signs of generalized disease, appear 2–6 months later.

In man, as well as in lower animals, asymptomatic carriers of kala-azar are known to have parasites in the dermis without visible alterations of the skin. These asymptomatic carriers play an important role in the transmission of the infection.

Pathology

The main organs involved are the spleen, the liver (Fig. 5.2), bone marrow, lymph nodes (Figs. 5.3–5.6) and the lymphatic tissue of the digestive tract (Figs. 5.7 and 5.8). In addition to spleno- and hepatomegaly (the latter less pronounced), there are no characteristic gross features of this infection.

Cutaneous lesions, as manifestations of a late form of kala-azar, have been described in India. This so-called 'cutaneous leishmaniasis post-kala-azar' is sometimes observed 1–2 years after a patient was thought to have been cured. The clinical late form shows spots which slowly enlarge and form nodules of a lepromatous aspect; usually, they do not ulcerate. The 'leishmaniomas' of the skin in the primary infection soon disappear, as noted above.

Clinically a differential diagnosis must be made, especially in infants, with generalized histoplasmosis capsulati. However, the latter is fatal in a very short time, whereas the progression of kala-azar is slower. In any splenomegaly occurring in endemic areas, kala-azar must be considered.

Histologically, parasites (amastigotes of *L. donovani*) are plentiful in the organs of the PMS. They are located in clusters inside histiocytes. Occasionally, they are also located in liver cells; here, they must be looked for with an electron microscope[13]. In the other organs, they are less numerous. It is a 'must' to rule out small yeast cells by application of special fungus stains. With HE, the parasites stain well. With the indirect immunoperoxidase method, they may be marked specifically[14].

Amastigotes of *L. donovani* are scarce in histiocytes in cases of chronic kala-azar (Figs. 5.9–5.11) and in the cutaneous nodules of the late form of the disease. It is most important to make a differential diagnosis with histoplasmosis capsulati in view of the characteristic location of the small yeast cells and the amastigotes inside histiocytes.

Tissue reaction in the visceral leishmaniasis is not characteristic. When numerous parasites are present in tissues, there is practically no tissue response. When parasites are scarce, marked infiltrates of lymphocytes and, above all, plasma cells, are seen with numerous typical Russell bodies (Figs. 5.12 and 5.13).

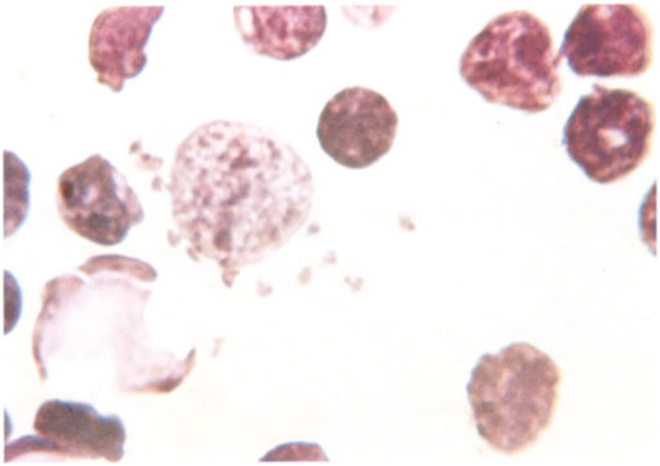

Fig. 5.1 Kala-azar. Numerous leishmanias (*Leishmania donovani*) are seen near a large cell nucleus in the smear of a human bone marrow. Kinetoplasts cannot be recognized. H&E

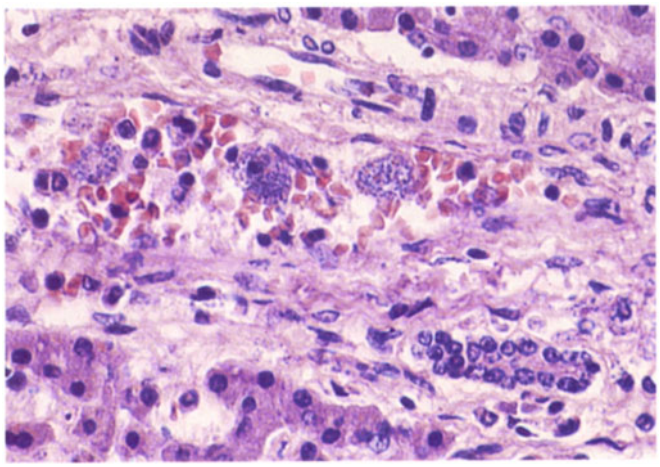

Fig. 5.2 Kala-azar. Nests of leishmanias (*Leishmania donovani*) are seen clearly in macrophages of a portal field in the liver in a case of an acute infection. H&E

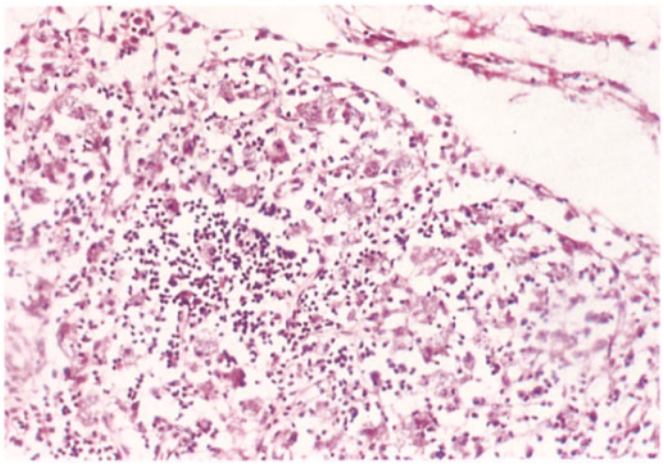

Fig. 5.3 Numerous intracellular nests of leishmanias (*Leishmania donovani*) in mesenteric lymph node of a human case at low power. H&E

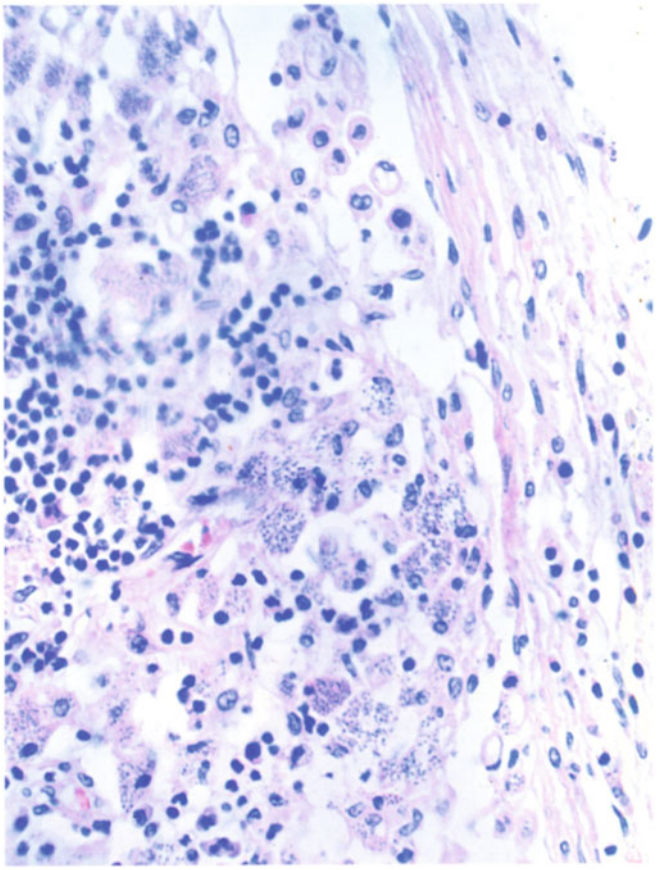

Fig. 5.4 Same case as Fig. 5.3 at higher magnification. H&E

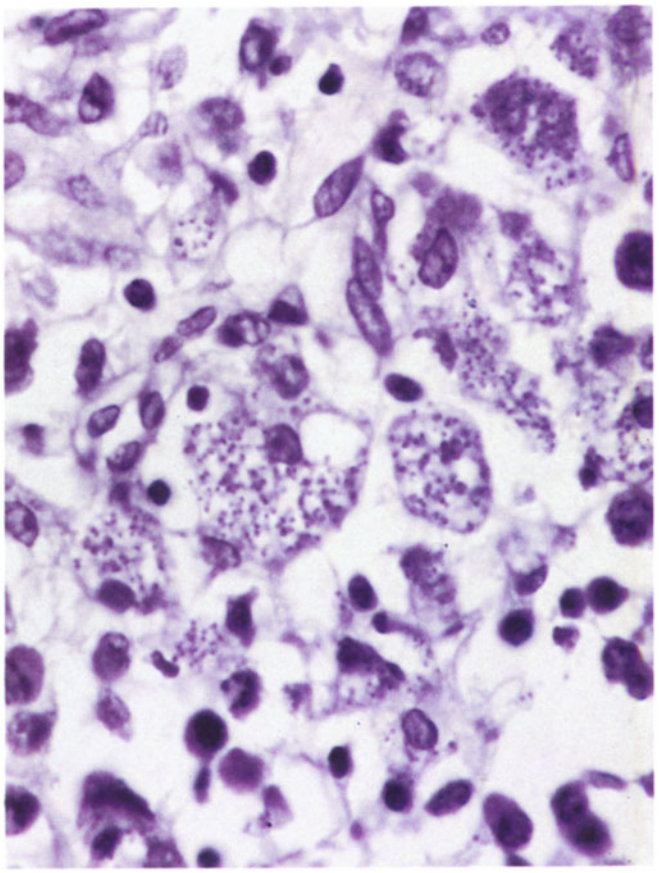

Fig. 5.5 Same case as Figs. 5.3 and 5.4 at higher magnification. H&E

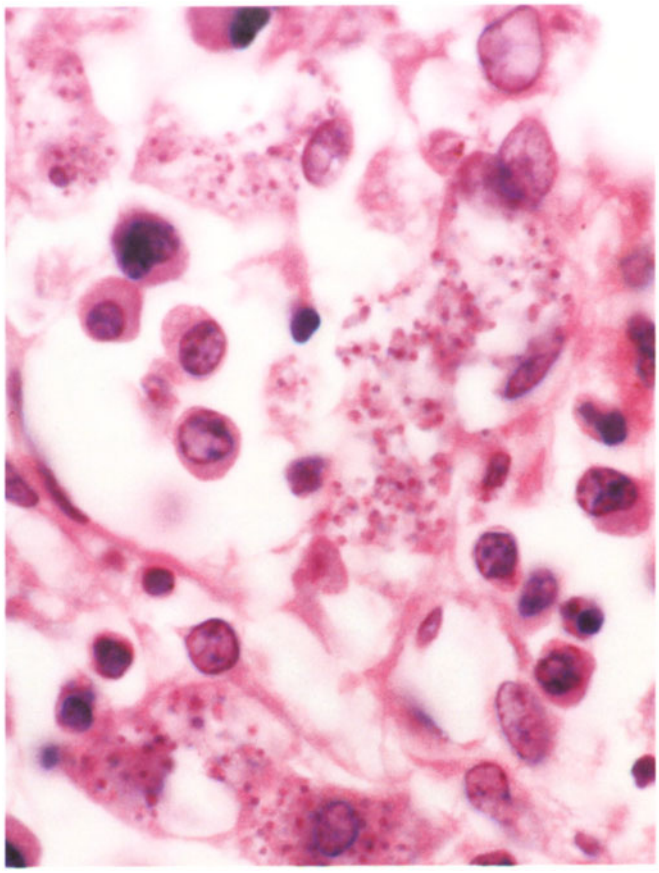

Fig. 5.6 Same case as Figs. 5.3–5.5 at a still higher magnification. Kinetoplasts, however, cannot be detected. H&E

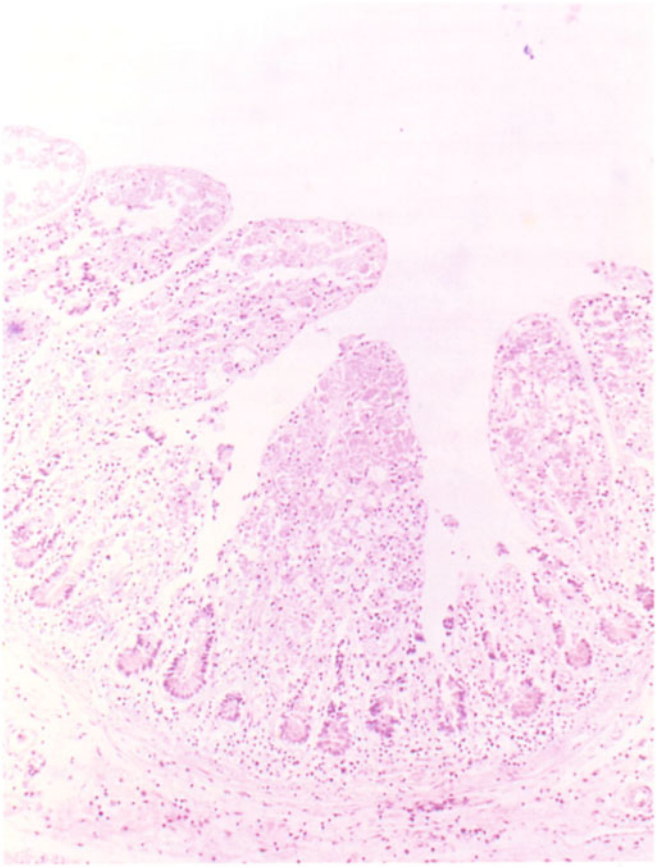

Fig. 5.7 Kala-azar. Human small intestine with thickened villi of the mucosa due to numerous leishmania-containing macrophages. H&E

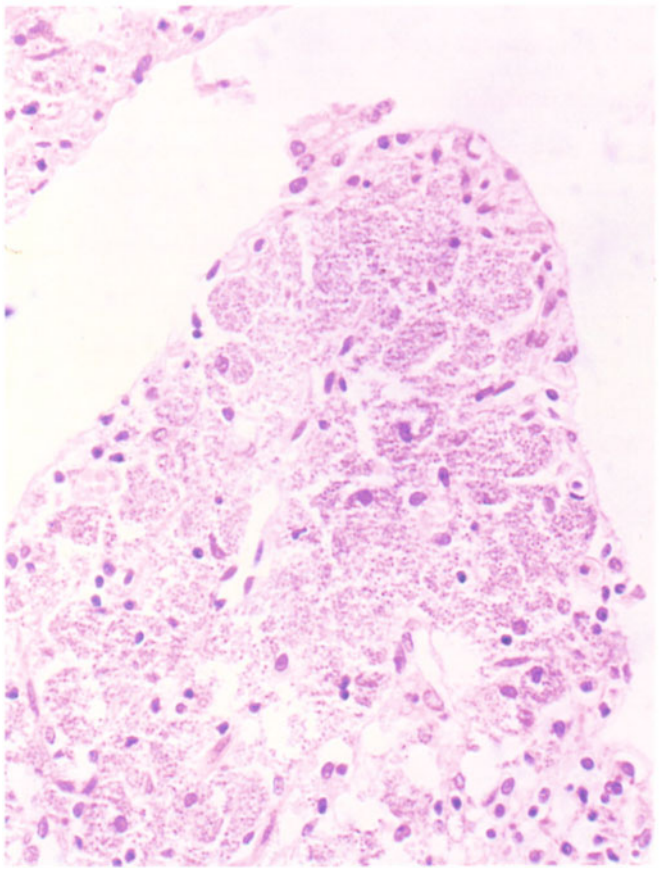

Fig. 5.8 Villus of the mucosa from Fig. 5.7 at higher power. Numerous intracellular organisms of *Leishmania donovani* may be seen. H&E

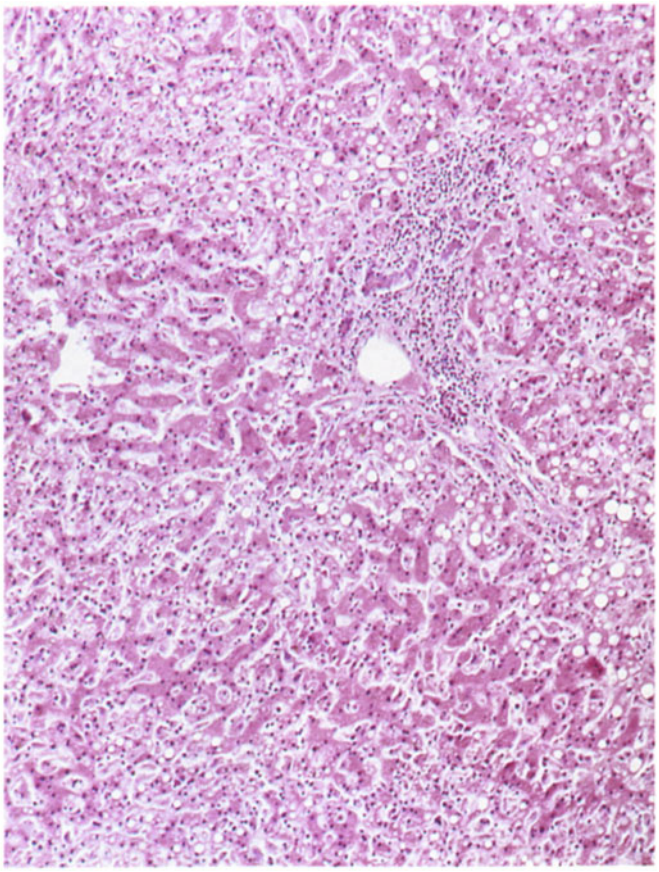

Fig. 5.9 Liver tissue in case of chronic kala-azar at low power. H&E

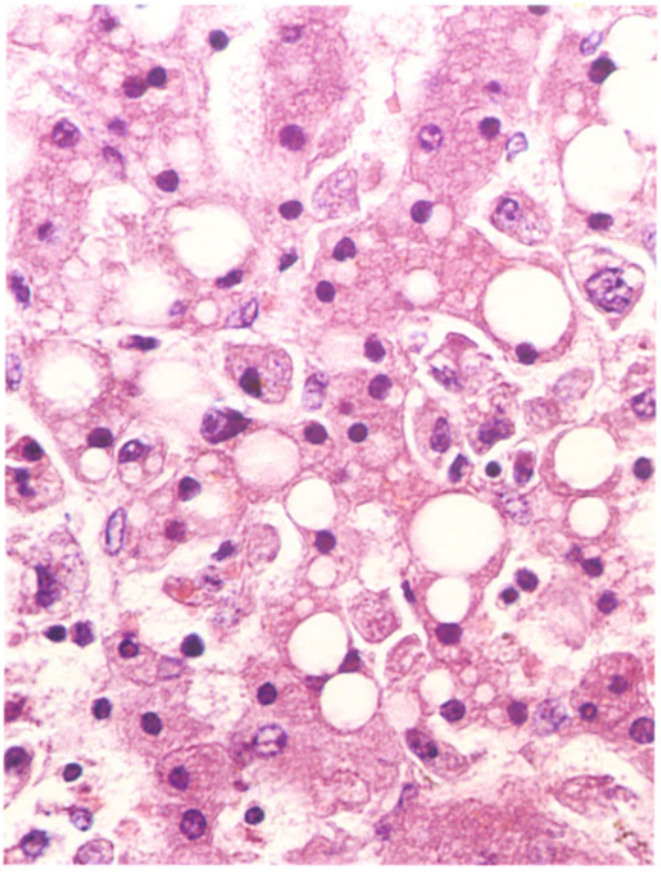

Fig. 5.10 Same case as Fig. 5.9 at higher magnification. Nests of leishmanias (*Leishmania donovani*) may be seen in Kupffer cells. H&E

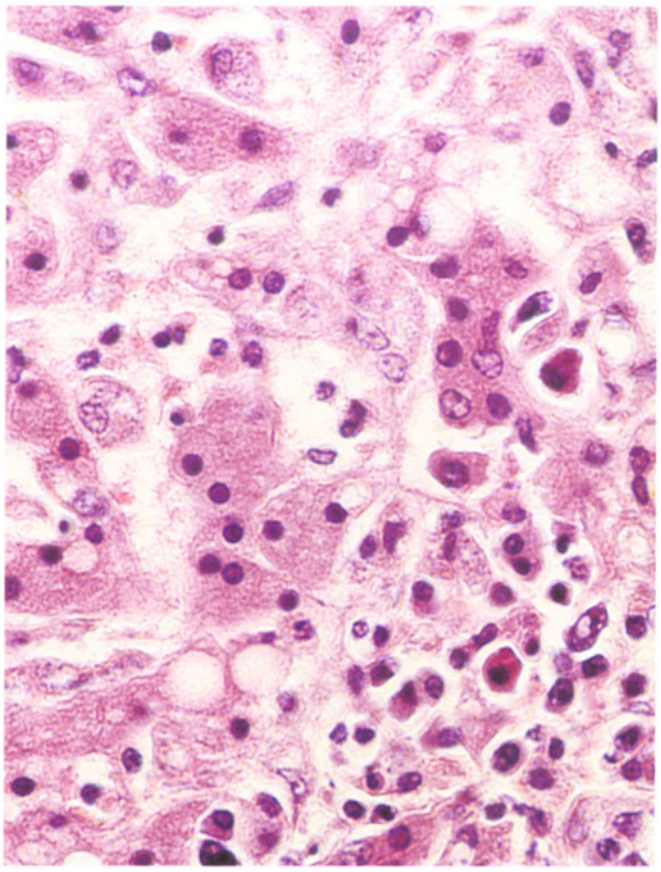

Fig. 5.11 Same case as Figs. 5.10 and 5.11 at a still higher magnification. Parasites and cell infiltrate may be recognized. H&E

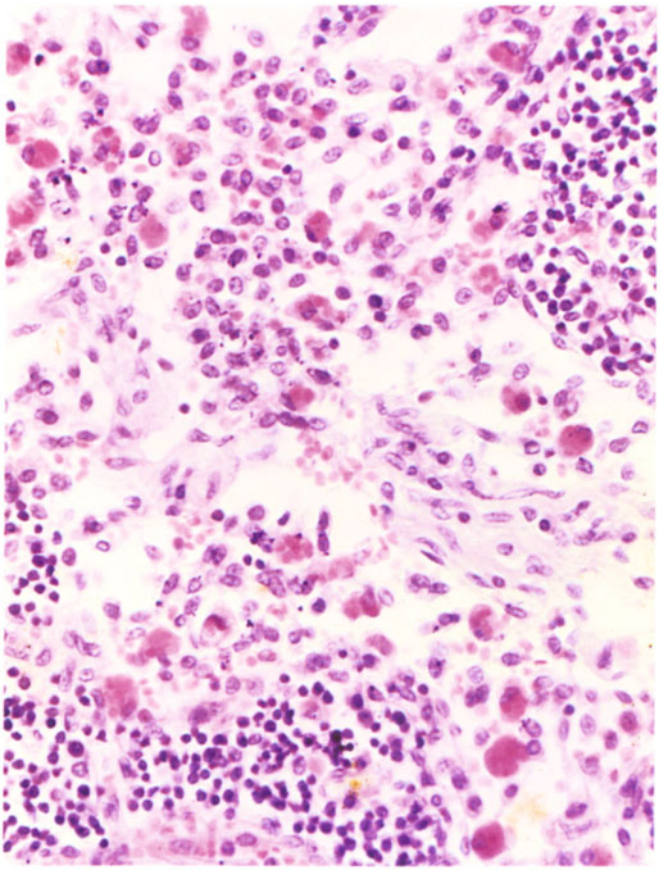

Fig. 5.12 Kala-azar. Human lymph node with numerous Russell bodies. H&E

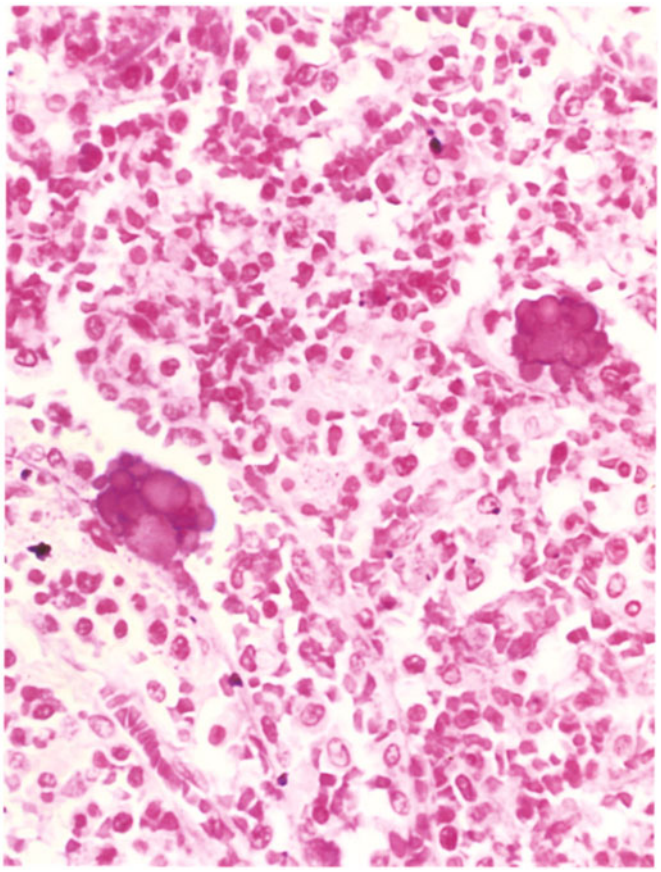

Fig. 5.13 Kala-azar. Human spleen with two large cells (plasma cells) containing Russell bodies. Parasites may not be discerned. Fibrine.

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6. GIARDIASIS

Introduction

A synonym for this parasitosis is lambliasis. The infection occurs worldwide; 2–10% of the population, mostly children, are infected in countries with a cold or temperate climate. In countries with a warm climate, the percentage goes up to 20–50%[1]. The prevalence among subjects at risk in certain countries is not negligible[2]. In Venezuela this intestinal infection is well known.

Dogs, cats and rodents are carriers of the parasite and may be used for experimental purposes[3,4].

Clinically, diarrhoea with yellowish fetid faeces are observed, occasionally accompanied by abdominal pain and cramps; also steatorrhoea[5] and malabsorption syndrome have been found. Prognosis is always favourable; there are no reports of a fatal outcome. Infection is symptomatic in children but usually asymptomatic in adults[6].

Clinical diagnosis is made by confirmation of mobile trophozoites in the duodenal contents[7]. Cystic forms of giardias are found in faeces. Giardiasis often occurs in combination with another bacterial or viral enteritis.

The parasite

Giardia lamblia or *Lamblia intestinalis* is a flagellated protozoan with vegetative (trophozoites) and cystic forms. The trophozoites are pear-shaped, measure 10–12 μm in length, 5–10 μm in width and are 3–5 μm thick. They possess 2 nuclei and 4 basal corpuscles near the round anterior pole. These 4 corpuscles, found between the two nuclei, are the origin of the 8 filaments which appear as 4 pairs of flagella outside the parasite. On the ventral surface, there is a so-called 'suction disc', visible only with an electron microscope, which corresponds to a circumscribed shallow part used by the parasite to attach itself to the intestinal mucosa.

The cysts of *Giardia lamblia* are oval and have 4 nuclei, situated at one pole. They measure 8–14 μm in length and 6–10 μm in width. In the interior of the cyst, one can see longitudinal filaments which reach beyond the membrane as short flagella. There are between two and four basal corpuscles shaped like a sickle or a banana. The cysts survive in a humid environment for weeks or months[8].

The giardias stain well with the Giemsa, H&E, and iron haematoxylin. They are Grocott-positive (Figs. 6.1–6.5). To see the structural details, fresh live giardias must be used with special methods of fixation and phase contrast.

Pathogenesis

The cystic forms of *G. lamblia* in the faeces of man or animal carriers contaminate water and foods, i.e. transmission occurs from man to man or animal to man directly and orally. Some insects and other small animals may also act as vehicles (seldom).

In the small bowel, cysts become trophozoites which attach themselves by means of the rims of the 'suction discs' to the superficial cells of the microvilli of the mucosa. Here they multiply copiously by fission but do not penetrate into the tissues.

It seems that it is the massive reproduction of giardias, with the resulting microlesions and the production of large amounts of mucoid substances, that leads to the enteritis. Other theories have also been put forward as pathogenic factors[9], e.g. the mechanical prevention of interchange of alimentary substances.

Pathology

The trophozoites of *Giardia lamblia* are found in the duodenum, sometimes in the jejunum and, occasionally, in the upper parts of the ileum. Location at other sites has not been confirmed. The cysts are located in the lower parts of the small intestine, in the large bowel and in formed faeces. Gross intestinal lesions have not been reported.

Histologically, the material obtained routinely in autopsies cannot be used for studying giardias or the lesions caused by these parasites. Biopsies must be performed and the material then examined under an electron microscope[10–12].

The attachment of the giardias to the surface of the mucosa apparently acts as an irritant with the subsequent inflammation. In acute cases, infiltrates of neutrophilic and eosinophilic granulocytes are found in the stroma of the upper parts of the mucosa and, in chronic cases, lymphohistiocytic ones are seen[13].

Ulceration of the mucosa, which is occasionally observed, is not thought to be due to giardias but to other infectious agents, such as bacteria or viruses.

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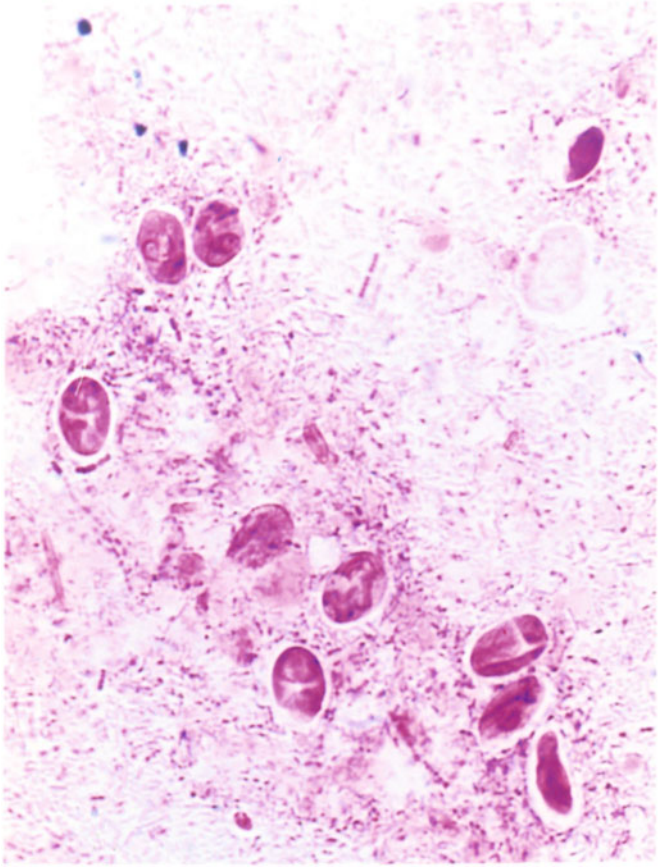

Fig. 6.1 Giardias in faecal smear of an autopsy. H&E

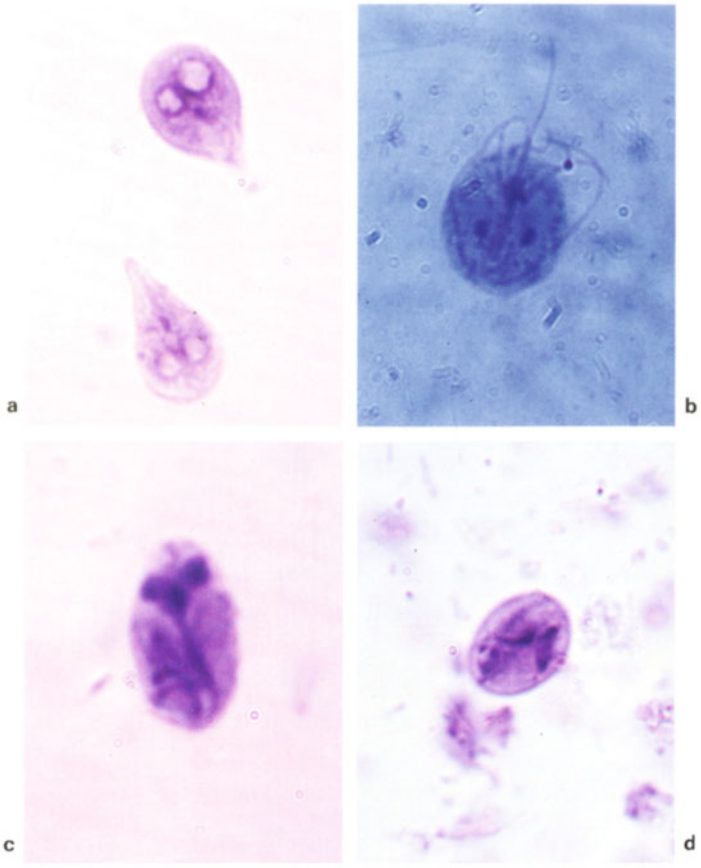

Fig. 6.2 Trophozoites (**a** and **b**) and cysts (**c** and **d**) of *Giardia lamblia*. Giemsa

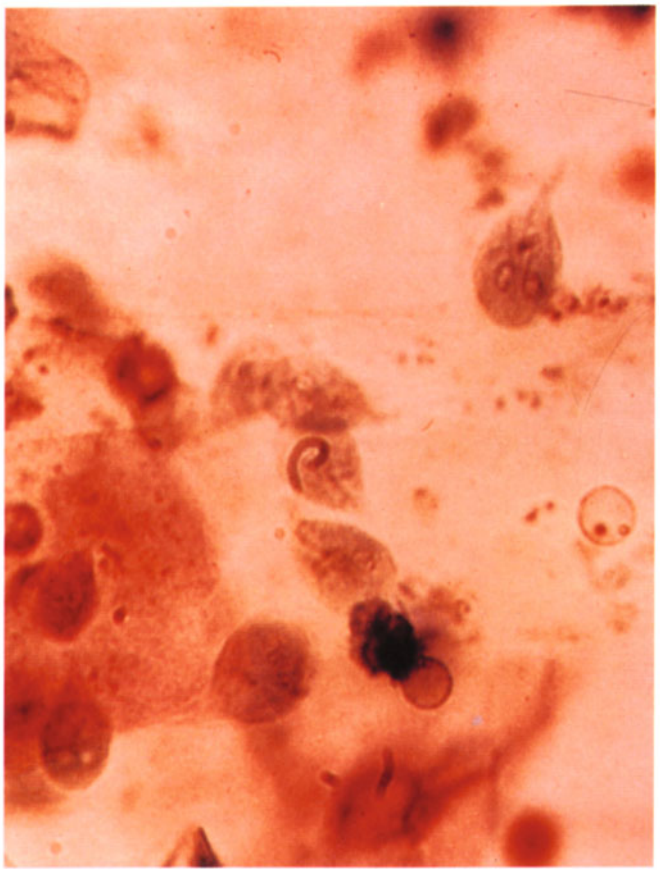

Fig. 6.3 Cluster of *Giardia lamblia* trophozoites in faeces. Trichrom

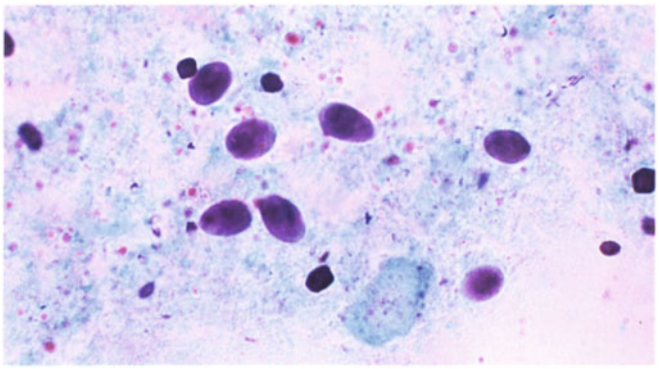

Fig. 6.4 Giardias in a smear of intestinal content. Autopsy material. Grocott

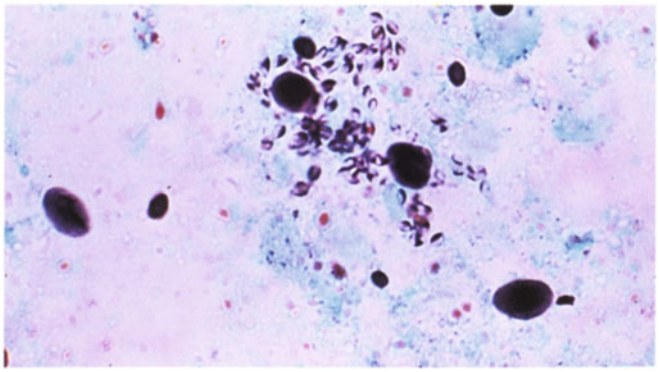

Fig. 6.5 Giardias and yeast cells (of *Candida* sp. ?) in smear of intestinal content. Autopsy material. Grocott

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7. TRICHOMONIASIS

Introduction

This disease (for which there is no synonym) is endemic all over the world. The infection is observed predominantly in promiscuous females but males may be infected[1]. Trichomoniasis is common in Venezuela.

Trichomonas vaginalis is not found in lower animals, although other species of this genus have been detected in squirrel monkeys[2], and systemic trichomoniasis has been found recently in squabs (young pigeons).

The infection is almost always asymptomatic or latent. Once symptoms appear, the infection remains active for a long time, causing complaints like vaginal flux and marked prurigo. Even with efficient therapy, patients are not always cured by a single treatment as relapses and reinfection are frequent. Clinical exacerbation, common during menstruation and pregnancy, has been ascribed to changes in pH of the vaginal milieu.

Clinical diagnosis may be confirmed by examination of vaginal, cervical or prostate secretions and/or urine; the typical mobile flagellates can be found in fresh specimens. In asymptomatic females, trichomoniasis is often found when cytological examinations (Papanicolaou) are made in order to rule out a carcinoma.

Trichomoniasis is quite often associated with previous bacterial, viral or mycotic infections of the urogenital tract and with carcinoma of cervix. It has been confirmed, however, that this parasitosis is not a carcinogenic risk.

The parasite

Trichomonas vaginalis is the only pathogenic species in this genus. Other flagellates found in the digestive tract and in the oral cavity of man are almost always apathogenic[3].

The parasite is almost always pear-shaped and measured 7–30 μm in length (on average 13 μm). The size varies with the type and strain, and the pH of the medium. At the anterior end, a blepharoplast is seen, and from this, originate 4 anterior flagella and one posterior flagellum with an undulating membrane. The nucleus is situated towards the rear end. In fresh preparations, the parasites can be seen moving with the aid of their flagella and the undulating membrane. They stain well with H&E, iron haematoxylin, Giemsa or the Papanicolaou method (Figs. 7.1 and 7.2). In cultures (Fig. 7.3), the parasites may phagocytose leukocytes, bacteria and red blood cells. Leukocytes and epithelial cells are able to phagocytose parasites[4].

Pathogenesis

An infected male transmits the infection from one female to another through sexual relations. The reservoir host of *Trichomonas vaginalis* is the infected female.

There may also be indirect transmission through sanitary installations or infected underwear. Infection of newborn babies is due to contamination during parturition.

Pathology

In the female, the parasite lives in the vagina, in the cervical channel, in the urethra and, occasionally, in the lower part of the bladder[5]. In males, they have been found in the urethra and the prostate; some observers have also noted them in the seminal vesicles[6]. Invasion of human tissues by *Trichomonas vaginalis* has not been reported. Gross lesions are not known.

The histological alterations which may be produced by these parasites can be summarized in the following manner:

1. The epithelial cells may degenerate and show cytoplasmic vacuolization (Fig. 7.4),
2. Regeneration and endocervical metaplasia of the epithelial cells may occur (Fig. 7.6),
3. Inflammation with lympholeukocytic and plasma cell infiltrates may be seen in the epithelium and in the stroma,
4. Erosions of the surface and mucopurulent exudation may occur (Fig. 7.5).

It is not entirely clear whether these alterations are due to the irritation produced by these parasites or due to a secondary bacterial infection. It has been established, however, that the parasites do not cause endometritis, puerperal infections, sterility, miscarriages or carcinoma.

The parasites may produce some alterations in tissue cells which could be confused with those seen in neoplastic processes. Therefore, if there is infection with this parasite, it must be taken into consideration when cytology and biopsy specimens are examined in order to avoid a false positive diagnosis of malignancy.

Cytologic differential diagnosis of the parasite must consider: artificial particles, as a result of contaminated slides, epithelial cells or their nuclei, and deformed granulocytes, histiocytes or degenerated parabasal cells.

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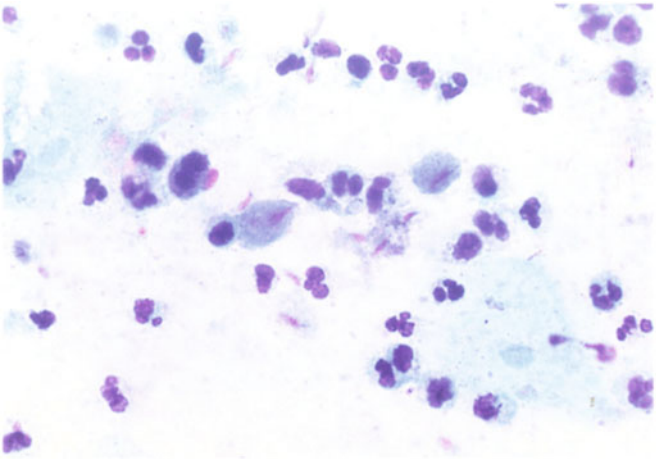

Fig. 7.1 Smear from the uterine cervix. Organisms of *Trichomonas vaginalis* and leukocytes. Papanicolaou

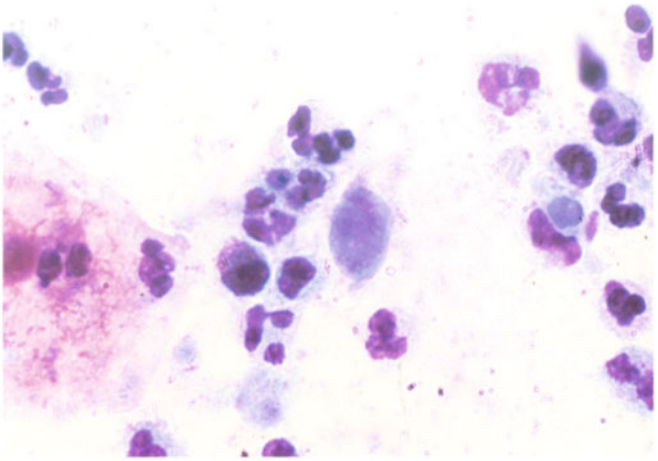

Fig. 7.2 Higher magnification of a smear from uterine cervix. Papanicolaou

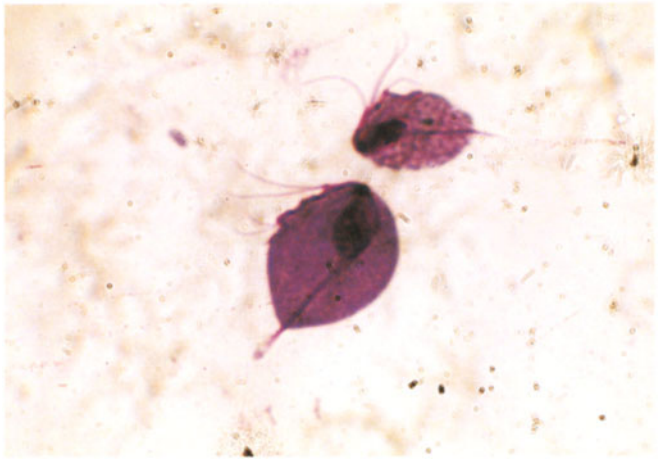

Fig. 7.3 Smear of cultured parasites with visible flagella. Giemsa

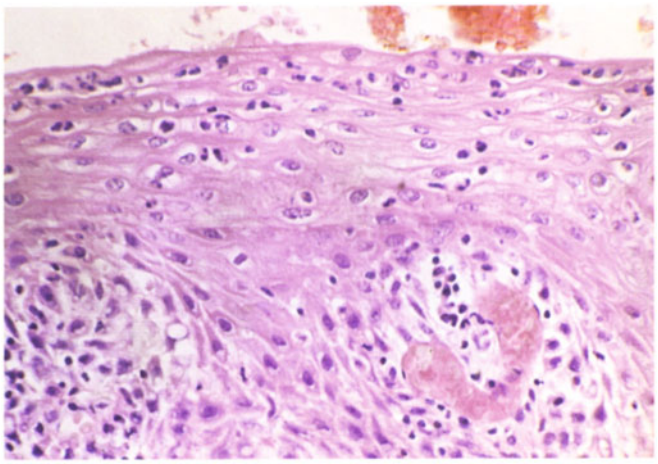

Fig. 7.4 Trichomoniasis. Vacuolic degeneration of epithelial cells and inflammation at epithelium and stroma. H&E

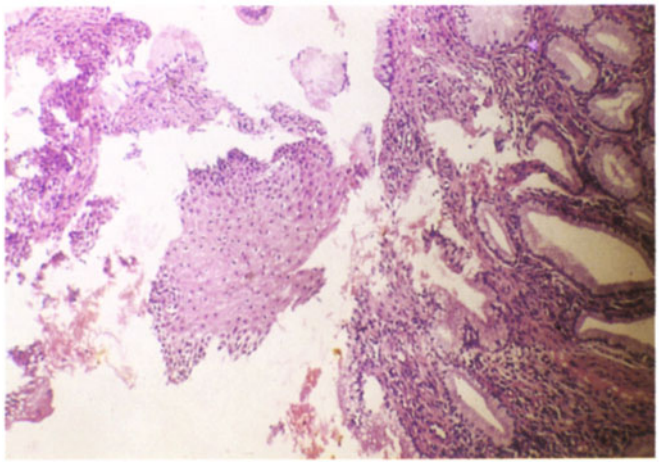

Fig. 7.5 Trichomoniasis. Islet of epithelial metaplasia (detached) at the endocervix. H&E

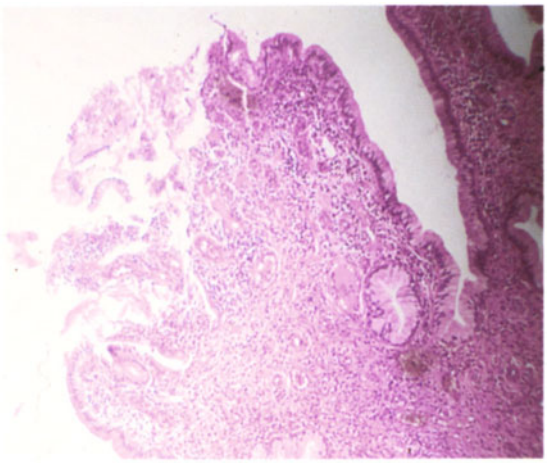

Fig. 7.6 Trichomoniasis. Erosion and marked inflammation at the endocervix. H&E

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8. AMOEBIASIS

Introduction

This disease is also called amoebic dysentery. It is still an important infection, with involvement mainly of the digestive tract. However, it may be treated successfully with modern drugs when an early diagnosis is made. When extraintestinal organs are involved, aetiological diagnosis is not easily made.

The prevalence of amoebiasis is approximately 10% of the world population and it occurs in practically all countries and all social groups[1]. The disease is frequently more severe in tropical and subtropical regions. Amoebiasis is endemic in Venezuela; in the fifties and sixties numerous fatal cases were still observed in Mérida/Venezuela[2,3]. Epidemic outbreaks may occur simultaneously with bacterial dysentery.

Natural *Entamoeba histolytica* infections are found in dogs, rats, pigs and monkeys but they are of no relevance to the human infection. Rats and other laboratory animals may be used as experimental models[4–7].

The dysenteric syndrome is characterized by blood and mucoid substances in the diarrhoeal stools. Relapses, after variable periods of apparent cure, are more frequent than definitive cures of an acute stage.

Clinical diagnosis is made by confirmation of the causative agent. This requires well-trained laboratory personnel since other species of amoebae must be ruled out and not all elements which are mobile in fresh preparations represent amoebae. Also, in biopsies and sections of cell blocks of a semiliquid material, amoebae are sometimes difficult to distinguish from tissue cells and other elements[8,9]. Serological diagnosis is indicated, above all, in patients with liver abscesses[10].

The parasite

Entamoeba histolytica is the only pathogenic species of the intestinal amoebae, i.e. this species may invade tissues. The other four species of intestinal amoebae (*Entamoeba coli, Endolimax nana, Iodamoeba buetschlii, Dientamoeba fragilis*) are non-invasive. Soil amoebae are mentioned in the next section, Acanthamoebiasis.

Two forms of *Entamoeba histolytica* exist, the trophozoites and the cysts. The former can be seen in diarrhoeal stools and in tissues; the latter are found in formed faeces and are able to survive outside the human organism representing an 'enduring form'.

The trophozoite measures 7–35 μm, is irregularly shaped and moves by typical amoeboid pseudopodia in fresh preparations. The nucleus is vesiculous or annular. In the cytoplasm, erythrocytes, fragments of tissue cells or engulfed leukocytes are often present (Fig. 8.1). After the division of trophozoites, cysts develop. These are amoebae with thicker membranes. The cysts are spherical and measure on average 11 μm. A young cyst has one nucleus; a mature one up to four nuclei.

We are more inclined to examine permanent and stained preparations than to look at unstained smears. For smears and tissue sections, the classical stain is iron haematoxylin (Heidenhain) which is somewhat better than haematoxylin–eosin. We prefer a modification of the PAS method[11] (Fig. 8.2). Amoebae also stain partially with the Grocott method.

Amoebae may be cultured in diverse artificial media. They may harbour HIV-1 but there is no transmission to human cells[12].

Pathogenesis

The amoebic cysts are the infectious elements of *Entamoeba histolytica*. They reach the human digestive tract through water, food or by other means, such as dirty hands or indirect vectors such as flies. The most important source of infection is man; a carrier of cysts is generally asymptomatic and apparently completely healthy. Patients with signs of amoebic dysentery are not the source of infection; they eliminate only trophozoites which die quickly.

When the cysts of *E. histolytica* reach the digestive tract, they transform into trophozoites which are able to penetrate the wall of the large bowel. They migrate through the mucosa and may reach the serosa through all the layers of the intestinal wall. The classical concept is that the vegetative forms (trophozoites) themselves produce proteolytic (cyto- and histolytic) enzymes (as indicated by the name *Entamoeba histolytica*) in order to facilitate the penetration of tissues but this has not been convincingly demonstrated. Be that as it may, the parasitic invasion leads to tissue necrosis. The action or co-action of bacteria, before and after formation of necroses, is also not clear. There is always an associated bacterial infection.

Lately, both in amoebiasis and in other pathological intestinal conditions, the necroses have been interpreted as being lesions similar to infarcts. This means that the necroses can be explained as the result of the formation of thrombi or microthrombi which produce ischaemic processes and consecutive infarcts. However, true thrombi have not been found in our series of cases of amoebic colitis.

The amoebae spread by contiguity, by lymphogenous and haematogenous dissemination from primary intestinal lesions (and from other sites). Haematogenous spread is the most frequent and important, while dissemination by contiguity or contact is less common, and lymphogenous spread is an exception. The large bowel and the liver are the preferential organs for amoebic infections. From the liver, they can also reach other viscera. Frequently, these amoebae are encountered in an organ or organ system distinct from the intestine, appearing to be an 'isolated disease' because the intestinal lesions have recovered and the patients may not even remember the diarrhoeal episodes which possibly occurred only once years ago.

Pathology

Intestinal amoebiasis. The incubation period has not been precisely defined. The superficial necrotic lesions in the mucosa soon detach and multiple large ulcerative areas remain in the mucosa, involving deeper layers of the intestinal wall (Figs. 8.3–8.6). These ulcers in the large intestine are deep crater-like defects with undermined rims and are called 'button-hole' ulcers (Figs. 8.7 and 8.8).

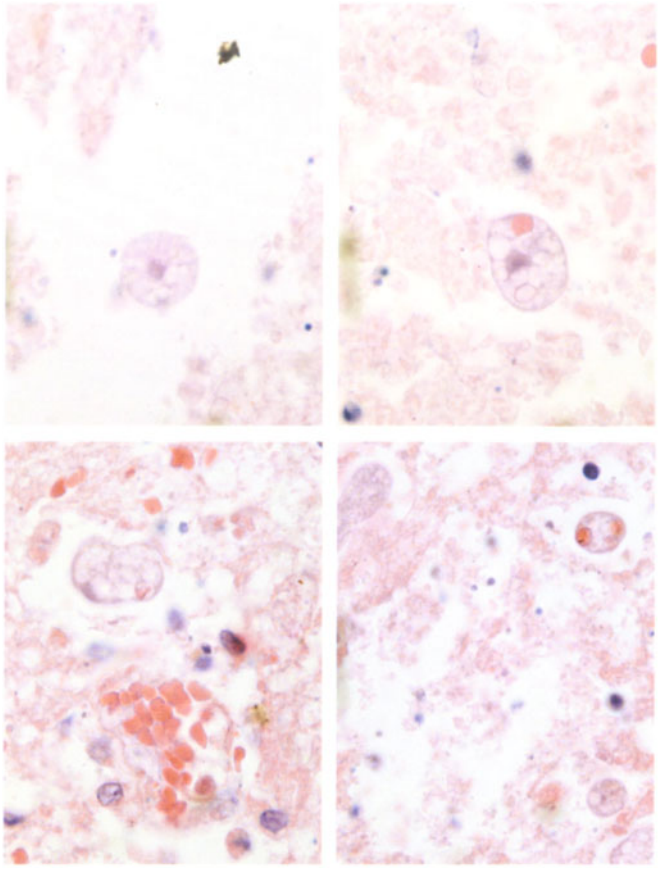

Fig. 8.1 Trophozoites of *Entamoeba histolytica* in tissues. H&E

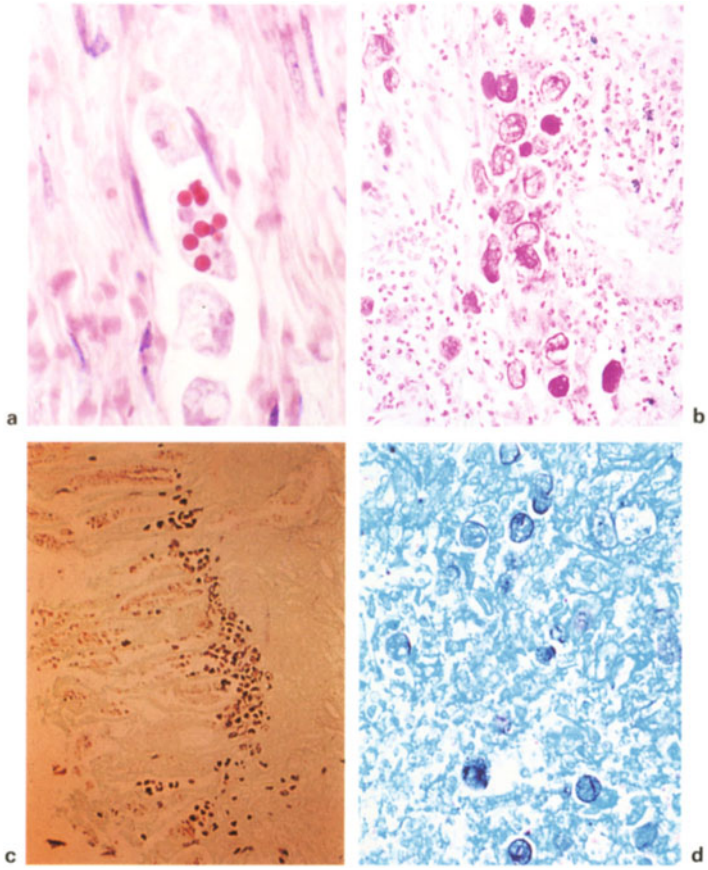

Fig. 8.2 Trophozoites of *Entamoeba histolytica* in tissues:
a. Erythrophagia. H&E
b. Special stain for amoebae. PAS
c. Marked amoebae at intestinal mucosa. Grocott
d. Amoebae. Grocott

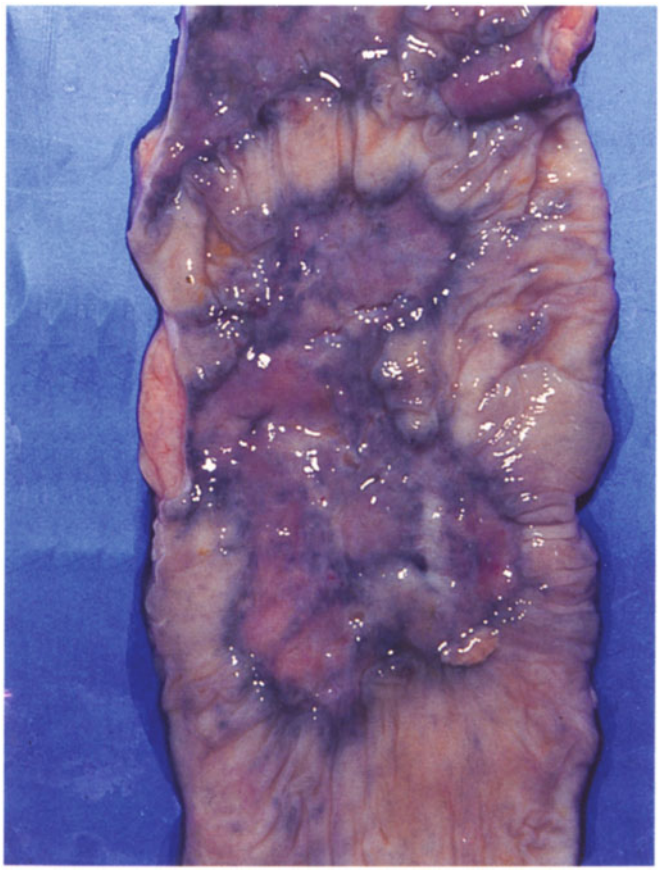

Fig. 8.3 Amoebiasis. Large intestine with deep mucosal ulcer showing thick borders

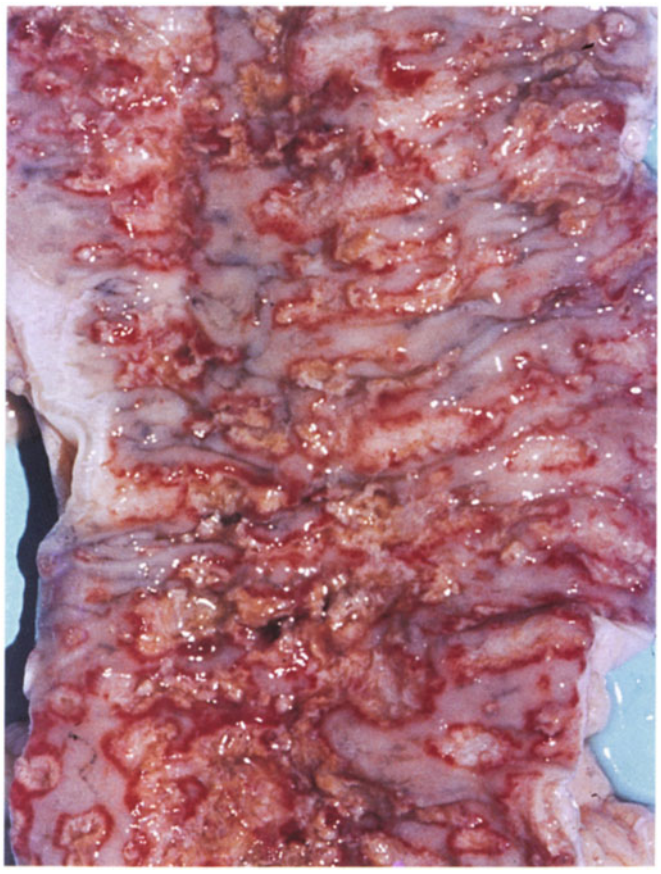

Fig. 8.4 Amoebiasis. Numerous necrotic foci and ulcers in the mucosa of the large intestine

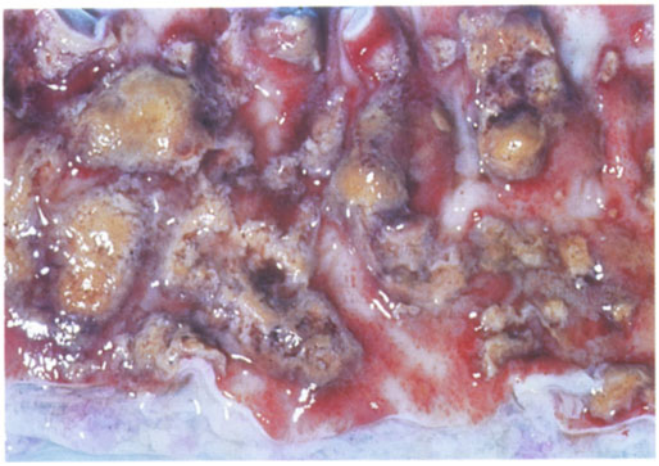

Fig. 8.5 Amoebiasis. Small and large necrotic foci in the mucosa of the large intestine

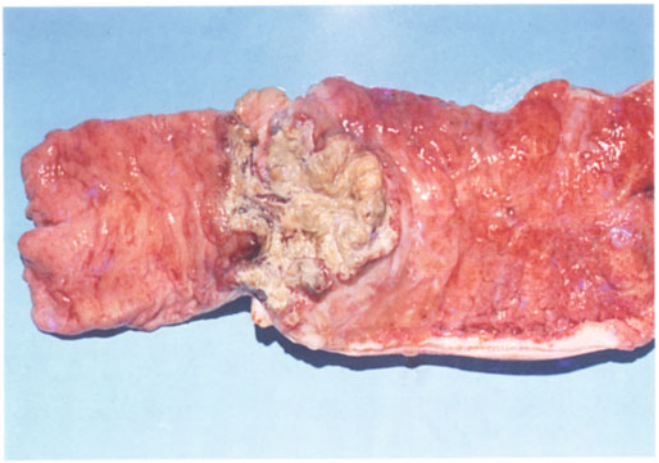

Fig. 8.6 Amoebic colitis with carcinoma of the colon transversum. Under the microscope in this lesion adenocarcinoma in addition to amoebae was found. It was not possible to determine which of these two processes was the initial one

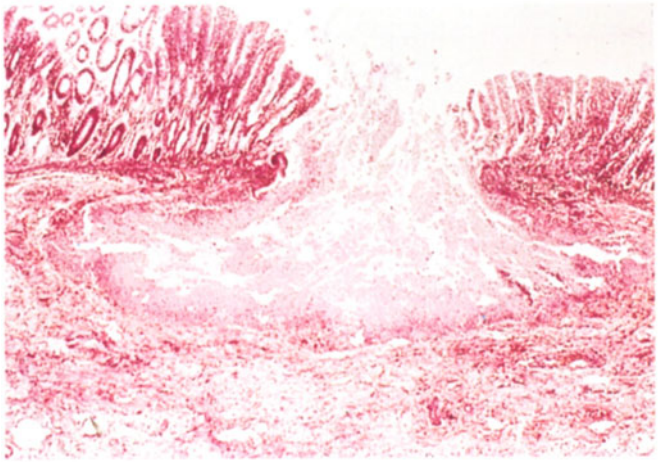

Fig. 8.7 Amoebiasis. A complete, characteristic 'buttonhole ulcer' of the large intestine comprising mucosa and submucosa with the borders of the entire circumference undermined. H&E

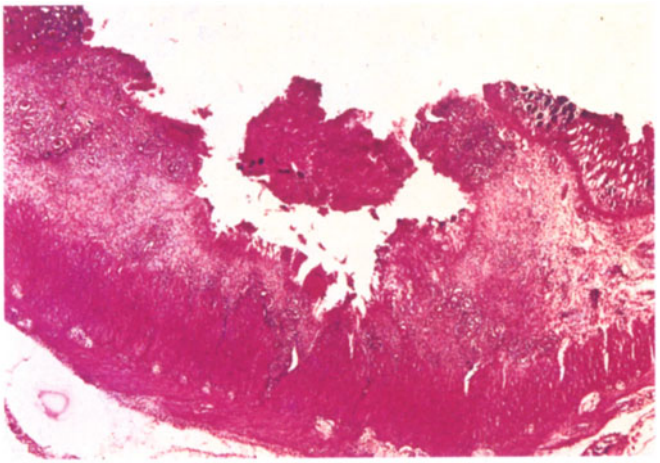

Fig. 8.8 Amoebiasis. A recently formed ulcer involving mucosa and submucosa of the large intestine in the form of a 'buttonhole'. H&E

Fig. 8.9 'Amoeboma' of the mesocolon. The opened large intestine presents an enlarged (cut) fistula connecting intestinal lumen with a large 'tumour' which shows a cavity (necrosis) on the central part. The 'tumour' is formed by chronic granulation tissue with numerous amoebae in the mesocolon

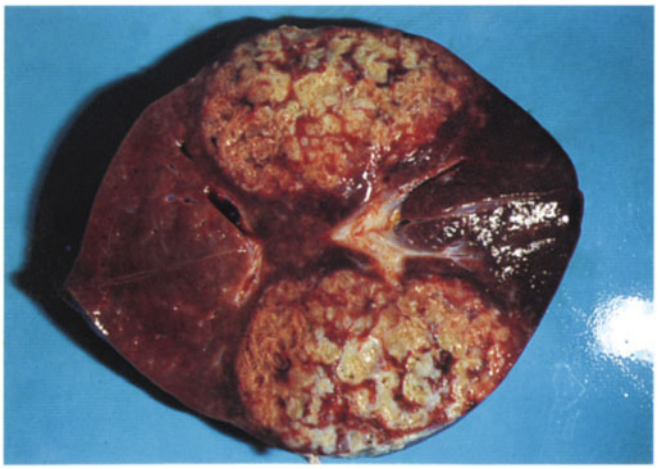

Fig. 8.10 Solitary amoebic liver abscess

However, it must be noted that ulcers with these characteristics are also found in bacterial dysentery and that they do not appear exclusively in amoebiasis. In our material, the ulcers of the amoebic colitis do not occur more frequently at any particular site in the large bowel: they occur in the caecum, both flexurae or in the rectosigmoidal region. Relatively frequently, the appendix showed amoebic lesions, although large grossly detectable lesions were not seen at this site. More frequently, the appendix and part of the ileum were involved, showing, microscopically, more or less extensive necrotic lesions in the intestinal wall, but almost never grossly visible ulcers. Isolated amoebic appendicitis is not common[13]. The amoebic ulcers in the large intestine, which are often deep, may reach the serosa where circumscribed peritonitis is provoked. Perforation of these ulcers with consequent diffuse peritonitis, which used to be a serious complication, is no longer seen, apparently because diagnosis is made earlier and/or therapy is more efficient.

Other important complications are peri-appendicular abscesses and colo-abdominal, colo-gastric or colo-hepatic fistulae. Fistulae between the colon and renal pelvis, rectum or bladder are rarer. Amoebic lesions of the spleen, pancreas and kidneys are formed rarely by contiguity.

In **chronic intestinal amoebiasis**, with or without mucosal ulcers, the mucosa becomes thicker and polypoid tumoral lesions form. These are the basis of the extensive chronic inflammation of the intestinal wall. Many authors believe that strictures, stenosis, scars of the intestinal wall and peritoneal adhesions are the result of chronic lesions caused by amoebae. However, we have looked for this type of sequel for many years in our autopsy and biopsy material and have never been able to attribute them to amoebic infections.

The lesions named 'amoebomas' and 'amoebic granulomas' need a brief discussion: Many cases described as 'amoebomas', 'amoebic granulomas', lesions of an 'X-ray aspect of a large tumour' or an 'inflammatory parasitic pseudotumour' do not merit this denomination. They correspond solely to a circumscribed thickness and/or dilatation of an intestinal segment in an amoebic infection, with the exception of a few cases, e.g. a reported amoeboma really similar to a carcinoma[14].

One of our cases showed amoebic alterations of a tumourous appearance and probably merits a denomination of resemblance to a tumour (see Fig. 8.9). An intestinal segment of the large intestine had been resected with the presurgical clinical diagnosis of carcinoma of the colon. In the surgical specimen, a large chronic paraintestinal abscess in the mesocolon was found which was in contact with the intestinal lumen through a 'not open perforation', i.e. a fistula. The central necrotic masses of this abscess, where numerous amoebae were found, were surrounded by a broad fibrotic shell. The 'large nodule' observed in this case was, in truth, very similar to a tumour or neoplasm and should be called a peri- or para-intestinal amoeboa.

In more than 30 years, only one case of amoebic colitis and an apparently coincident carcinoma of the colon has been seen in our material. However, primary carcinomas at this site are rare in our autopsy and biopsy material in comparison with other countries.

Hepatic amoebiasis. The amoebae reach the liver, usually by means of the portal vein, and there they produce either single (Fig. 8.10) or multiple (Figs. 8.11 and 8.12) pylephlebitic abscesses. The solitary amoebic abscesses previously described as being typical are the exception rather than the rule[15]. The amoebae first cause necrotic foci which may be confused with tumours or tumour metastases. Later, the foci become soft in the central parts and, on the whole, look like abscesses with a semiliquid necrotic content, often similar to chocolate because of massive old haemorrhages. The formation of a liver abscess by contiguity due to amoebiasis of the colon transversum is exceptional. Diagnosis of amoebic liver abscesses may be difficult[15].

Hepatomegaly (Fig. 8.13) in cases of amoebic colitis may occur without amoebic abscesses in the liver. In this case, the enlargement of the liver is due to an infectious–toxic reaction of this organ or a secondary infection, but not necessarily to the parasites. The hepatomegaly has been called 'amoebic hepatitis', but this name does not seem appropriate since, in these cases, amoebae are not present in the liver. The name 'reactive hepatitis' may be more suitable. When amoebae invade hepatic tissue, they produce abscesses but not amoebic hepatitis. Complications which may occur in this type of cases are:

1. Peripherical abscesses may perforate Glisson's capsule and drain towards the abdominal cavity, the stomach, pancreas, spleen or through the abdominal wall (Fig. 8.14).
2. Skin fistulae may originate in the right upper abdominal region.
3. Subdiaphragmatic or subphrenic abscesses may result which may perforate the diaphragm and cause pleural and pulmonary lesions with formation of abscesses or hepato-bronchial fistulae, mostly in the lower lobule of the right lung (Figs. 8.15 and 8.16). Amoebic abscesses in the lower lobule of the left lung are less frequent and are the result of amoebic lesions in the left lobe of the liver.
4. Rarely, amoebic lesions have been found in the pericardium and in the oesophagus.
5. Sometimes, metastatic abscesses resulting from haematogenous dissemination are formed in the lung.
6. Rarely, *E. histolytica* produces brain abscesses (Fig. 8.17). In the two latter instances, the point of departure may have been an amoebic lesion in the large intestine or an amoebic liver abscess.

Rare extraintestinal amoebic lesions. In addition to the relatively frequent extraintestinal lesions mentioned above, the parasites may also cause lesions in less common sites: the lymph nodes; the skin of the perineal region; the uterine cervix; and the penis.

1. Amoebae in the lymph nodes (Figs. 8.18 and 8.19) do not cause important inflammatory reactions. If lymphadenitis is present in cases of amoebiasis, it is usually due to a secondary infection rather than to the amoebae.
2. Amoebic lesions of the rectum may lead to amoebic cutaneous perineal alterations (Figs. 8.20–8.22) which may originate by contiguity and cause recto-cutaneous fistulae and lesions similar to cutaneous tumoral manifestations. This type of lesion naturally heals rapidly when specific anti-amoebic drug treatment is applied. Making a differential diagnosis between cutaneous amoebiasis and Meleney's synergistic gangrene is discussed in reference 17.
3. Sometimes, we have come across amoebic lesions at the exocervix (Figs. 8.23 and 8.24) which resemble cauliflower-like growths and are virtually impossible to differentiate from cervical carcinomas. If, in regions endemic to amoebiasis, these exocervix lesions are seen with extensive necrotic areas and carcinomatous cells are not immediately found, it is recommended that amoebae are looked for using the special staining methods. Amoebae may be found in routine Papanicolaou smears: in one case, in a patient wearing an

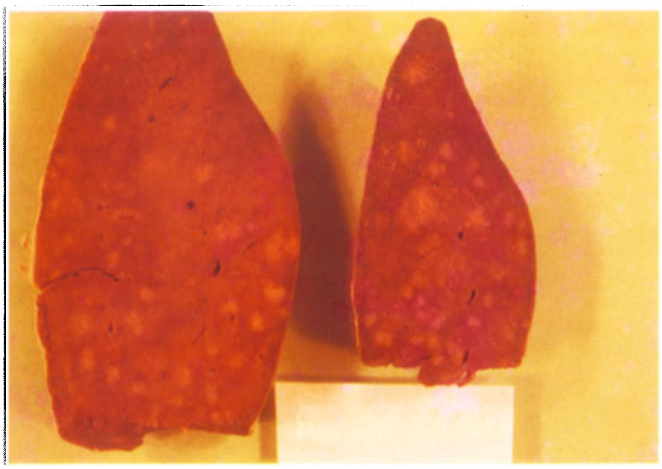

Fig. 8.11 Multiple hepatic abscesses due to amoebic infection in a 4-month-old infant

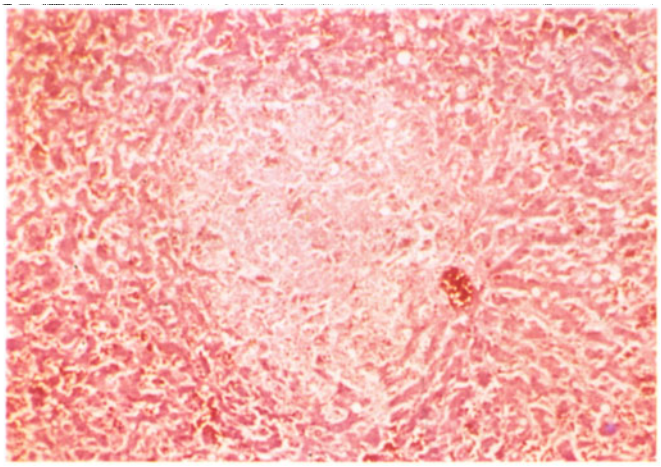

Fig. 8.12 The histological picture of a liver 'abscess' of the case shown in Figure 8.11. Parasites are not seen at this magnification and staining method. H&E

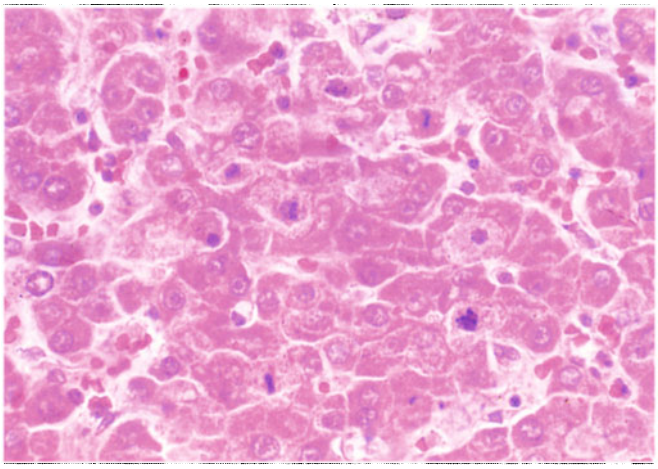

Fig. 8.13 'Reactive hepatitis' with hepatomegaly in an amoebic infection. Numerous mitoses of hepatocytes are seen, but never amoebae. H&E

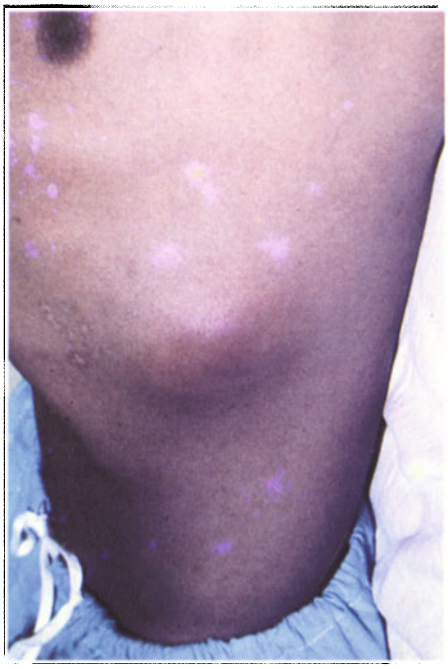

Fig. 8.14 The protuberance seen in the skin of the right-hand upper quadrant of the abdomen is produced by an amoebic liver abscess

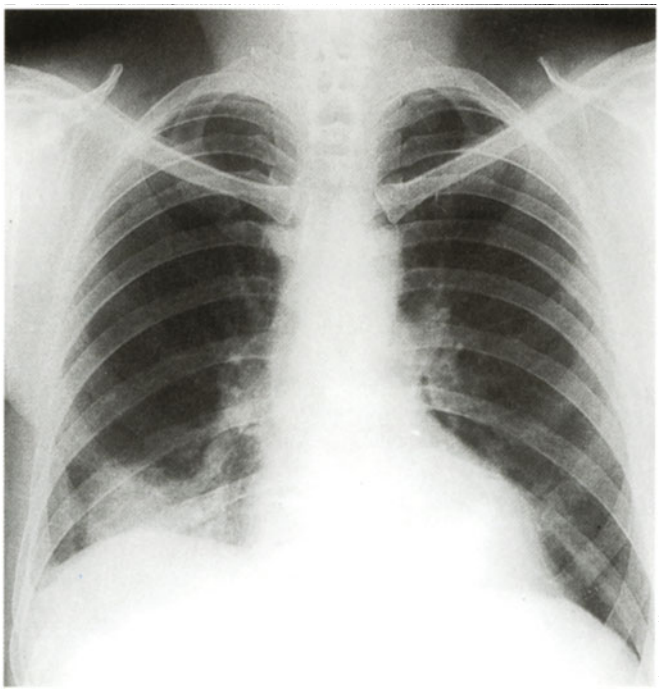

Fig. 8.15 Amoebic abscess in the lower lobule of the right lung

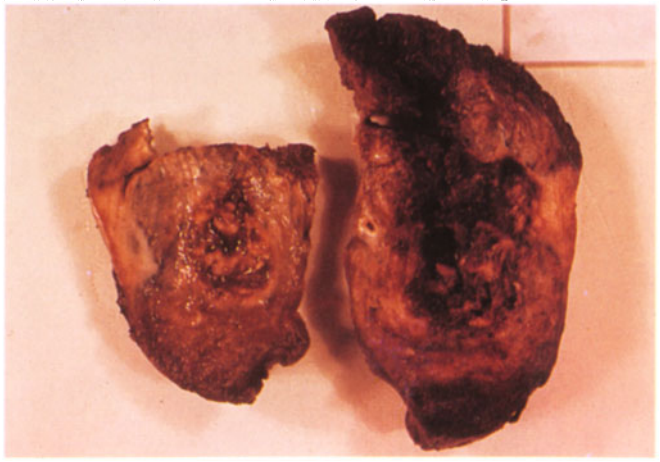

Fig. 8.16 This surgical specimen is the resected lung abscess of Fig. 8.15

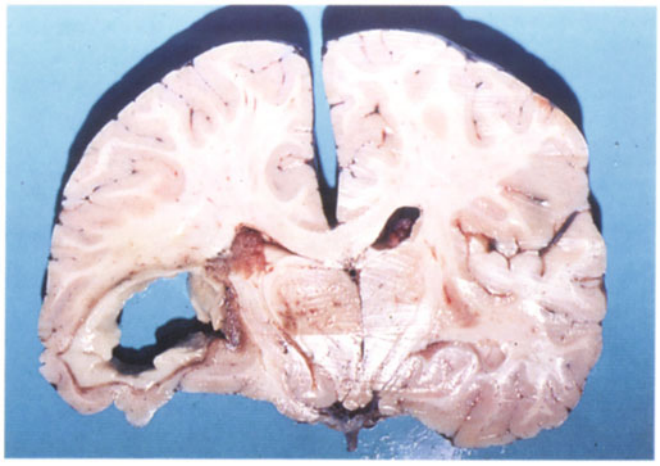

Fig. 8.17 Amoebic brain abscess. The central nervous system is very rarely attacked by *Entamoeba histolytica*

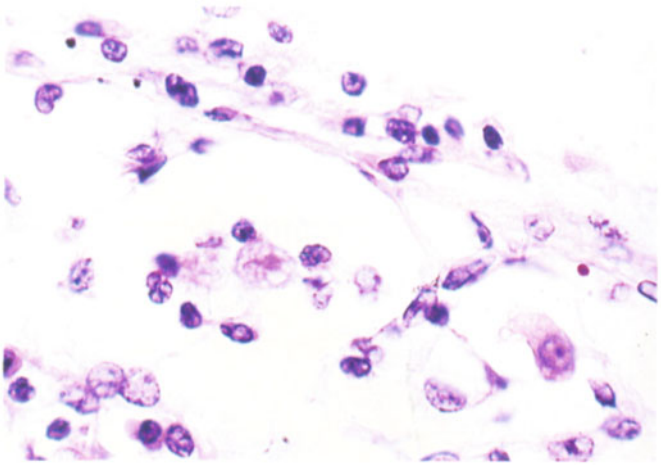

Fig. 8.18 Two structures are seen in the marginal sinus of a mesenteric lymph node which represent amoebae. This organ is rarely invaded by amoebae. H&E

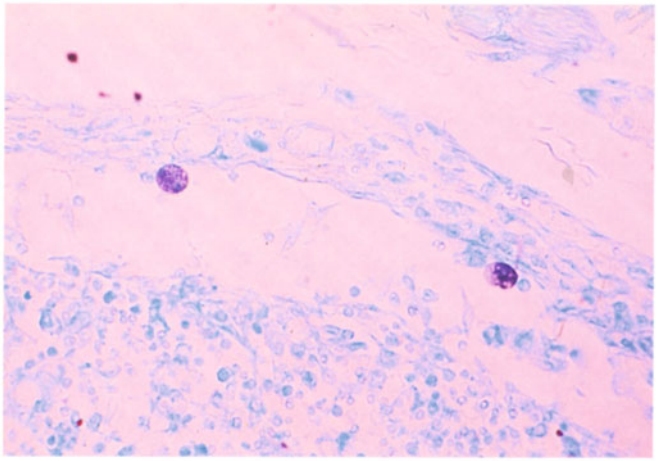

Fig. 8.19 The same lymph node as in Fig. 8.18 with parasites positive in this staining method. Grocott

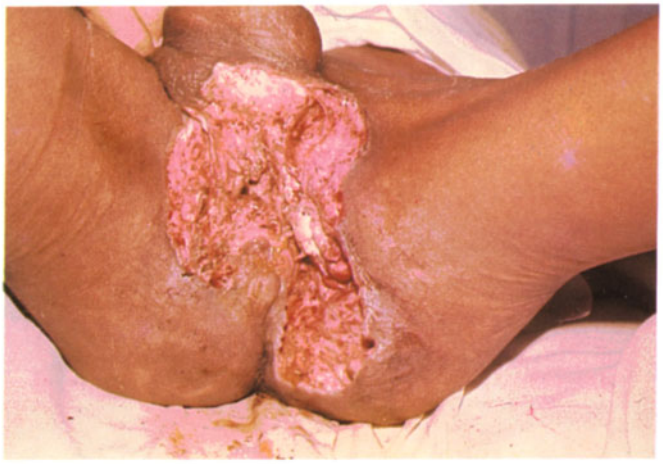

Fig. 8.20 Amoebae were found in the biopsy of this extensively ulcerated perineal region. Cutaneous carcinoma had been the clinical diagnosis previously

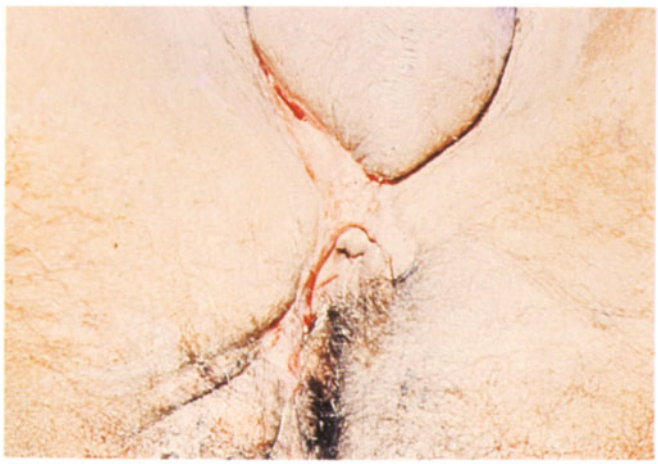

Fig. 8.21 The same case as in Fig. 8.20 three months after specific treatment

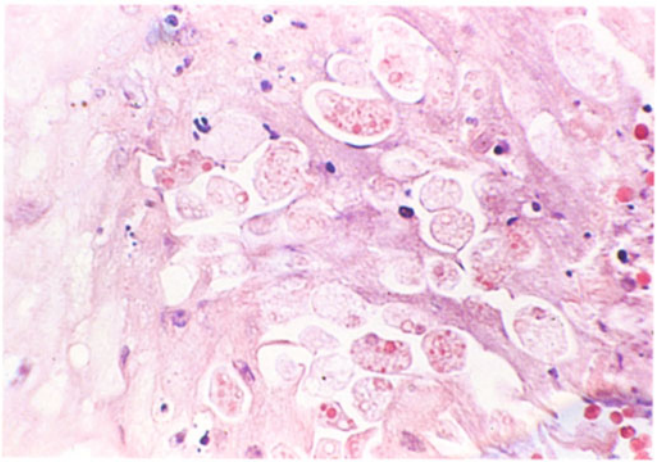

Fig. 8.22 Histology of the case of Fig. 8.20. Numerous organisms of *Entamoeba histolytica* are seen. H&E

intrauterine contraceptive device[18], and, in another, in association with a cervical squamous cell carcinoma[19]. Amoebic infections of the uterine cervix without mucosal defects are an exception.

4. Amoebic lesions of the penis (Fig. 8.25) are rare and originate only from sexual relations with an infected individual.

Histopathology. In order to obtain good results in the diagnosis of the amoebae, it is indispensable to have personal experience. Personnel who have no appropriate training and do not examine this sort of specimen daily run the risk of making too many false positive diagnoses. Histological diagnosis of amoebiasis may be difficult[20]. Figs. 8.26–8.28 show intestinal lesions at low power.

The trophozoites of *E. histolytica* can be seen in the intestinal content and in the tissues of the intestinal wall at various stages of preservation. If the results of the H&E or iron haematoxylin staining methods are not satisfactory, the PAS and Grocott methods give good results and bring the parasites out clearly, above all in cases with few parasites and in slides with badly preserved ones. Often, amoebae contain red blood cells, a phenomenon called erythrophagia (Fig. 8.2a). Occasionally, numerous amoebae are situated in the lumen of blood or lymphatic vessels, without signs of vasculitis (Fig. 8.29). Sometimes, it is difficult to distinguish amoebae from macrophages or ganglion cells (Fig. 8.30). The problem of differential diagnosis of the different species of amoebae may arise in smears of the intestinal content but does not exist in tissue sections since only *E. histolytica* is found in tissues.

The amoebae may be present in the cavities of abscesses, in the liver or in other viscera, but, more often, they perish and disappear before being recognized by the pathologist. However, amoebae are more likely to be encountered in the granulation tissue on the periphery of an abscess. Nevertheless, in spite of the use of special staining methods, false negative diagnoses are also possible when pus of liver abscess is obtained by puncture. Recently, we have achieved good diagnostic results by examining sections of cell blocks of this material stained with the PAS method. In these sections, only a few necrobiotic amoebae, difficult to recognize as such, were detected with the H&E stain but they came out clearly with the PAS method (Fig. 8.31).

The tissue reaction consists mainly of lympho-histiocytic infiltrates; occasionally eosinophilic leukocytes are present. Neutrophilic leukocytes and fibrine exudation are scarce or absent and, when they are present, are due to a secondary associated bacterial infection.

In the liver, the so-called abscesses are not true ones because their content consists of semiliquid necrotic material without leukocytes. The above mentioned reactive hepatitis, present in the hepatomegaly of amoebic colitis, shows infiltrates of lympho-histiocytes and a certain number of granulocytes in the hepatic sinus and in periportal spaces. Also, a marked oedema and, occasionally, numerous hepatocytes showing mitoses can be observed.

'Amoebic granulomas' have been mentioned above when 'amoebomas' were discussed. It must be emphasized that the terms granulomas or granulomatous reactions refer to histological features. Neither granulomas nor granulomatous reactions are found in amoebic infections.

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9. ACANTHAMOEBIASIS

Introduction

Free-living 'soil' or 'water' amoebae may cause severe diseases of the central nervous system with meningitis or meningo-encephalitis. Species of the genus *Naegleria* cause primary amoebic meningo-encephalitis (PAME), while *Acanthamoeba* species cause chronic granulomatous amoebic encephalitis (GAE). Keratitis due to acanthamoebae has also been observed[1–5]. *Naegleria* is not an acanthamoeba. Acanthamoebiases are really 'infections due to free-living amoebae'.

In the world literature, no more than 200 cases have been recorded, mostly in small groups of children or adolescents. They have been found in Australia and New Zealand, in European countries, in the USA and single cases have been observed elsewhere. In Venezuela, we know of three reported cases[6,7].

Spontaneous acanthamoebiasis, to our knowledge, has been reported only exceptionally in lower animals[8]. Experimentally, amoebic infections of this kind may be produced by various routes in mice, rabbits and monkeys. Rhinitis, encephalitis and pneumonia (after direct inoculation) have been achieved in these animals[9–11].

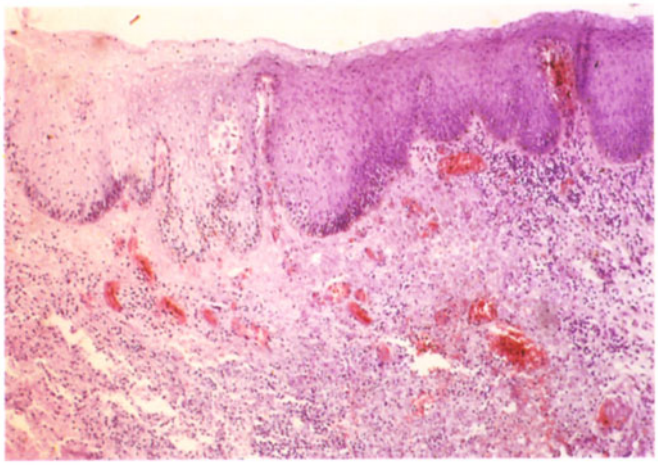

Fig. 8.23 Amoebic cervicitis. Parasites are not seen at this magnification and with this staining method. H&E

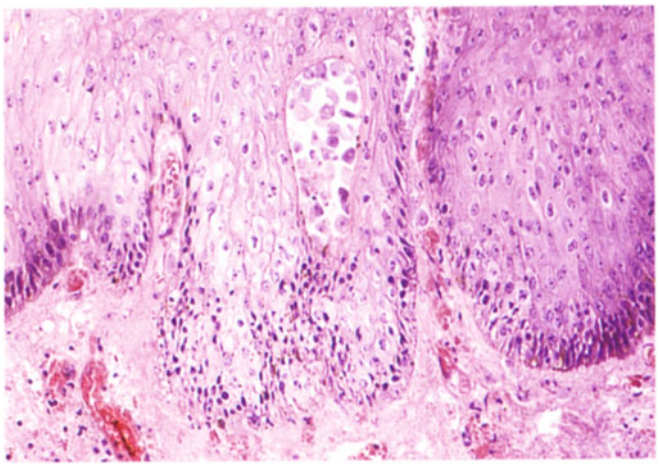

Fig. 8.24 Higher power of Fig. 8.23. A nest of amoebae may be recognized easily in this field. H&E

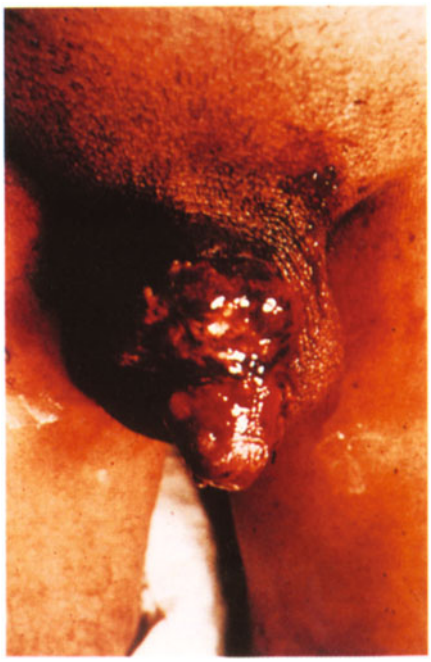

Fig. 8.25 Amoebic lesions of the penis with extensive ulceration and haemorrhages

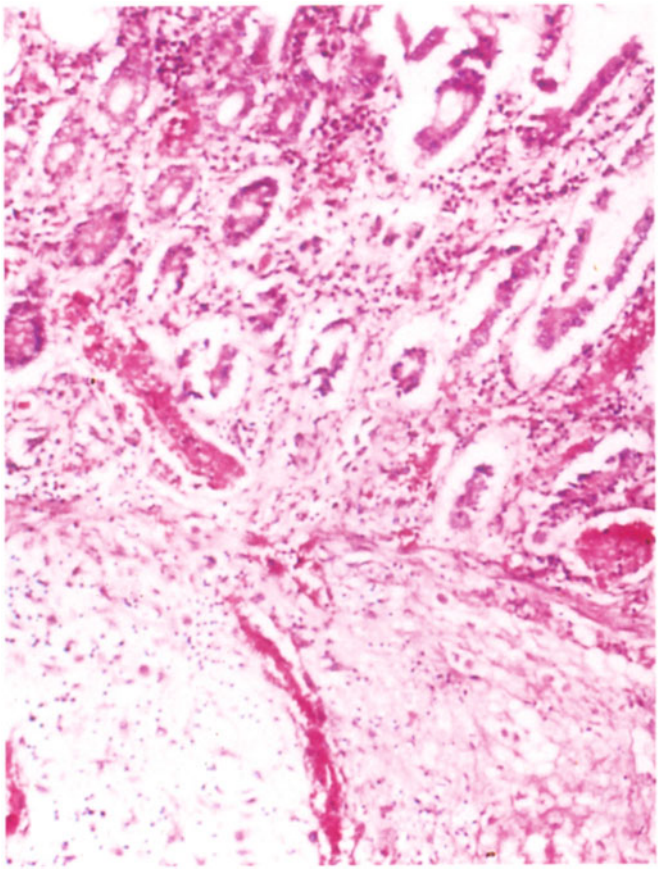

Fig. 8.26 Low power view of early amoebic colitis. H&E

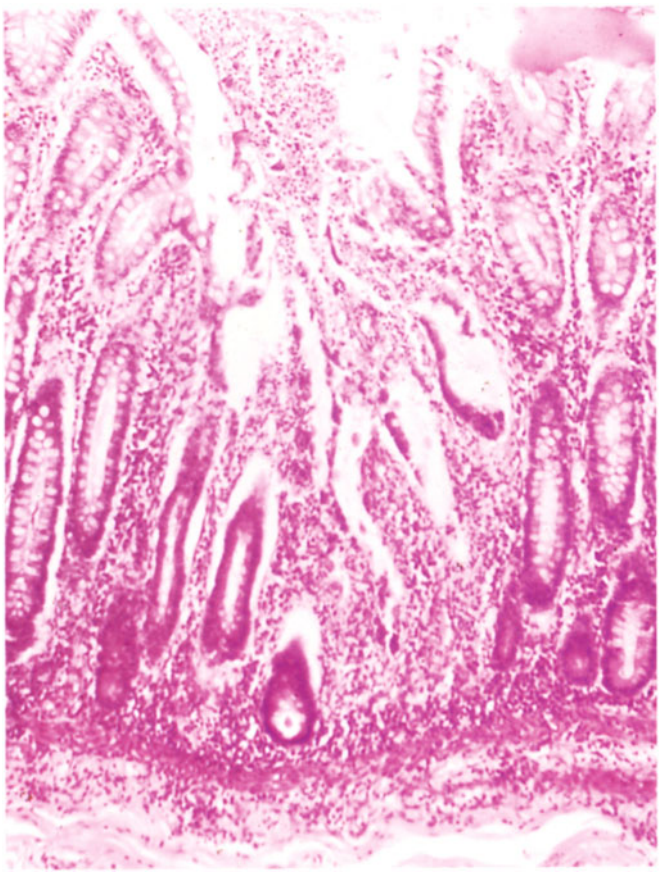

Fig. 8.27 Early circumscribed necrosis of the mucosa of the large intestine due to infection with amoebae. Parasites are not seen at this magnification. H&E

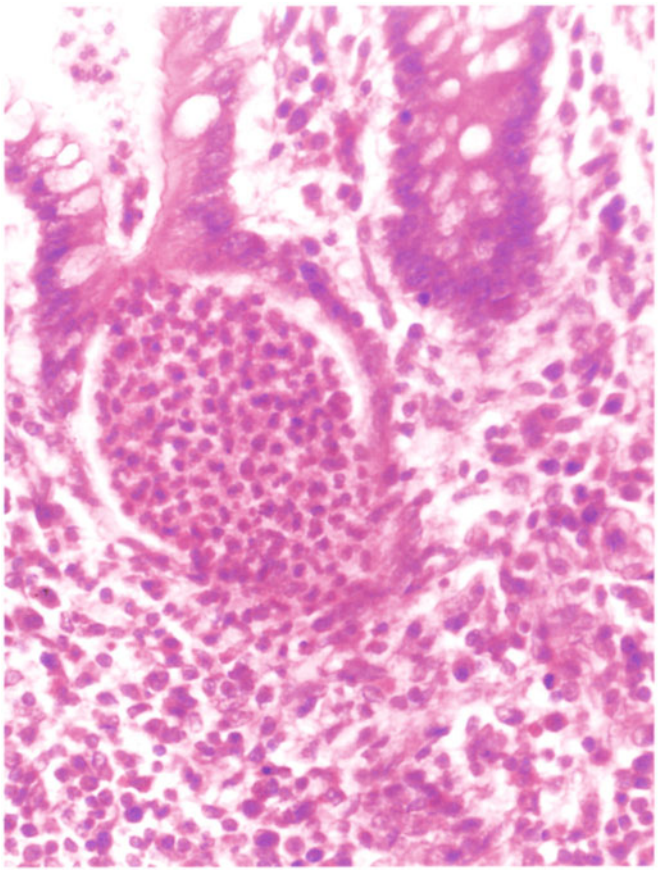

Fig. 8.28 Early lesion of amoebic colitis with an abscess at a crypt. The leukocytic exudate is probably due to secondary bacterial infection. H&E

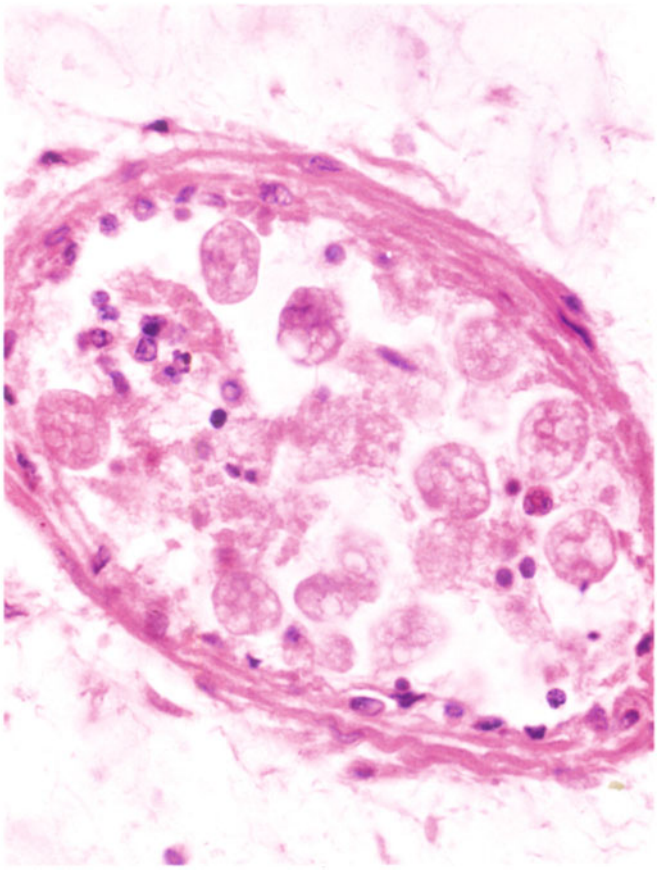

Fig. 8.29 Numerous amoebae present in the lumen of a vein in the submucosa of the large intestine. The wall of the vein is intact. H&E

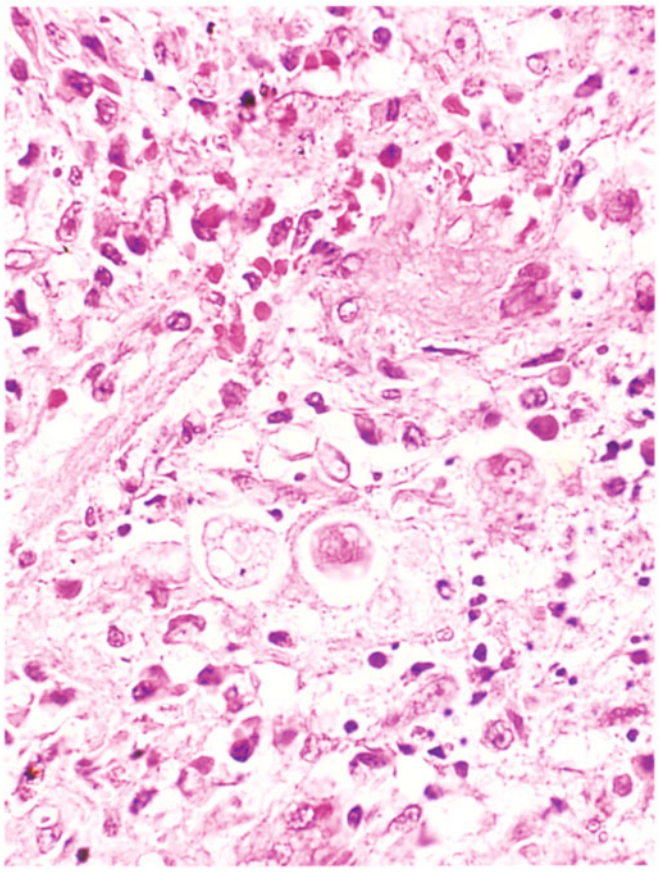

Fig. 8.30 *Entamoeba histolytica* seen in tissues with the routine stain. H&E

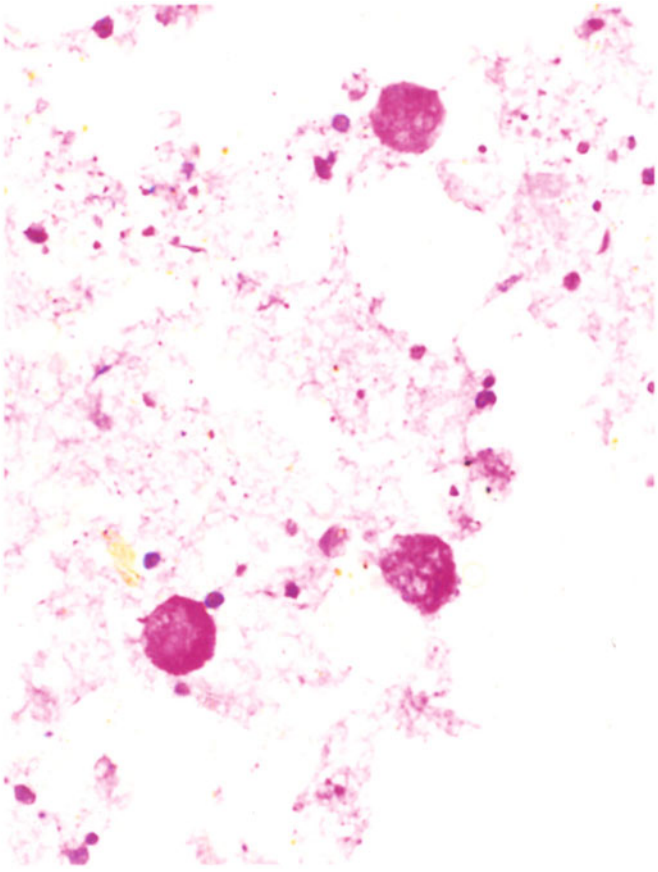

Fig. 8.31 Amoebae in section of a cell block from contents of a liver abscess are seen clearly with this staining method. PAS

The disease produced by *Naegleria* species (PAME) is reported with increased frequency after swimming in pools, lakes and rivers. This form shows an acute fulminating course and is always fatal. In addition, PAME occurs more frequently than GAE.

The amoebic infection caused by *Acanthamoeba* species, on the other hand, occurs as an opportunistic infection[12] in clinically ill or immunosuppressed patients and without contact with water. It has been reported in AIDS patients[13,14] and has a more chronic course but nevertheless, a fatal outcome.

Clinical diagnosis of this disease is made by detecting trophozoites in the spinal fluid (Fig. 9.1) by culture of spinal fluid or tissue specimens of the brain in the NNN medium. Immunofluorescence techniques and the complement fixation test are performed in special research laboratories.

The parasite

The important pathogenic species for man and mouse are *Naegleria fowleri, Naegleria australiensis, Acanthamoeba castellanii* and *Acanthamoeba culbertsoni*. The terms *Limax* and *Hartmanella* as genera are now obsolete. Non-pathogenic species of soil or water amoebae do exist but will not be described here in detail.

The *Naegleria* and *Acanthamoeba* species measure between 10 and 40 μm. The former are somewhat smaller, about 7–22 μm, and the latter, 15–45 μm. Trophozoites and cysts may be distinguished. Trophozoites are seen in tissues. They are spherically shaped and almost always possess only one nucleus, but, exceptionally, up to three may be present. They stain with H&E, iron haematoxylin and trichromic methods and are PAS- and Grocott-negative. In spinal fluid, trophozoites measure 8–10 μm and are motile.

Cysts (persistent forms) of these amoebae are found ubiquitously, e.g. in dust, water and in the upper respiratory tract of man[15–18]. They are uninuclear and have a thick membrane which may be irregular. Acanthamoeba cysts may show stellate or polyhedral structures; they have been described in tissues by García Tamayo *et al.*[7].

In the presence of bacteria, amoebae may be cultured easily in Nelson medium and also in Bacto agar Difco in horse serum. Axenic cultures have been achieved too. In tissue cultures, soil amoebae have a typical cytopathic effect. In plate cultures *Naegleria* species have 2 flagella.

Pathogenesis

In *Naegleria* infections, the portal of entry of the parasites is probably the nasal mucosa. Here an inflammatory process develops. The amoebae penetrate the mucosa and migrate along blood vessels and nerve fibres (*N. olfactorius*) centripetally, through the cribriform plate of the ethmoid into the subarachnoid space and, from there, into the brain. This movement of amoebae has been shown experimentally after intranasal administration of culture material[19].

By haematogenous dissemination, the amoebae, apparently, reach internal organs and may cause inflammatory processes. However, the parasites soon perish in these viscera and have not been confirmed at these sites. Involvement of lung, kidney, liver, pancreas, lymph nodes and myometrium has been described but without the presence of parasites in these viscera.

Acanthamoeba species may use the upper respiratory tract as portal of entry. The CNS is probably secondarily affected from another active focus, e.g. the lungs or the skin[20].

Pathology

Meninges and brain are affected by species of *Naegleria*, and the brain (occasionally also the meninges) by *Acanthamoeba* species. Further, the cornea may be involved.

In human beings, lesions produced by these species of amoebae have been reported in liver, lungs, kidneys, pancreas, lymph nodes and myometrium, but the parasites themselves have not been described in internal organs outside of the CNS (possibly they perish soon after haematogenous dissemination).

Grossly, the purulent and haemorrhagic necrotizing meningitis and the purulent foci in cortical and subcortical areas of the brain, as well as the keratitis, do not present special or specific features. They may be easily confused with the suppurative meningitis or necrotizing haemorrhagic encephalitis of any other aetiology. Specific gross lesions of internal organs are not known. Circumscribed tumour-like lesions have been observed in the brain in the GAE form of the disease[21].

Histologically, we have seen, personally, only three cases, one from Venezuela, one from Czechoslovakia and one from the USA, all of the PAME variety. The amoebae in the tissues of these cases were almost solely trophozoites. Only a few large parasites seemed to be cysts, some with thin membranes and others with thicker and irregular membranes. García Tamayo *et al.* have illustrated cysts in tissues in an *Acanthamoeba* infection[7]. On an average, the amoebae measured 10 μm and were of a spherical shape, or occasionally amoeboid or polyhedral. The majority of amoebae had one small nucleus, centrally located; only few amoebae had several nuclei, but no more than three nuclei could be seen in one amoeba. Often, the cytoplasm of the amoebae was clear, similar to a vacuole, but inhomogeneous or granular inclusions were also seen in variable amounts in the cytoplasm. The amoebae in these cases stained weakly with the H&E, iron haematoxylin and the Goldner stain (Fig. 9.2) but did not stain at all with the PAS or Grocott methods. Often, large nests of numerous amoebae showed necrobiotic changes of the amoebae themselves. Figs. 9.3–9.5 show brain tissue with soil amoebae at low power, Figs. 9.6 and 9.7 at medium power and Figs. 9.8–9.10 at high power with the H&E stain.

The parasites were mostly located, often in clusters with numerous amoebae, in perivascular clear spaces or in nests, localized in the parenchyma without any alterations or showing clear spaces. This means that there is focal destruction of brain tissue. Frequently, the walls of large blood vessels were invaded by amoebae. Often, parasites situated in dense cell infiltrates, e.g. granulocytes, were recognized only with difficulty. They were seen clearly inside nerve cells (neuronophagy) (Fig. 9.9), a feature which previously had been erroneously interpreted by us as phagocytosis (amoebae engulfed by macrophages). Tissue reaction in these cases consisted of oedema, colliquative necrosis, exudation of fibrin and extensive cellular infiltrates with a predominance of neutrophilic leukocytes. Often, the latter were densely packed with necrobiosis of these cells, i.e. a real suppurative lesion was present. In addition, thromboses of small and medium blood vessels and extensive haemorrhages were observed. Perivascular infiltrates with granulocytes, lymphocytes and plasma cells were found in the parenchyma, without amoebae. In some areas, a marked proliferation of astrocytes was noted.

A granulomatous reaction with giant cells was described in the GAE form[22–24]. We have not seen lesions of this kind personally.

Histological differential diagnosis of amoebae is relatively easy. In tissues, intestinal amoebae (*Entamoeba*

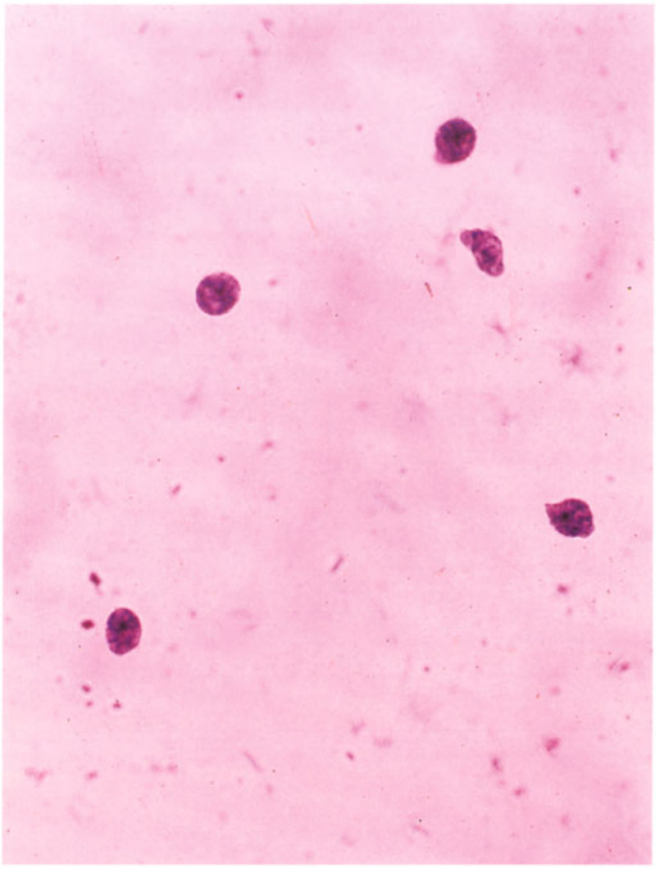

Fig. 9.1 *Naegleria fowleri* in smear of spinal fluid. H&E

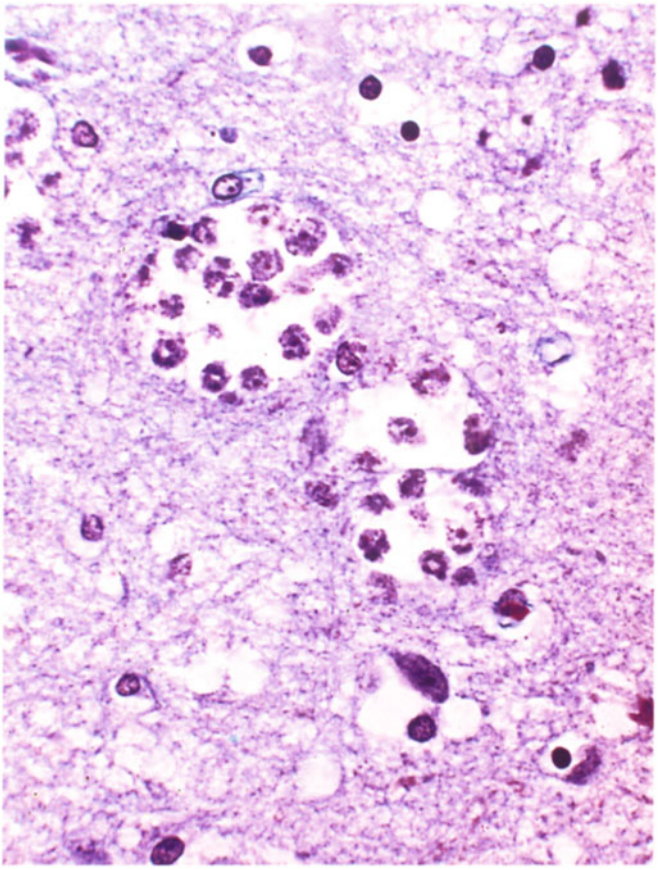

Fig. 9.2 Clusters of soil amoebae in brain. Goldner

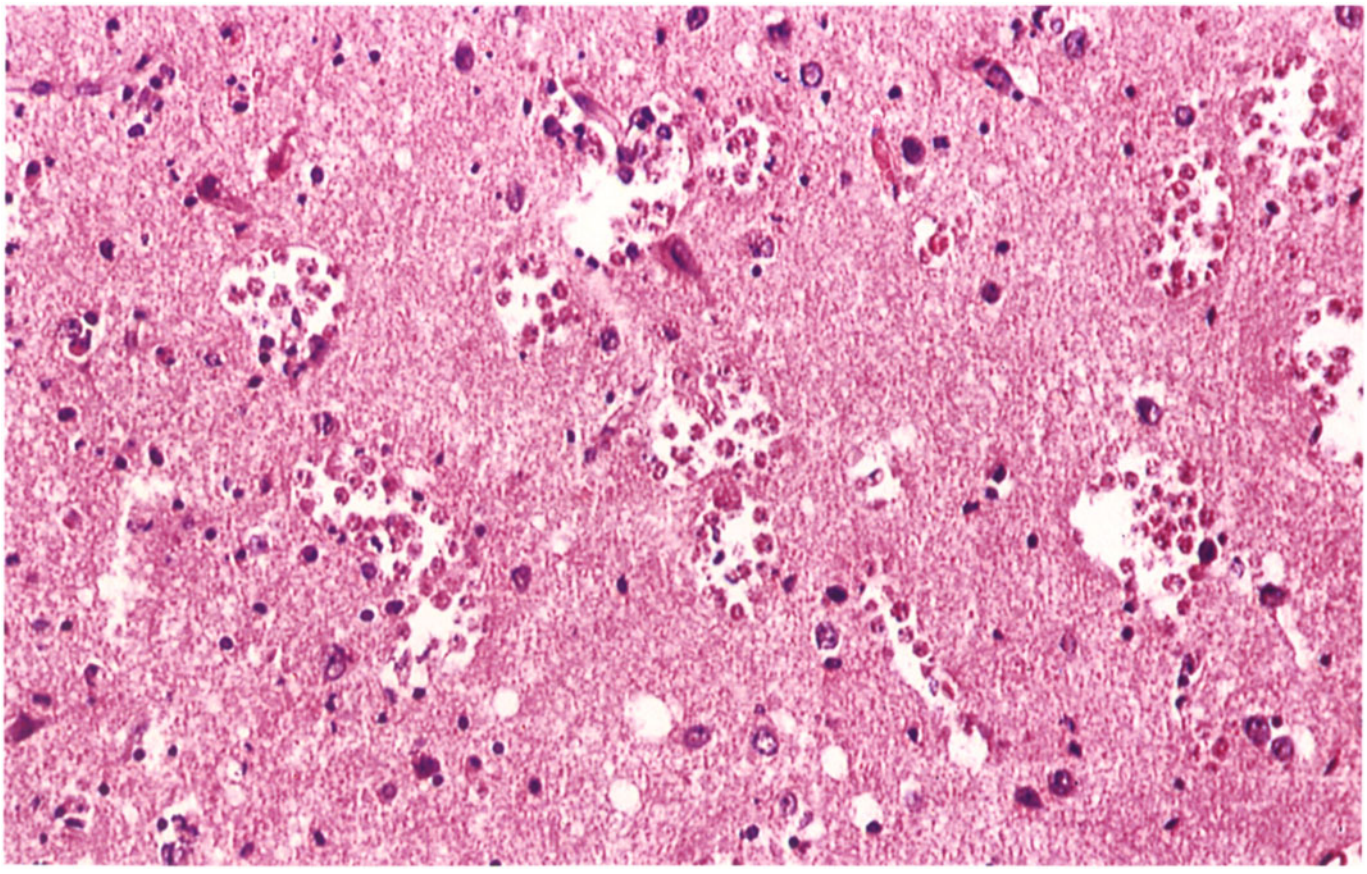

Fig. 9.3 Low power view of brain tissue with numerous clusters of soil amoebae. H&E

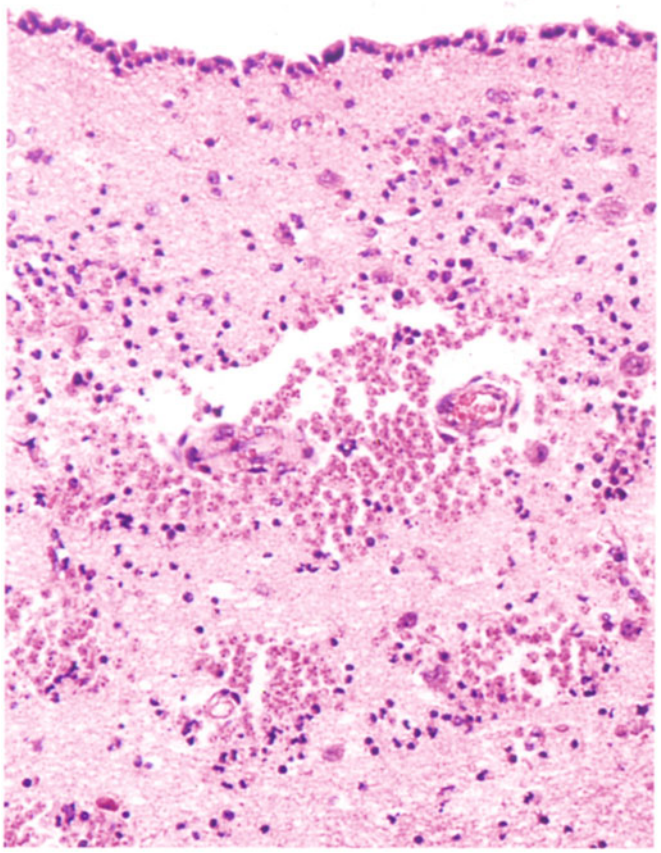

Fig. 9.4 Numerous soil amoebae in brain tissue with inflammation. H&E

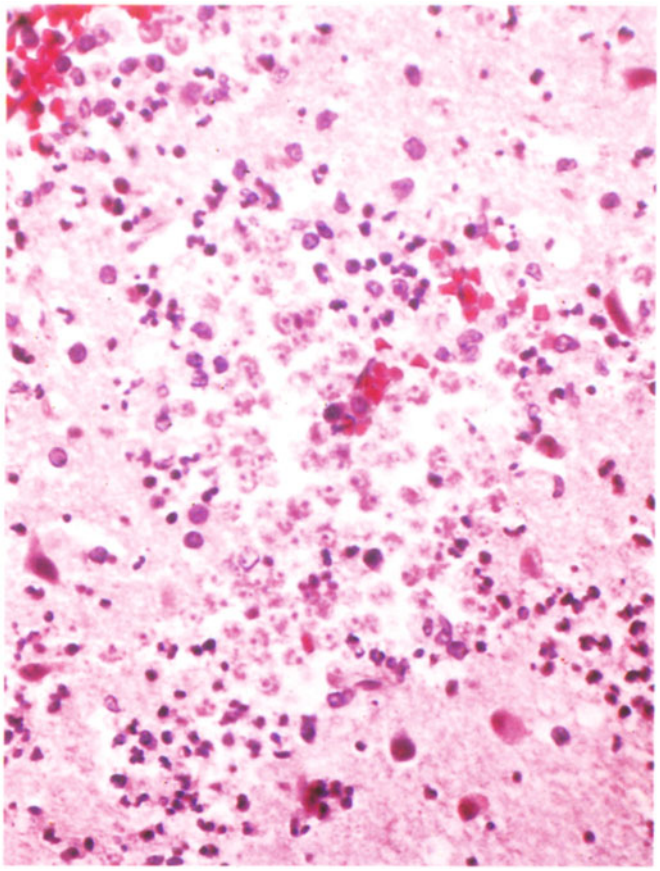

Fig. 9.5 Acanthamoebiasis. In addition to parasites, marked leukocytic inflammation is seen. H&E

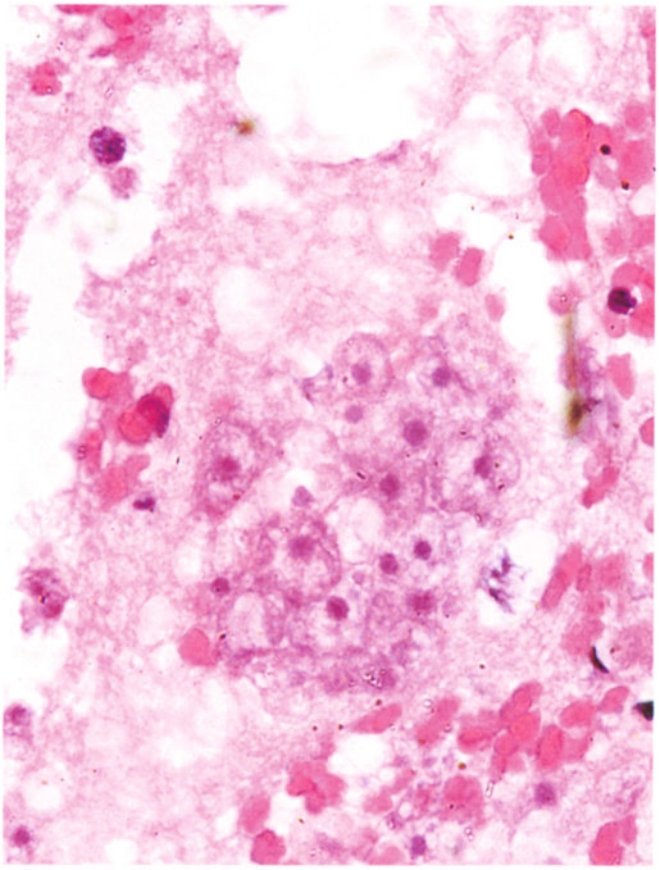

Fig. 9.6 Nest of soil amoebae in necrotic brain tissue. H&E

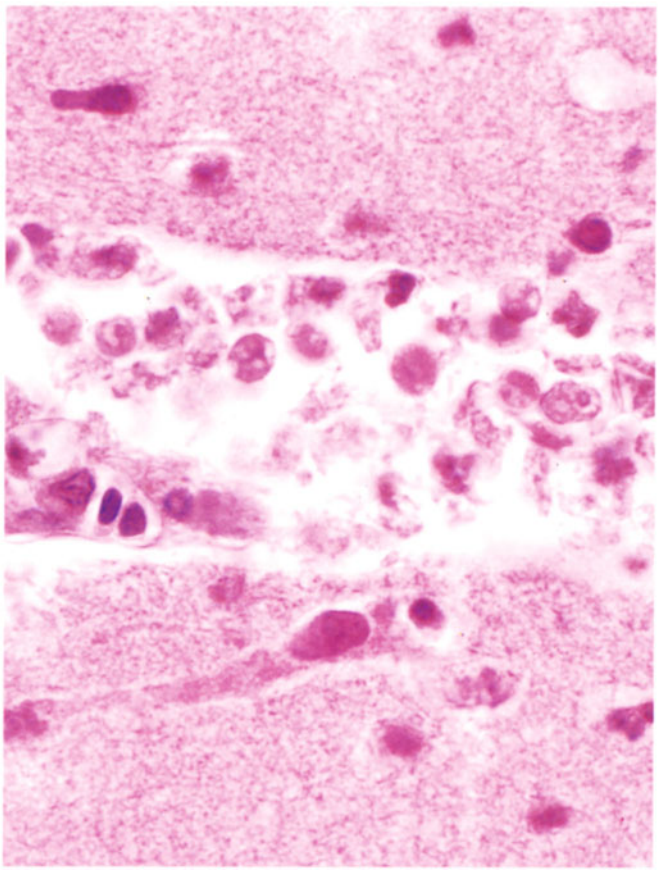

Fig. 9.7 Cluster of soil amoebae in a perivascular space. H&E

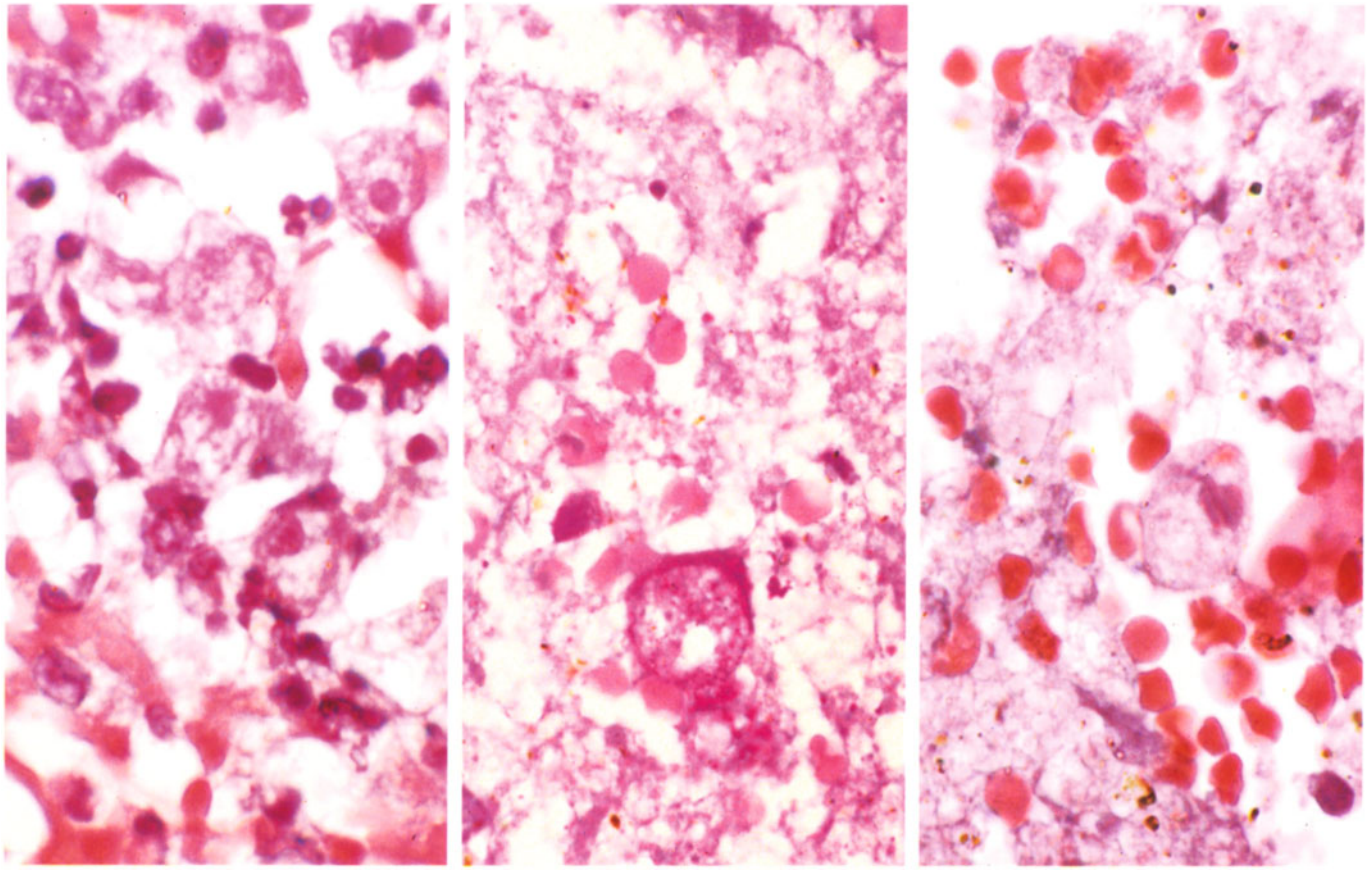

Fig. 9.8 Soil amoebae in brain tissue at high magnification. H&E

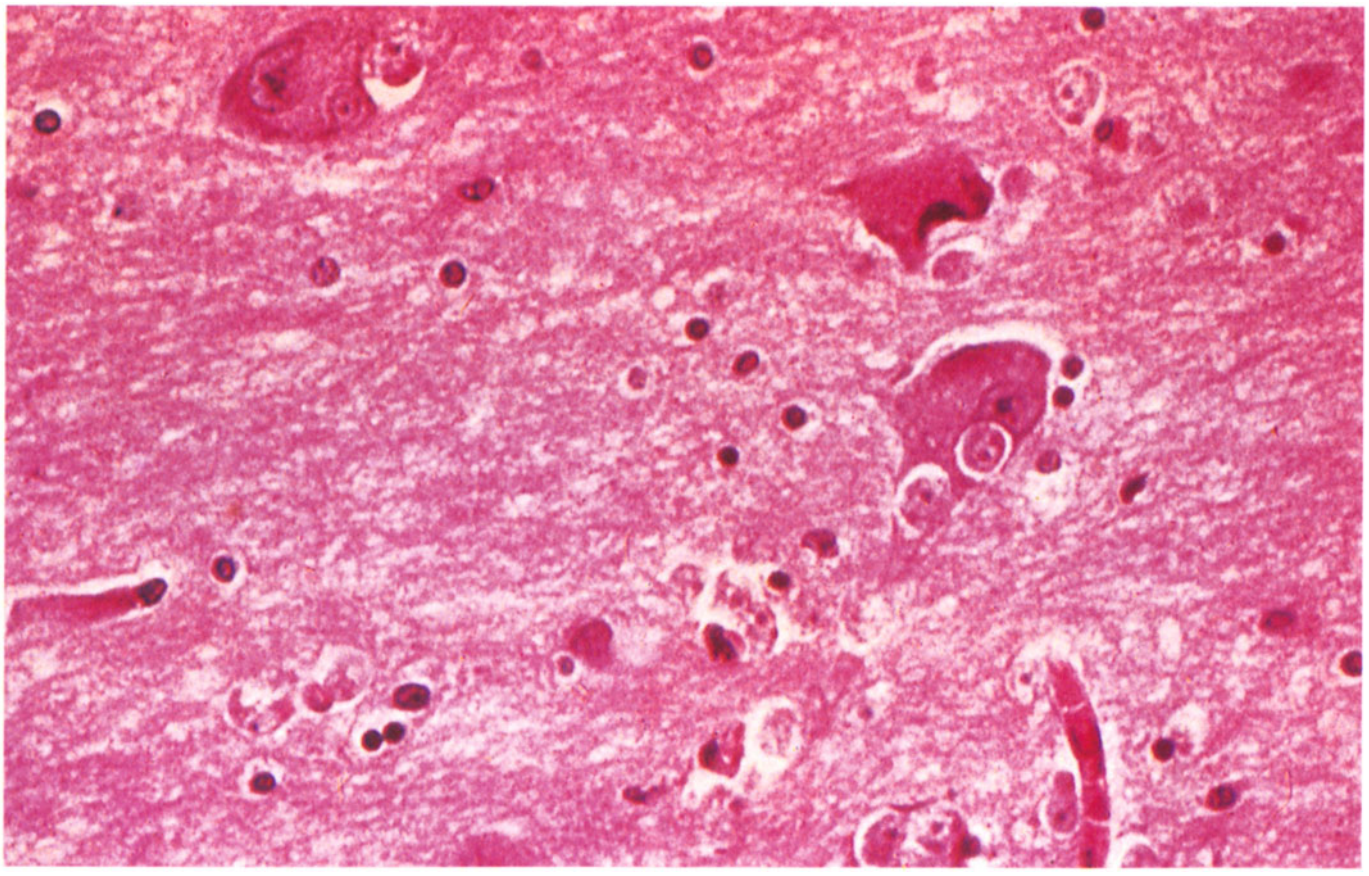

Fig. 9.9 'Soil amoeba' in the brain are phagocytising nerve cells (neurophagy). H&E

histolytica) are pleomorphic, show erythrophagia and stain with PAS and weakly with Grocott. In addition, the purulent tissue reaction in acanthamoebiasis is not found in the lesions produced by *E. histolytica*.

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10. BLASTOCYSTIS INFECTION

Introduction

Blastocystis hominis was accepted, previously, as an intestinal yeast, harmless for humans[1]. From the sixties on, it was considered a protozoan[2] and, now, its pathogenicity for man is being discussed[3,4]. It has been found worldwide with an average prevalence of 10–15% in people with enteric symptoms; also in Venezuela[5].

Whether *Blastocystis hominis* occurs naturally in lower animals we do not know. Experimentally, diarrhoea may be produced in guinea pigs and non-human primates[6] when these species are infected with *B. hominis*.

Enterocolitic symptoms, flatulence, diarrhoea, abdominal cramps and pain as well as anorexia, are thought to be caused by this parasite. Clinical diagnosis may be made by examining fresh stools prepared with physiological saline and seen with the light or phase-contrast microscope. However, a single examination of a stool is not sufficient. When, in stools with neutrophilic leukocytes, pathogens are not found, *B. hominis* should be looked for.

The parasite

Blastocystis hominis, known since the beginning of this century as a yeast, has been considered a protozoan, belonging to the Sporozoa, since 1967. The vacuolated cell is spherical in shape and, characteristically, shows a large central vacuole which occupies 75% of the cell volume and is surrounded by a narrow rim of cytoplasm containing nuclei and some inclusions. It has a signet ring appearance (Fig. 10.1). Commonly, there is one nucleus situated in the peripherical membranous-like structure but, occasionally, there are 2–4 nuclei. Mitochondria are confined to the thin peripheral band of cytoplasm between the membrane surrounding the voluminous inner body and the outer membrane[7,8]. There are wide variations in size. *Blastocystis hominis* may measure from 5–120 μm in diameter. This parasite reproduces rapidly in human serum as culture medium.

In cultures, three morphological forms may be observed: vacuolic, amoeba-like with pseudopodes, and granular. In faecal specimens, the vacuolated and amoeba-like forms are found.

Parasites may be stained permanently with iron haematoxylin (Fig. 10.2), Goldner or the Giemsa method; with the last, they stain weakly. The parasites are Ziehl–Nielsen-, Gram- and Grocott-negative. Their vacuoles do not stain with Lugol. At Mérida, we have achieved good results by staining sections of cell blocks (Figs. 10.3 and 10.4). Reproduction of parasites takes place by binary fission or sporulation.

Pathogenesis

The mechanisms of the pathogenic actions of this parasite have not been elucidated. Massive infections with *Blastocystis hominis* may lead to enterocolitic symptoms. Whether this is due to the parasite alone or to the interaction of *Blastocystis hominis* with other organisms has yet to be resolved.

Invasion of tissues by this parasite has not been confirmed convincingly. In one case, a human death due to these organisms was reported because evidence of mucosal invasion of the colon was shown postmortem[9].

Pathology

Parasites are found in the lower ileum and caecum. Descriptions of gross or microscopic lesions do not exist

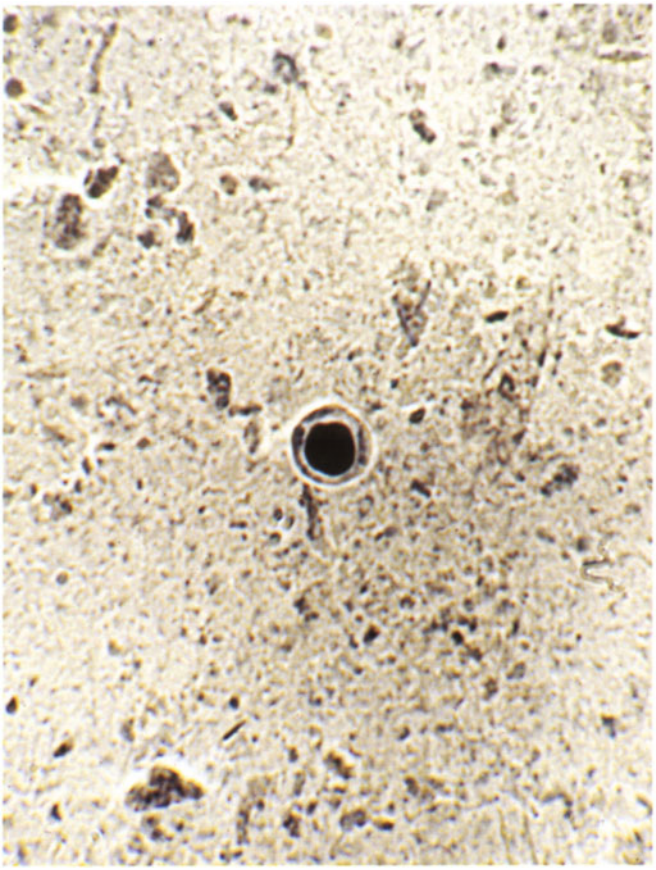

Fig. 10.1 *Blastocystis hominis* cell in smear of faeces. Note the cyst-like appearance with the nucleus in the 'cyst wall'. Iron–haematoxylin

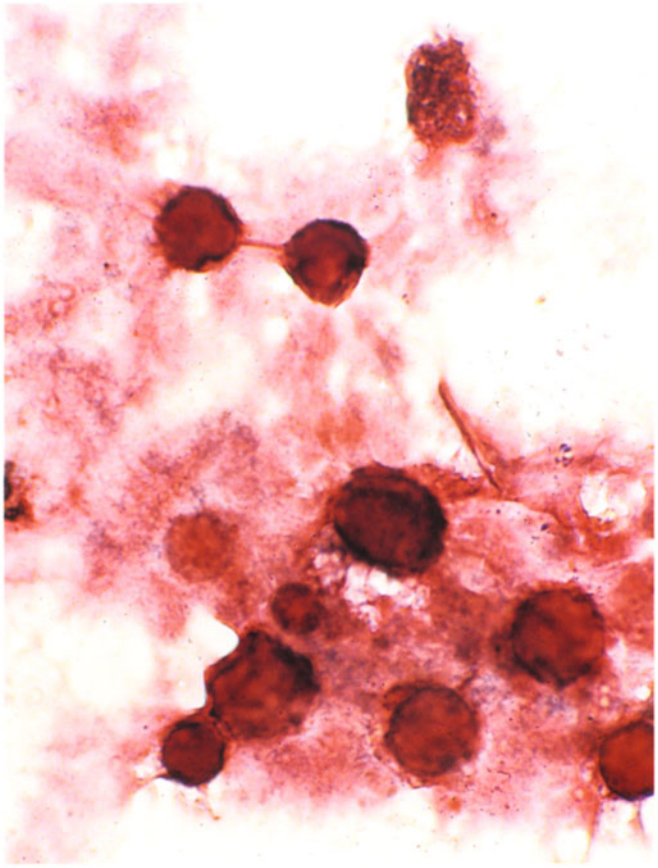

Fig. 10.2 *Blastocystis hominis* cells in smear of faeces. Iron–haematoxylin

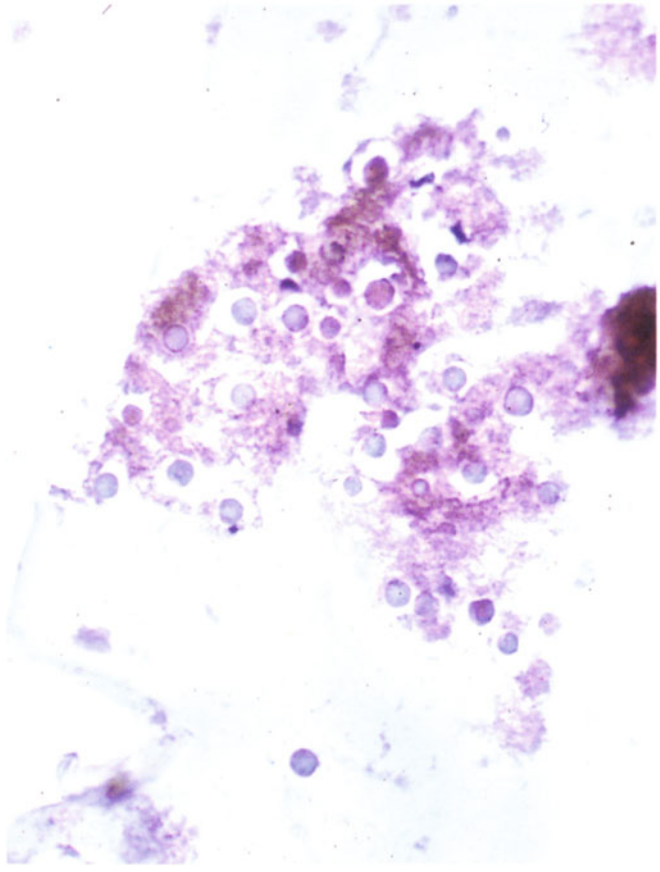

Fig. 10.3 *Blastocystis hominis* cells in section of cell block of faecal material. Iron–haematoxylin

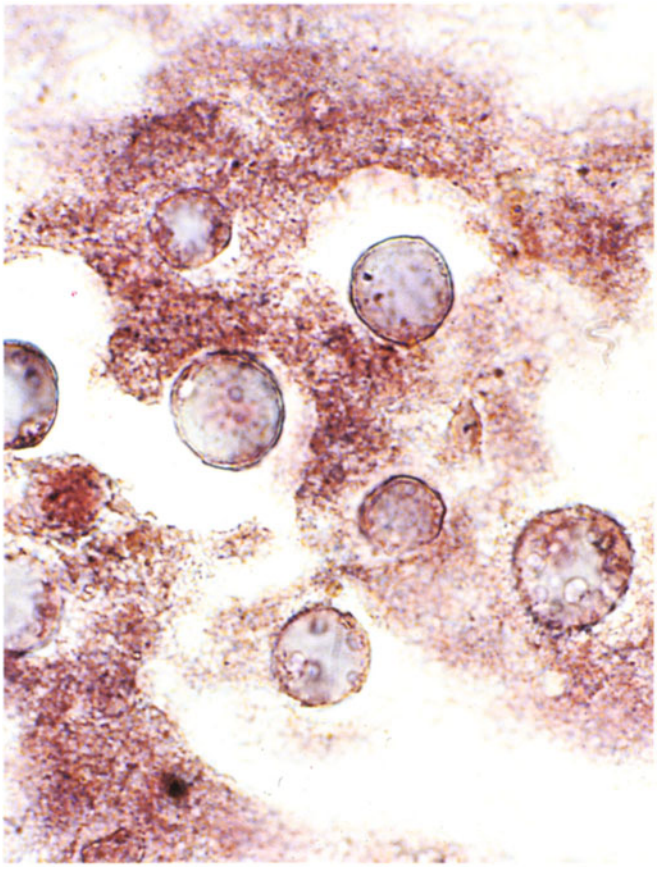

Fig. 10.4 High magnification of Fig. 10.3

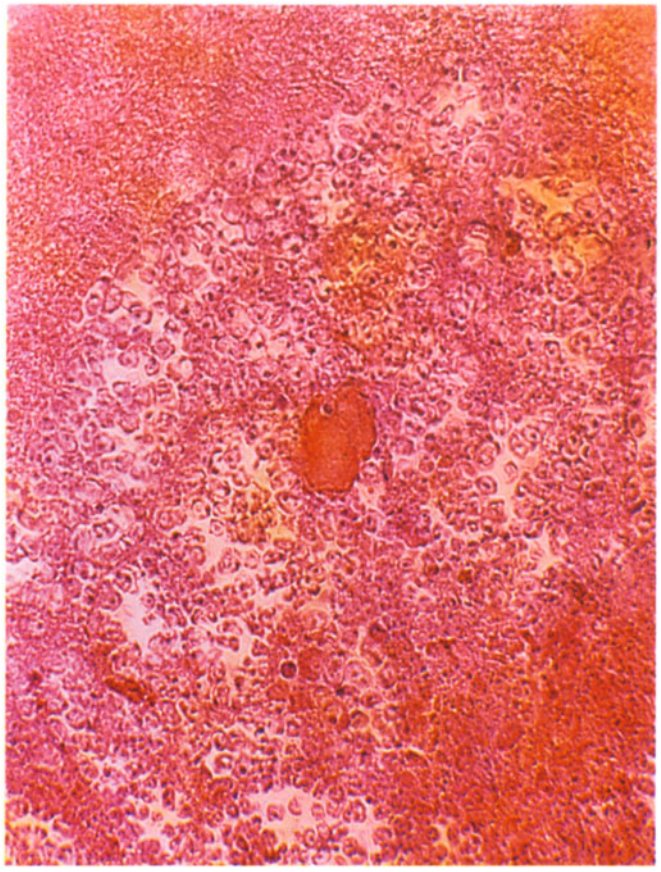

Fig. 10.5 Numerous *Blastocystis hominis* organisms on the surface of the necrotic mucosa of the large bowel. Diffuse pseudomembranous colitis post surgery of carcinoma of the oesophagus. (Case of Dr J. Boehm, München, Germany). Low power. H&E

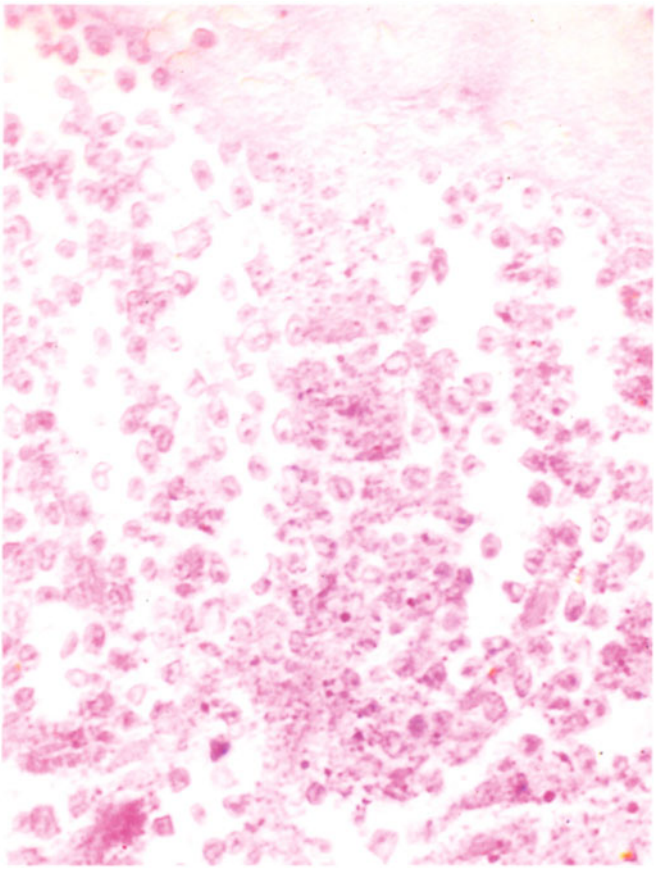

Fig. 10.6 Same case as Fig. 10.5. PAS

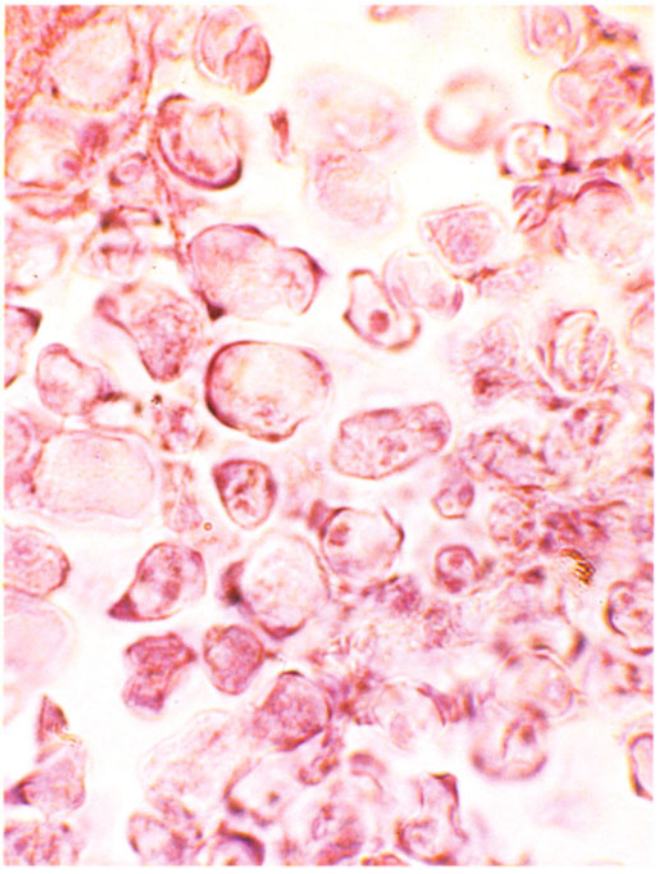

Fig. 10.7 Same case as Figs. 10.5 and 10.6. Nuclei are situated in the peripherical annular-like cytoplasm. This signet-ring appearance cannot be seen. High power. H&E

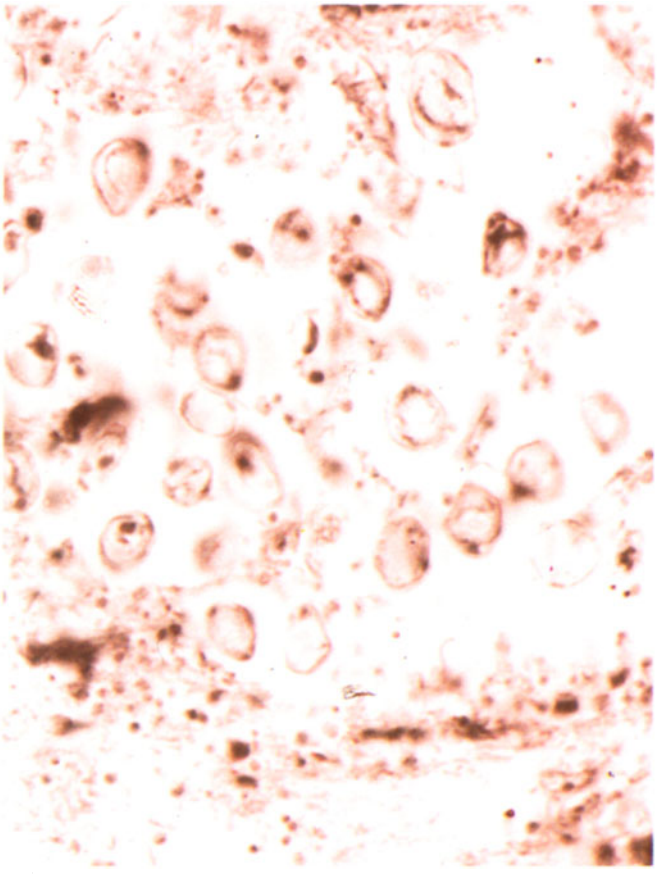

Fig. 10.8 Same case as Figs. 10.5–10.7. High power. Iron–haematoxylin

in the literature. While some reports stress the potential pathogenicity of *Blastocystis hominis*[4,10], there are others who deny that this parasite has a pathogenic role[11]. Recently, an autopsy case was kindly provided from Germany, showing numerous organisms of *Blastocystis hominis*, arranged colony-like and attached to the surface of the large bowel, apparently as secondary non-invasive opportunistic infection in an immunodeficient patient (Figs. 10.5–10.8).

Blastocystis hominis is the most important source of error in stool examinations for protozoa. These parasites may be confused easily with cryptosporidians or intestinal amoebae.

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11. TOXOPLASMOSIS

Introduction

Toxoplasmosis is an important infectious disease. Infection with *Toxoplasma gondii* is, worldwide, of a high prevalence. Latent symptomless infections are far more frequent than disease. Symptomatic disease, on the other hand, is rare but may be severe in man. Today, this is one of the prominent opportunistic infections in immunodeficient persons and typical in AIDS patients[1–5]. The proportion of infected persons is progressively higher in older age groups. More than 80% of the adult rural population in Venezuela[6] was found to have positive serology to *T. gondii*.

The cat (*Felis domestica*) is the specific definitive host for *Toxoplasma gondii*[7–10], although almost all mammals are non-specific intermediate hosts[11,12]. In dogs, lately, toxoplasma-like cyst-forming sporozoans which could not be identified have been reported in nervous tissue[13,14]. Mice and other animal species may be used for experimental purposes[15,16].

There are two principal clinical forms of toxoplasmosis:

1. An extrauterine infection acquired in a natural way. Opportunistic infections with disseminated disease, lymphadenitis and ophthalmitis are of special interest in this group.
2. An intrauterine toxoplasmotic infection in a pregnant woman and the transplacental infection of the fetus.

Clinical diagnosis is only confirmed by the demonstration of the parasite or specific antibodies. Microscopically, the toxoplasmas may be found in the spinal fluid, body and organ fluids as well as organs. Inoculation of specially sensitive *Toxoplasma*-free white mice and special rats may be used for diagnostic purposes.

The parasite

Toxoplasma gondii is the most important of the four coccidian species; the other three are *Sarcocystis bovihominis, Sarcocystis suihominis* (Section 13, Sarcosporidiosis) and *Isospora belli* (Section 14, Isosporosis). The Coccidia belong, together with the Plasmodia, to the class of Sporozoa.

It goes through a heteroxenous cycle of evolution, i.e. with several hosts. The final host is solely the cat which becomes contaminated after eating infected mice. In the feline digestive tract, the three phases of the coccidian cycle take place and end with the expulsion of oocysts. After sporulation outside the animals, new infections of mice, domestic animals, e.g. sheep etc., and cats occur. Table 3 shows the cycle of evolution.

The parasite has a half-moon form or is sickle-shaped. It measures 2–5 μm when observed in liquid smears and fresh histological preparations, above all as single organism. However, when seen in clusters intracellularly, i.e. in cysts or pseudocysts, they always appear as spherical elements in tissue sections. This is of practical importance because the observer, in order to make a diagnosis, should not look for sickle-shaped structures in tissue sections (Figs. 11.1–11.5). When the parasites reproduce inside tissue cells (muscular, glial or endothelial cells and in macrophages), they form numerous young parasites which fill the cell, pushing cytoplasm and other elements to the periphery, thus forming the already mentioned pseudocysts. Two types of *Toxoplasma* are distinguished by their manner of multiplication: the tachyzoites in pseudocysts, in the acute phase of the disease; and the bradyzoites in cysts with membranes, in the chronic form. However, these two types do not always show visible structural differences. Both types may appear in nests, but the bradyzoites tend to do this more often because they frequently present as cysts which are PAS-positive. This PAS-positivity seems to be related to the storage of glycopolysaccharides.

Artificial culture media which induce growth and multiplication of *Toxoplasma gondii* are not known; however, they may be kept alive in tissue cultures. These parasites can also be preserved in a viable condition for many months by adding glycerol or DMSO and then deep freezing in liquid nitrogen[17].

Pathogenesis

1. The extrauterine and acquired natural infection occurs in two ways:
   a) Raw meat (pigs, sheep or cows) harbouring parasitic cysts may be eaten.
   b) Oocysts or sporocysts of *Toxoplasma gondii* stemming from faeces of a cat may reach the human digestive tract. (Oocysts are highly resistant and may remain viable for longer than a year.)

   From the human gut, haematogenous dissemination takes place. The musculature and the central

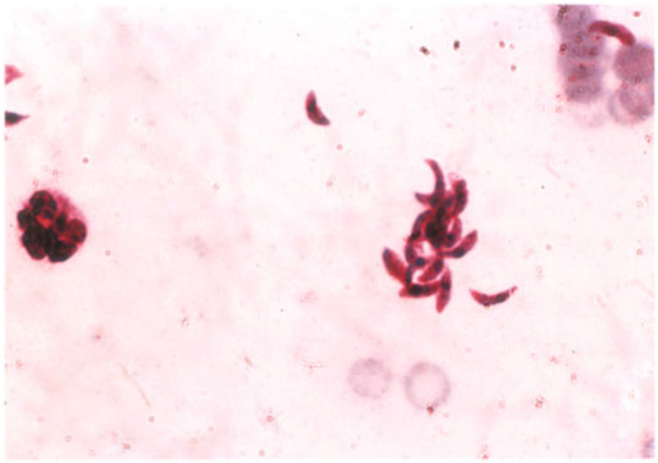


Fig. 11.1 *Toxoplasma gondii* in smear of human bone marrow showing half-moon-shaped parasites. Giemsa

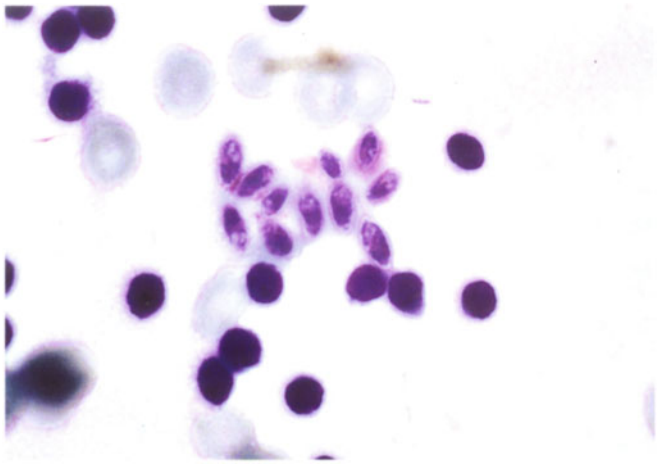


Fig. 11.2 Suspected toxoplasms in smear of pleural exudate, later determined as contaminating fungus cells; here shown as differential diagnostic elements. Giemsa

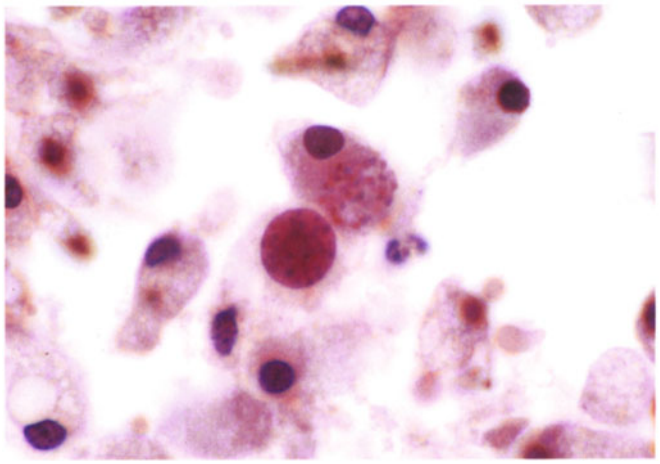


Fig. 11.3 Cyst and pseudocyst with *Toxoplasma gondii* parasites. The densely packed organisms are located in a cyst (dark) and the scarce parasites in a pseudocyst. Section of a toxoplasmic encephalitis. H&E

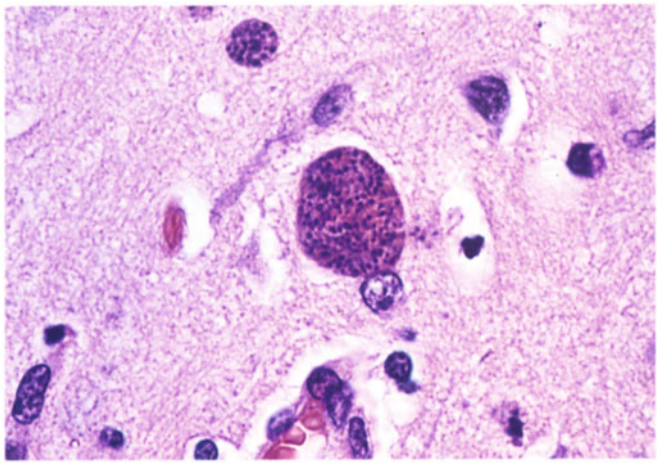


Fig. 11.4 Toxoplasmic cyst without inflammatory reaction at high power in the brain. H&E

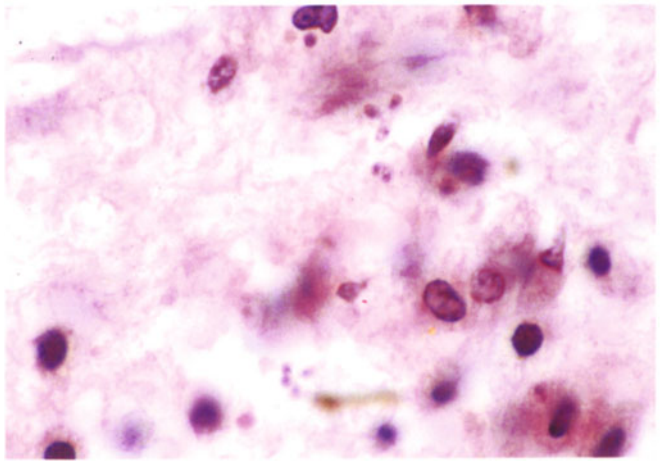


Fig. 11.5 Single, extracystic toxoplasms in tissue section of brain. H&E

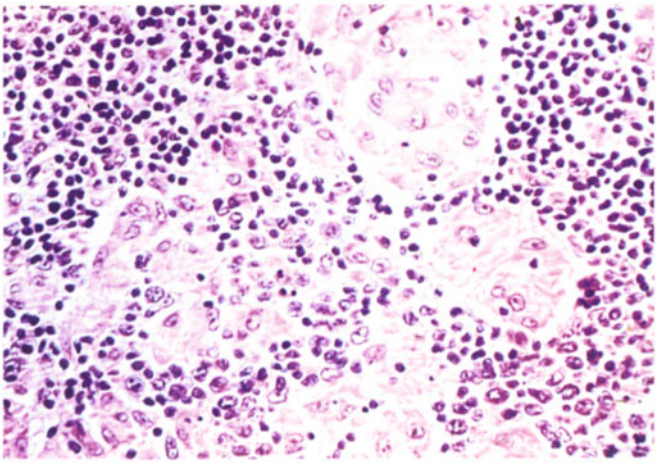
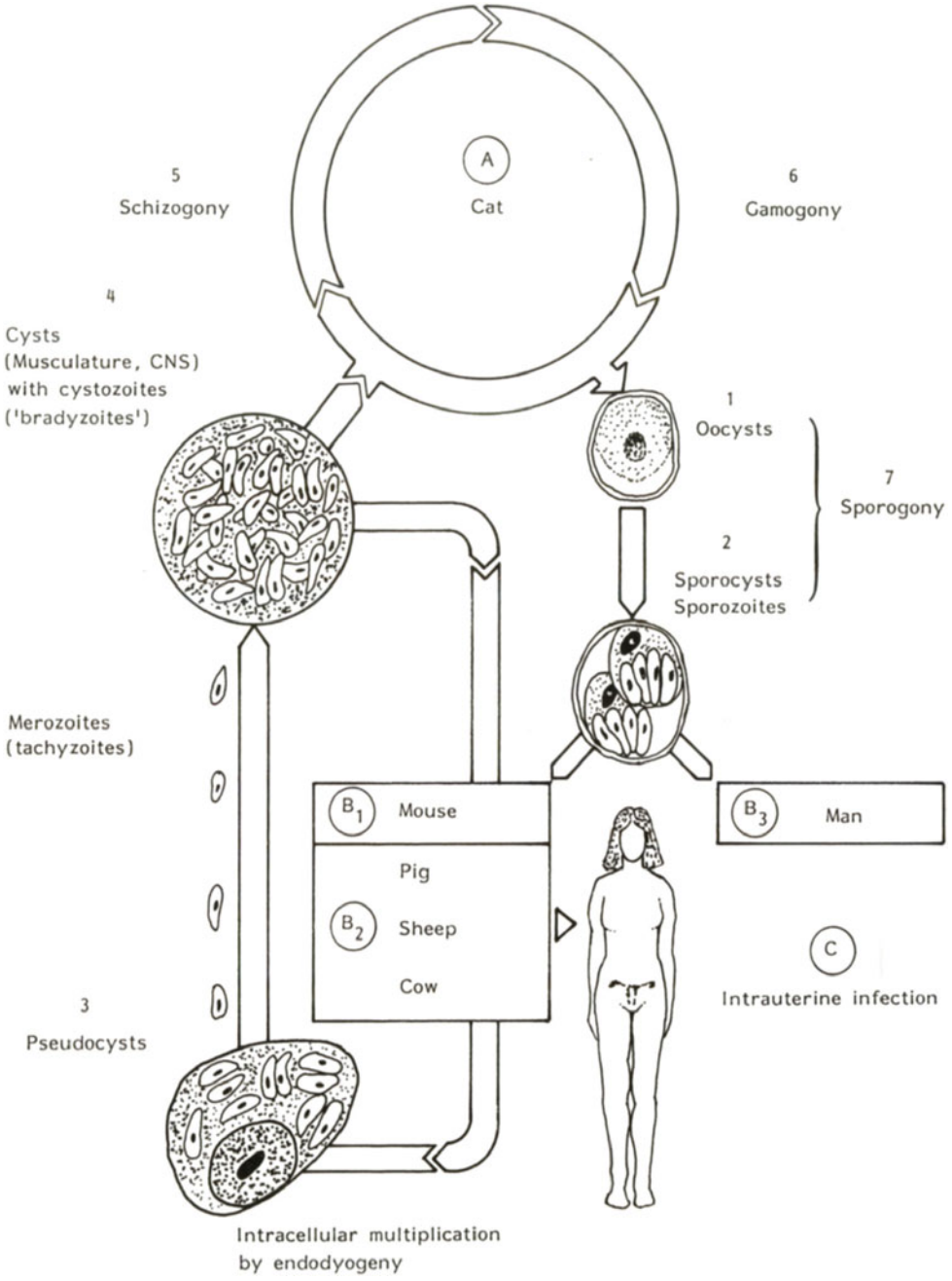


Fig. 11.6 Piringer–Kuchinka lymphadenitis. Cell nodules of variable sizes made up of histiocytes, reticular and epithelioid cells. Parasites of *Toxoplasma gondii* are found only occasionally in this kind of lesion. H&E

Table 3 The evolutionary cycle of *Toxoplasma gondii* (G. Piekarski, *Medical Parasitology*, 1989)



A Cats, the specific definitive hosts, excrete *Toxoplasma gondii* oocysts in their faeces (*1*). Almost all mammals (non-specific intermediate hosts) can be infected B by the sporocysts (each with four sporozoites, *2*).

B For example, mouse B_1, pig, sheep, cow B_2, and man B_3.
The parasites multiply intracellularly asexually (*acute phase*) (*3*) and form cysts (*chronic phase*) (*4*). These lead to renewed infection in meat eaters. After a cat is infected, the organisms first multiply asexually in the small intestinal epithelium (schizogony *5*). Thereafter, they form gamontes and gametes (gamogony, *6*). Development of oocysts and sporocysts, each containing four sporozoites (sporogony, *7*), occurs after fertilization.

C Intrauterine infection; congenital toxoplasmosis
Infection pathway in man B_3:
a. Through oral ingestion of sporulated oocysts (*2*)
b. Consumption of raw cyst-containing meat from sheep, pig or cow
c. Intrauterine transmission

nervous system are the favoured sites of lesions (tropism). The incubation period is 2–3 weeks. The fact that, all over the world, the prevalence of serologically positive reactions is so high and yet the number of clinical symptomless cases is so low indicates that there must be numerous asymptomatic or subclinical latent infections.

2. If the infected person (see 1) is a woman who is pregnant at the time of the first infection with *T. gondii*, she may miscarry or transmit the parasite through the placenta to the fetus with resulting congenital (better described as connatal or prenatal) toxoplasmosis[18–20]. In this situation, the *Toxoplasma gondii* infection seems to be more virulent than when the infection is through the digestive tract. Transplacentally-induced toxoplasmosis has also been detected in fetal pigs[21].

3. Indirect transmission, for example through flies or other insects contaminating food with oocysts, does occur but is in practice of minor importance.

Pathology

The musculature, central nervous system and eyes[22] and lymph nodes[23,24] are the favoured sites of involvement. In cases of disseminated toxoplasmosis, mostly the lungs, liver and adrenal glands are involved. In massive infections, all organs may show lesions.

Natural acquired infection. It is rarely that this infection is followed by an acute or chronic course and fatal cases are even more rare. Gross lesions are of an entirely non-specific nature. It seems appropriate to discuss in this context toxoplasmotic lymphadenitis, toxoplasmotic ophthalmitis and toxoplasmosis due to opportunistic infection.

Lymphadenitis. In adolescents and young adults, toxoplasmotic lymphadenitis appears predominantly in the rear cervical region, or manifests itself as generalized lymphadenopathy. The histological lesions, first described by Piringer-Kuchinka in 1958, have been confirmed by other observers[25]. They are characteristic but not specific for infection with *Toxoplasma gondii*.

Isolated multiple histiocytes or nodules of histiocytic cells, similar to reticular or epithelioid cells, are found. In the cell nodules, neither necrosis nor giant cells are observed (Fig. 11.6), and they do not have the features of true granulomas. Proliferation of this sort of cell and the formation of cell nodules are also seen in Hodgkin disease, infectious mononucleosis and in Whipple disease. The described histological lesions should be confirmed by a positive serological test. Parasites are rarely seen in the lymph node lesions of this aetiology; therefore, definitive aetiological diagnosis should be based on the histological lesions, together with positive results of the serological test.

Ophthalmitis. If toxoplasmotic aetiology has been established, there is a possibility that this is the late consequence of a neonatal infection, or it may be due to haematogenous infection. In toxoplasmotic ophthalmitis, the choroid may present non-specific or necrotizing inflammation, or, less frequently, granulomatous reactions. The nests or cysts of parasites, when present, may show necrobiotic lesions (Figs. 11.7–11.10).

In practice, there is always a problem, in a case of chorioretinitis, in differentiating between toxoplasmotic, histoplasmotic or tuberculous infections or diagnosing an immunological process of undetermined aetiology. It is estimated that a third or a quarter of all cases of chronic ophthalmitis is due to a toxoplasmotic infection (Figs. 11.11–11.13).

Opportunistic infection. Toxoplasmosis, when an infection of this sort, is an acute form of this disease which has, lately, been frequently observed in debilitated patients who have suffered for a long time from various types of chronic diseases. It has been encountered especially in patients treated with cortisone or by irradiation, in individuals with organ transplants, treated with immunosuppression, or in persons with congenital or acquired immunological defects, for instance AIDS. It is thought that this sort of toxoplasmosis did not previously exist but that *Toxoplasma gondii* 'made use of the opportunity' in the above-mentioned conditions to attack without having been present earlier in man as a saprophyte or a facultatively pathogenic micro-organism, as in *Pneumocystis carinii, Candida* sp., etc. This opportunistic infection by *T. gondii* presents neither particular nor specific pathological alterations. Furthermore, this toxoplasmosis is not limited to the central nervous system, as expressed in some books, but, on the contrary, is generalized (Figs. 11.14–11.21). The pathological lesions in the brain are not very extensive, as in neonatal toxoplasmosis (Fig. 11.22). Often, there are other opportunistic infections, for instance cytomegaly, simultaneously present in one patient.

Histologically, when routine tissue sections are reviewed, the parasites are spherical or ovoid in shape. They may be found inside or outside cysts or pseudocysts. We have not always been able to distinguish clearly between these two sorts of intracellular nests of parasites. In cysts, the organisms are densely packed and PAS-positive. The cyst membranes are PAS-positive also. Generally, they do not produce an inflammatory reaction. In pseudocysts, on the contrary, organisms are less numerous and their PAS-positivity is weak. *Toxoplasma* organisms are scarce in tissues because they perish rapidly when outside the nests and when in tissue zones where necrobiosis is occurring. This means that the search for single *Toxoplasma* organisms may be difficult and false negative reports are frequent.

The electron microscope is superior to the light microscope in the identification of *Toxoplasma* organisms: their intracellular localization, type of division, lack of budding and absence of a kinetoplast, are all clearly recognizable ultrastructurally.

Small yeast-like fungus cells of numerous species and *P. carinii* are differentiated from *Toxoplasma* by their Grocott-positivity. Protozoan organisms of other species must be differentiated from *Toxoplasma* on the grounds of their intra- or extracellular localization, presence of kinetoplasts and situation in determined organs or tissues.

Tissue reaction does not show either typical or specific features. Circumscribed necrotic foci, common in toxoplasmotic lesions, may also be observed in other acute infections with massive generalization. In the central nervous system, the parasites are found in necrotic lesions, in cell nodules or situated in areas of undamaged parenchyma. There are numerous coagulative and colliquative necrotic foci. In the periphery of these lesions, proliferation of capillaries and a 'status spongiosus' may be seen. Also, accumulation of foamy cells may be noted.

The glial reaction is characteristic: it consists of the presence of gemistocytic forms of astroglia, some glial nodules and 'naked' astroglial nuclei. The latter mimic Alzheimer nuclei type II, and confusion may also occur with so-called liver-glia seen in the so-called hepatogenic encephalopathy.

Around the small blood vessels are found macrophages (lipophages) and focal infiltrates of scarce lymphocytes and monocytes (Figs. 11.23–11.32). It is not clear whether the necrotic foci are due to damage directly produced by the parasites or whether they are the result of circulatory disorders with hypo- or anoxaemia. The necrotic foci in the brain later become scars and calcification may also occur.

Intrauterine infection. In pregnant women, *Toxoplasma gondii* reaches the uterus after primary infection by haematogenous dissemination and produces placentitis. This does not occur, apparently, in women with latent chronic toxoplasmosis, infected before the beginning of pregnancy. If infection occurs during the first few months of pregnancy, a miscarriage may be the consequence of the placentitis. However, the frequency of miscarriages due to toxoplasmotic infections is lower than was earlier assumed. Only a minimal percentage of all miscarriages can be attributed to infection by *Toxoplasma gondii*. Furthermore, the toxoplasmotic infection has nothing to do with so-called habitual, multiple or repeated miscarriages, as was believed for many years. The inflammatory reaction in the placenta is neither marked nor characteristic, and the parasites are difficult to recognize in the infected placenta (Figs. 11.33–11.35). We, ourselves

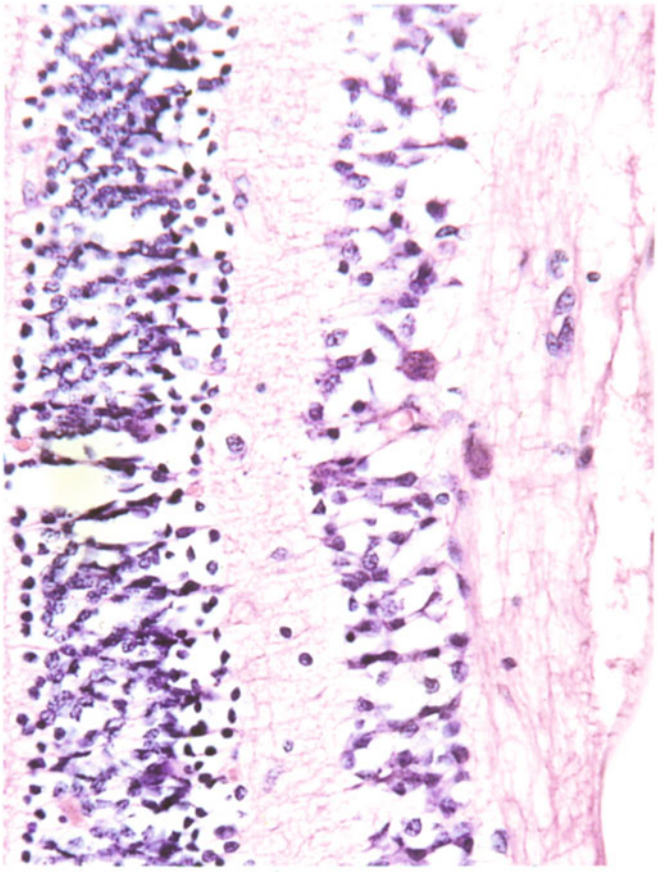


Fig. 11.7 Toxoplasmic chorio-retinitis. Two cysts are seen without inflammatory reaction in this field. H&E

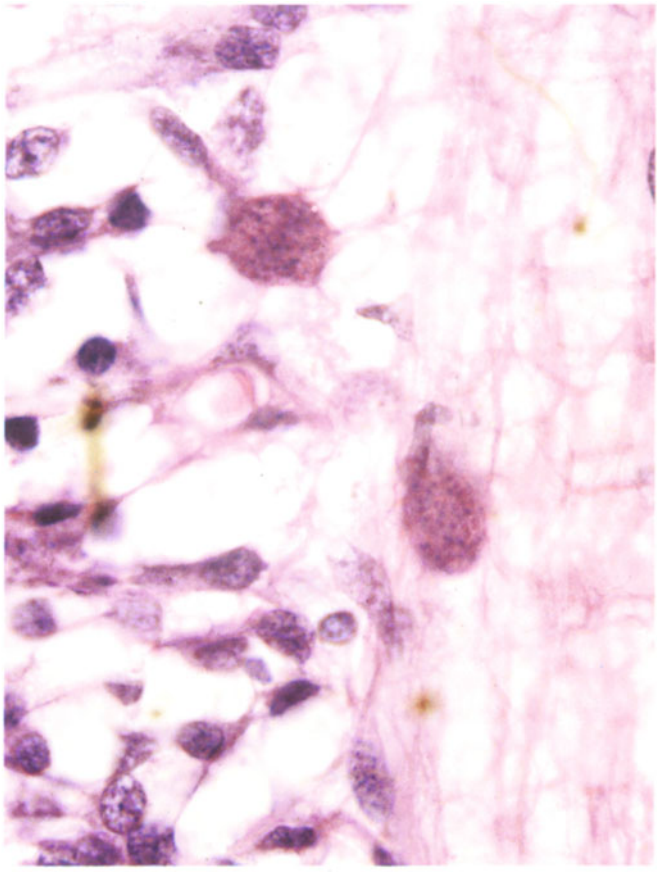


Fig. 11.8 Higher magnification of Fig. 11.7

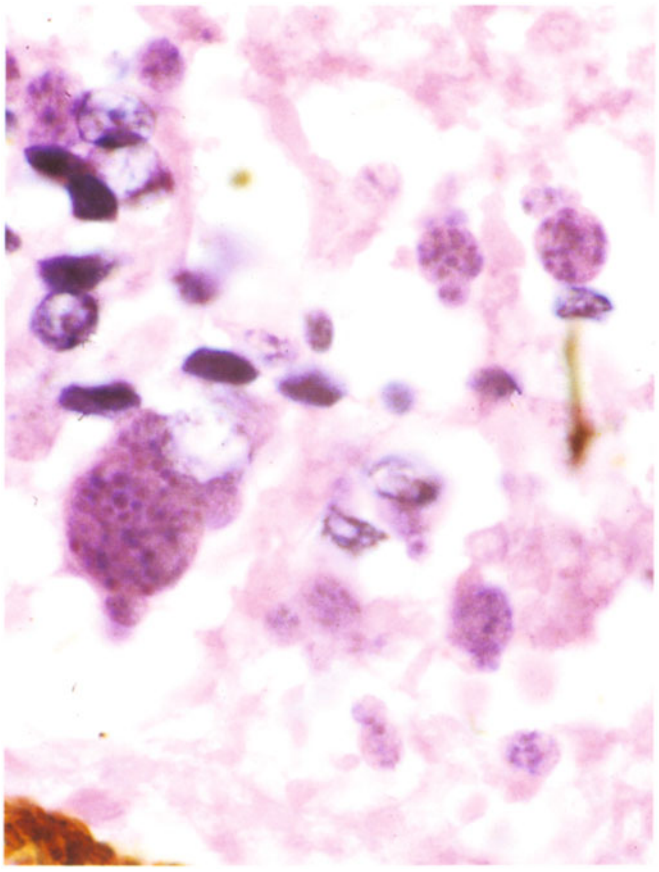


Fig. 11.9 Toxoplasmotic chorio-retinitis with necrobiosis and several parasitic cysts of variable sizes. H&E

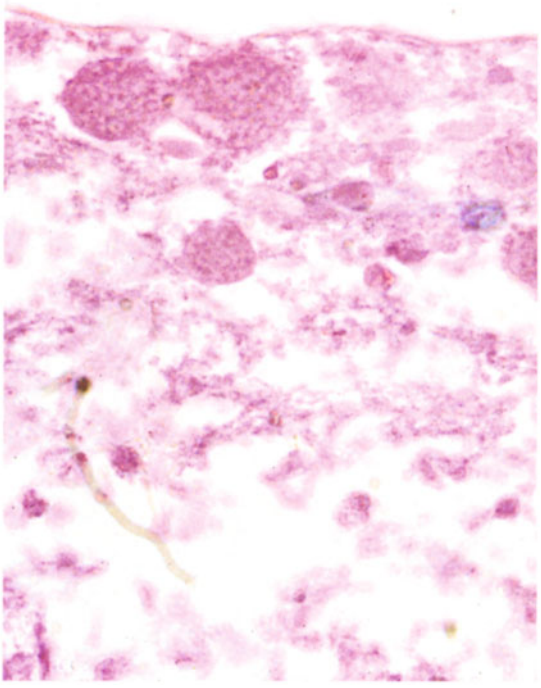


Fig. 11.10 Toxoplasmotic chorio-retinitis with marked necrobiosis of cysts or pseudocysts which are hardly recognizable as such. H&E

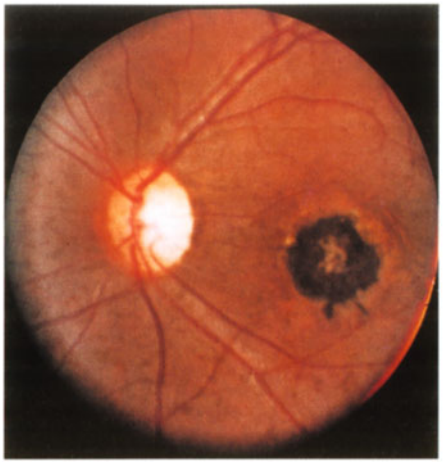


Fig. 11.11 Toxoplasmotic ophthalmitis. Fundus of the eye with an isolated, relatively fresh alteration and a superficial central defect

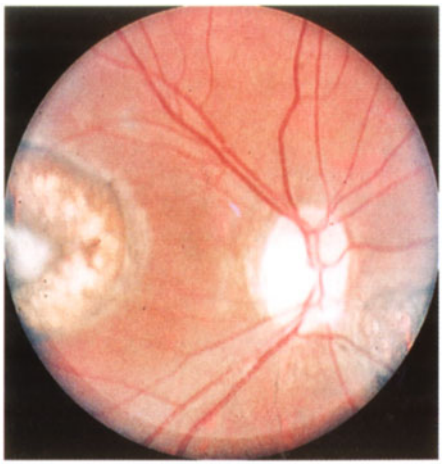


Fig. 11.12 Toxoplasmotic ophthalmitis. Fundus of the eye with an advanced lesion showing a necrosis with a well-defined central ulcer

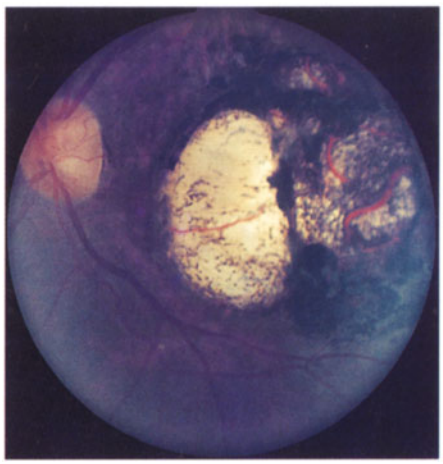


Fig. 11.13 Toxoplasmic ophthalmitis. Fundus of the eye with an extensive chronic lesion. Hyperaemia indicates active inflammation

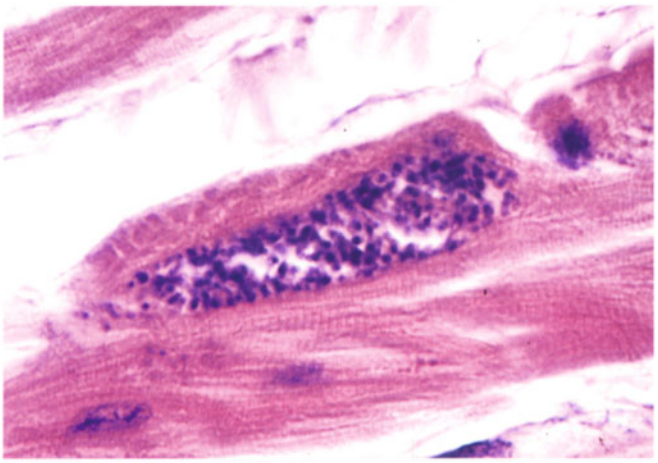


Fig. 11.14 Nest of toxoplasms in a myocardial fibre without inflammation in the vicinity. Originally, this nest was confused with amastigotes of *Trypanosoma cruzi* forming a pseudocyst. H&E

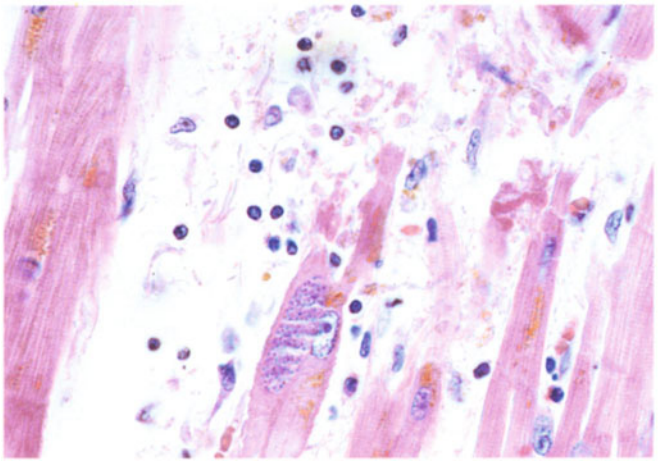


Fig. 11.15 Focal toxoplasmotic myocarditis in a case of generalized toxoplasmosis. The nest of toxoplasms is faintly visible. H&E

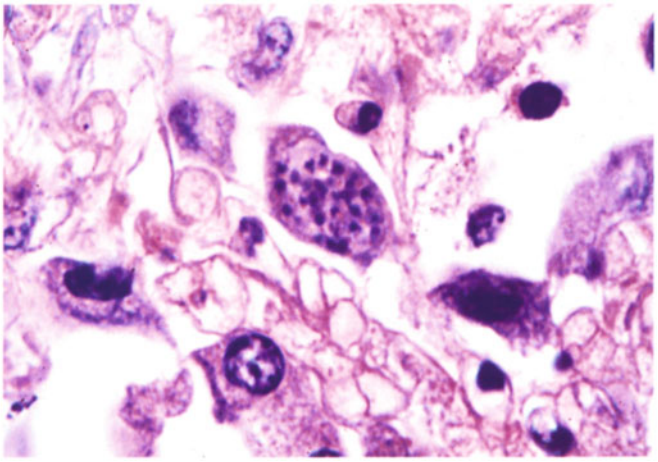


Fig. 11.16 Toxoplasmotic cyst or pseudocyst in lung section. H&E

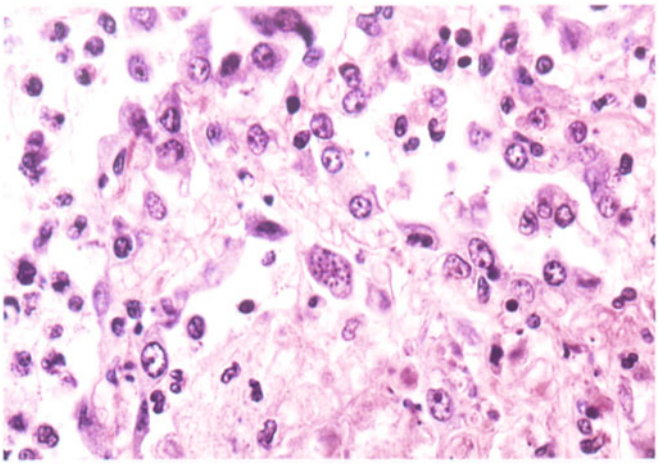


Fig. 11.17 Toxoplasmotic pulmonary lesion. Necrobiosis and acute inflammation are seen together with a small parasitic cyst. H&E

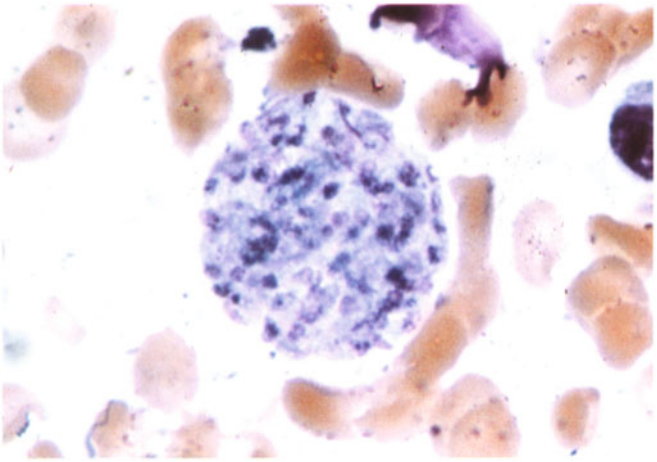


Fig. 11.18 Cyst with *Toxoplasma gondii* organisms in the pleural fluid of a patient with an acquired, disseminated and fatal toxoplasmosis. H&E

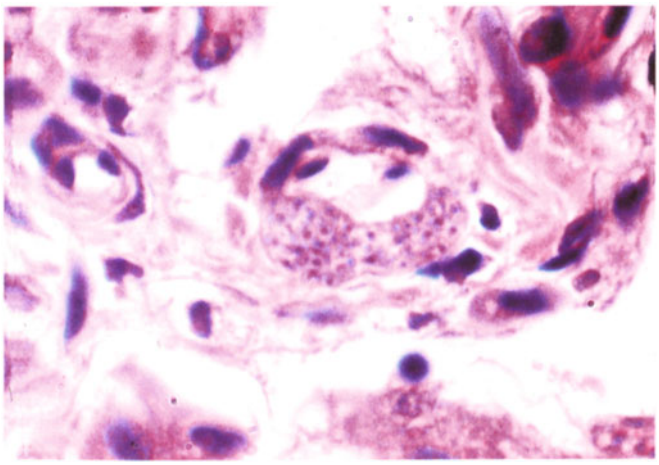


Fig. 11.19 Small toxoplasmotic cyst or pseudocyst in a muscular fibre or endothelial cell of a small blood vessel in the portal space of the liver in a case of generalized toxoplasmosis. H&E

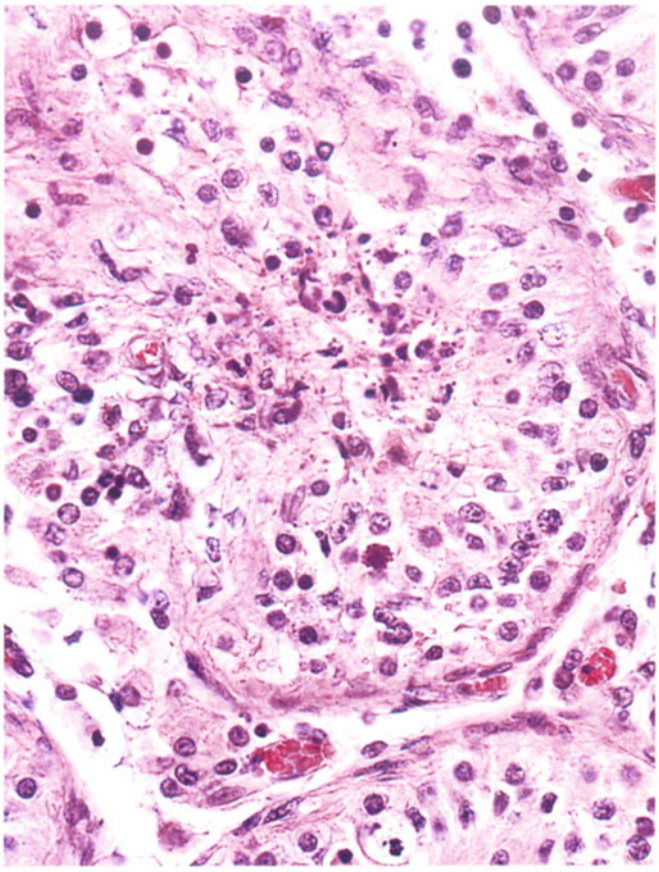


Fig. 11.20 Toxoplasmotic orchitis with necrobiotic changes at a seminiferous tubule and interstitial inflammation. H&E

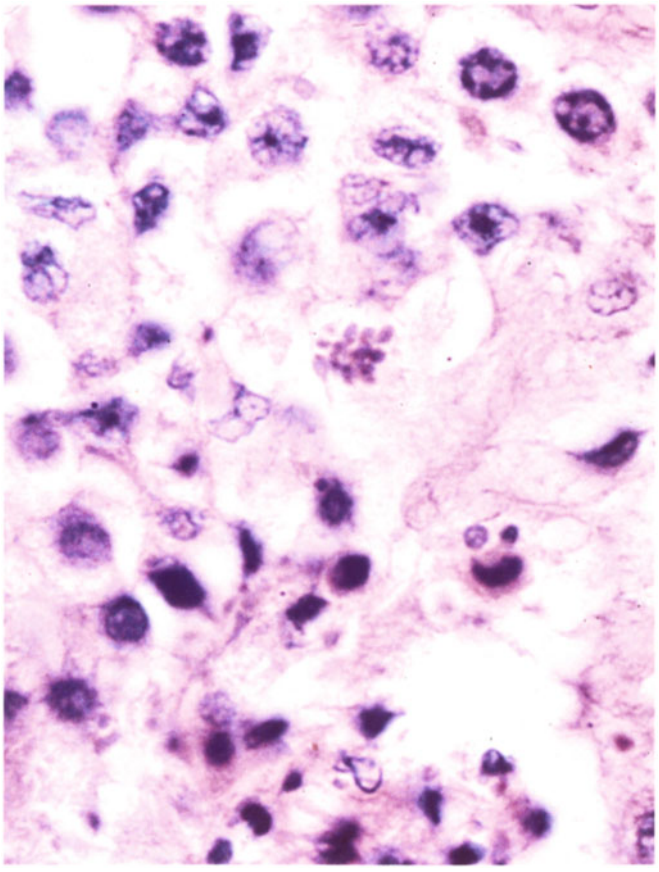


Fig. 11.21 Higher magnification of Fig. 11.20. A cluster of toxoplasms may be discerned. H&E

Fig. 11.22 Circumscribed necrotic focus in the medulla oblongata in a case of neonatal toxoplasmosis

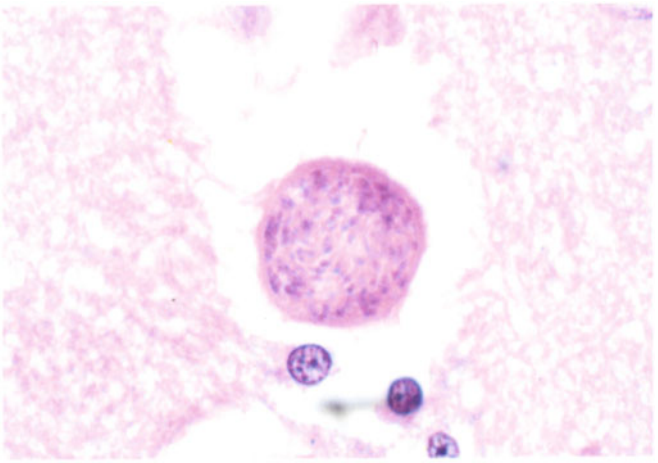


Fig. 11.23 Toxoplasmotic cyst or pseudocyst without inflammatory reaction in the brain. H&E

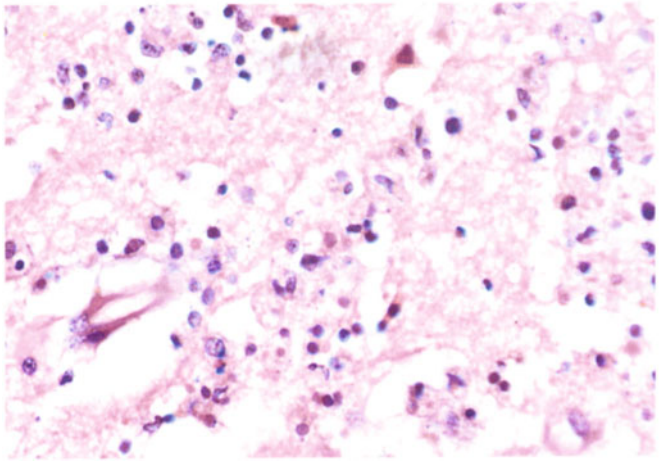


Fig. 11.24 Necrobiotic lesions and inflammation in toxoplasmotic encephalitis. H&E

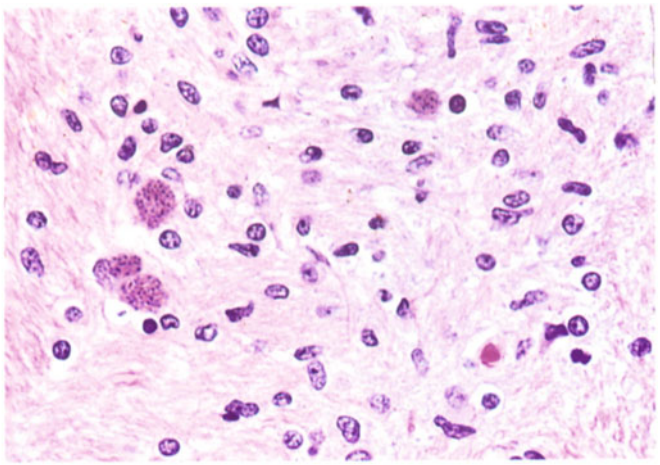


Fig. 11.25 Cell nodule in the brain with several toxoplasmotic cysts or pseudocysts visible in this field. H&E

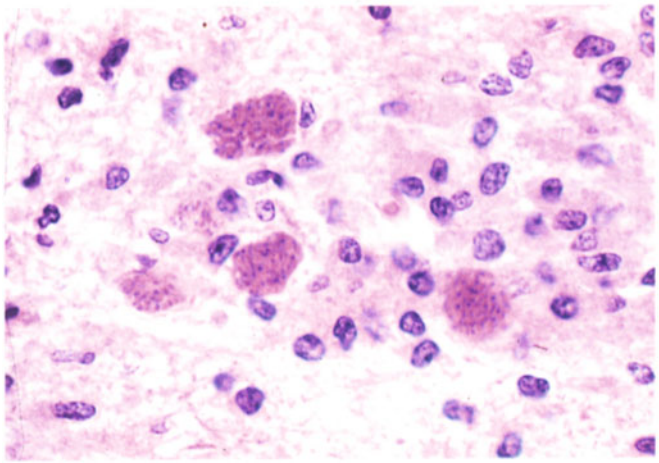


Fig. 11.26 Toxoplasmotic cysts or pseudocysts in cellular infiltrates of the brain with necrobiotic lesions. H&E

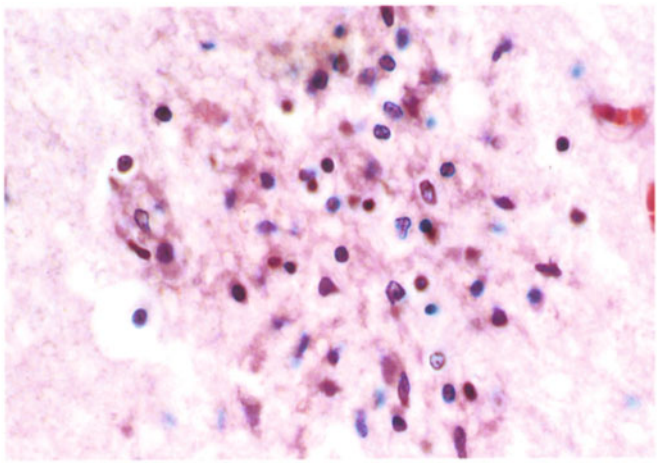


Fig. 11.27 Toxoplasmotic encephalitis with cell nodule not showing parasites. H&E

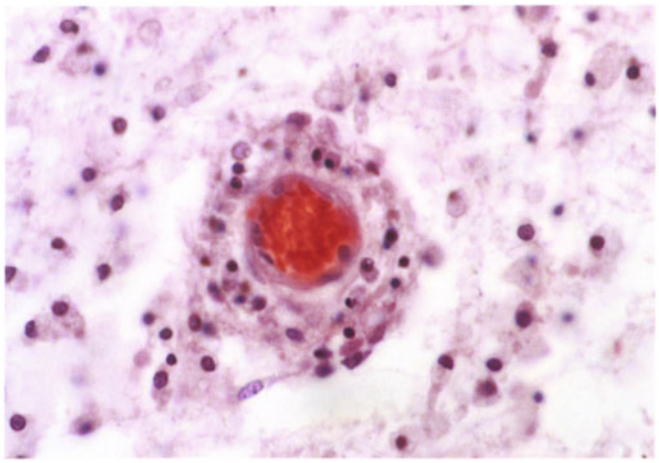


Fig. 11.28 Perivascular infiltrates in toxoplasmotic encephalitis. Parasites are not seen in this field. H&E

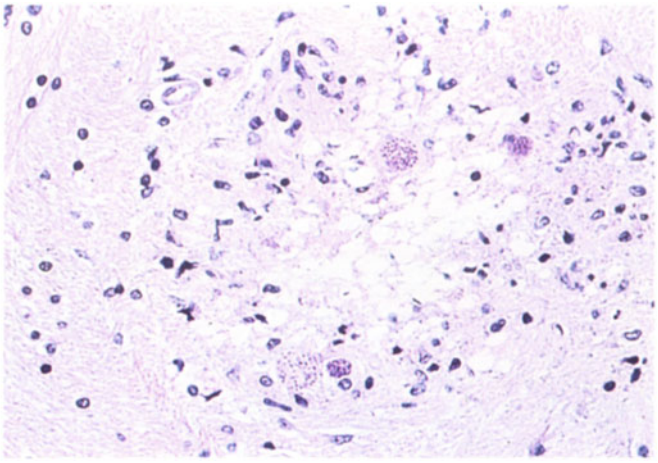


Fig. 11.29 Brain with focal necrobiosis and cell infiltrates. Some toxoplasmotic cysts or pseudocysts faintly visible. H&E

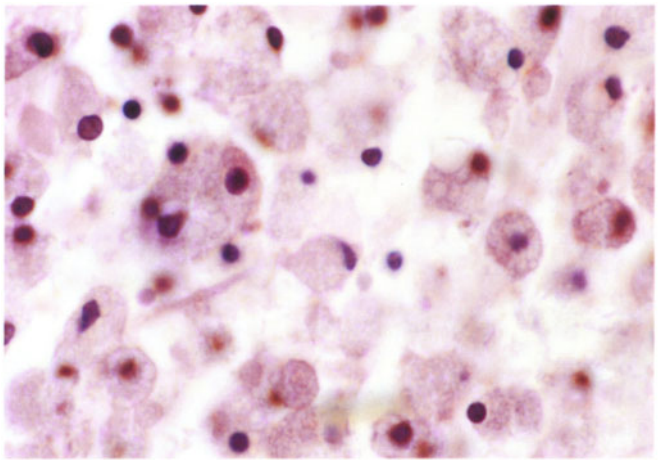


Fig. 11.30 Toxoplasmotic encephalitis with numerous foamy histiocytes in this field. Parasites are not seen in this area. H&E

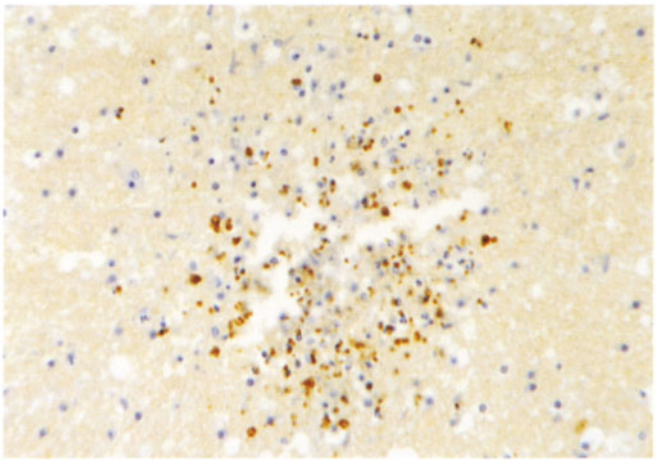


Fig. 11.31 Toxoplasmotic encephalitis. The brownish particles indicate antigen–antibody reaction. Immunoperoxidase

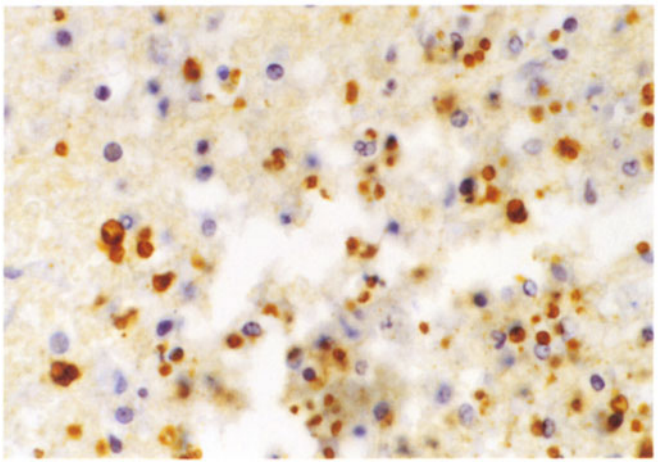


Fig. 11.32 Higher magnification of features in Fig. 11.31

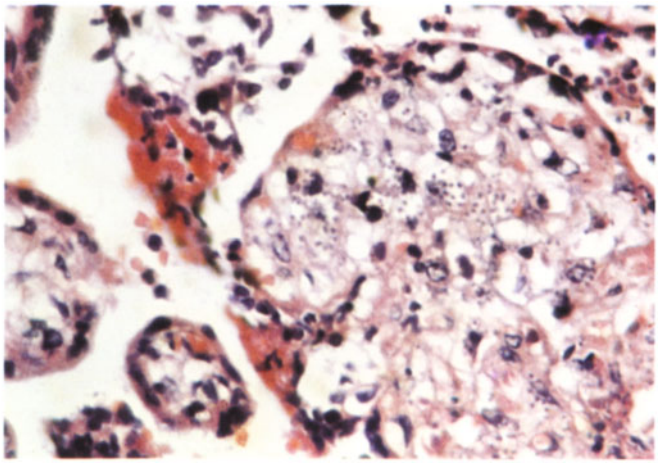


Fig. 11.33 Placenta with several nests of parasites. H&E

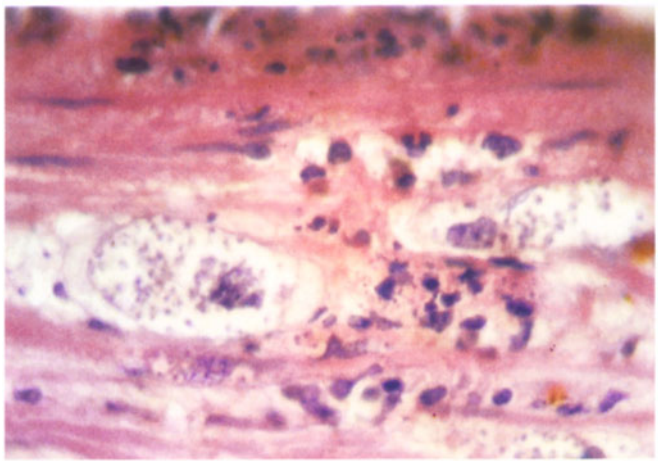


Fig. 11.34 Nest of parasites clearly visible in the placental tissue. H&E

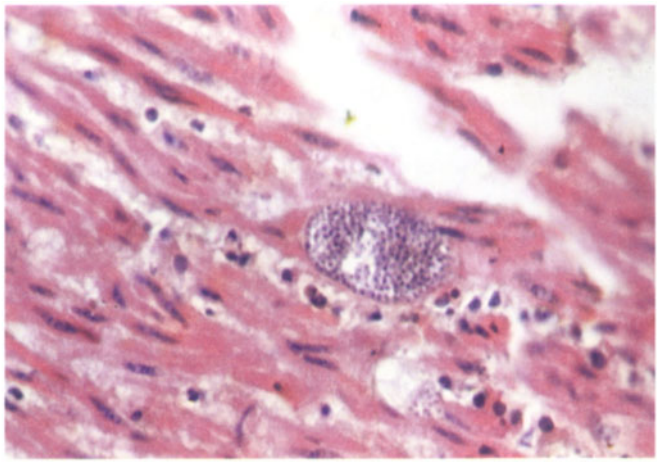


Fig. 11.35 Toxoplasmotic cyst in the umbilical cord. H&E

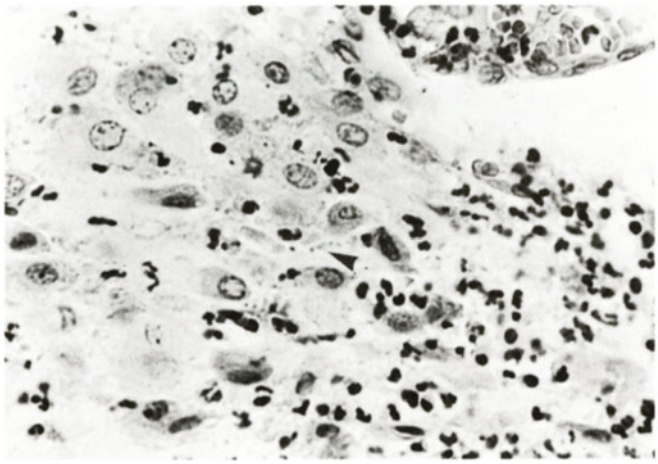


Fig. 11.36 Toxoplasmotic placentitis. The small granular elements in the decidua (arrow) were interpreted by experts as *Toxoplasma gondii* organisms. H&E

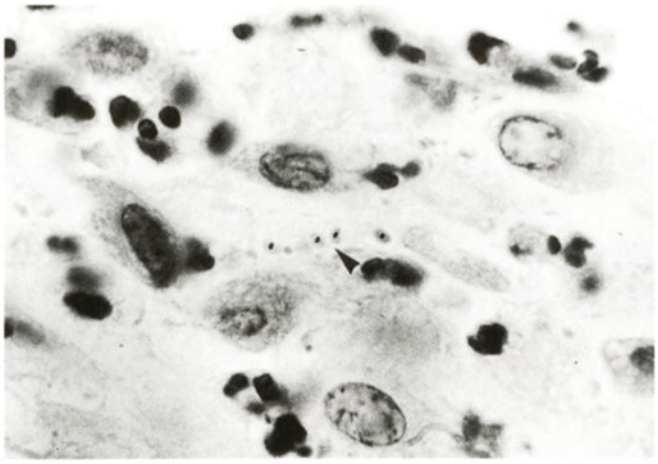


Fig. 11.37 Higher magnification of Fig. 11.36. The toxoplasms are marked with dots. H&E

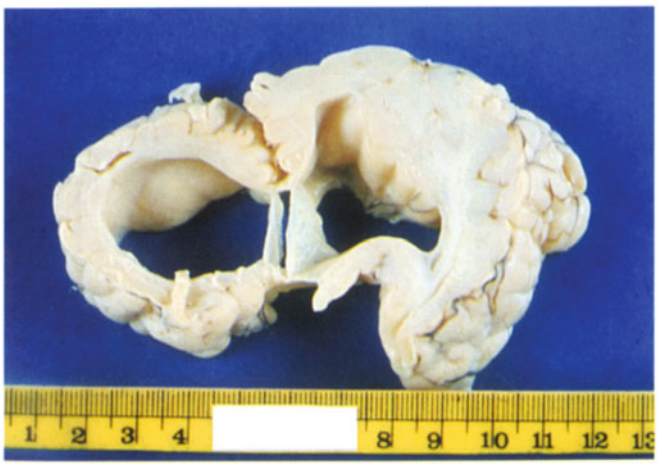


Fig. 11.38 Marked hydrocephalus with cortical atrophy in case of neonatal infection with *Toxoplasma gondii*

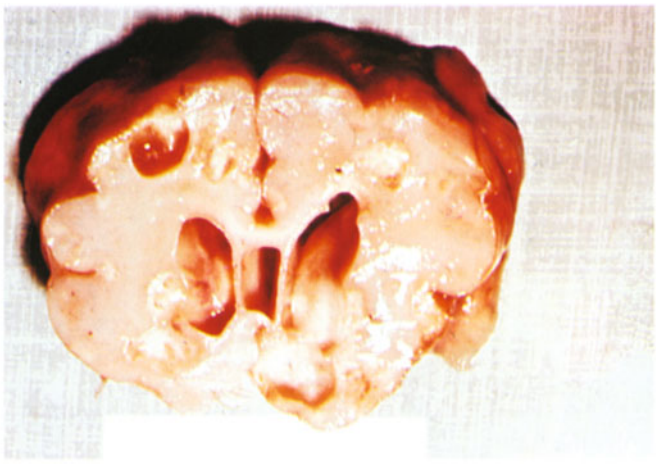


Fig. 11.39 Cut surface of brain with marked oedema, cavities and internal hydrocephalus in case of neonatal toxoplasmosis

were not able to detect parasites in the tissue sections of our cases, although expert parasitologists could demonstrate the micro-organisms in the same cases (Figs. 11.36 and 11.37).

If the toxplasmotic infection occurs during the second three months of pregnancy, premature or still birth may be provoked.

Finally, when infection with *T. gondii* takes place in the last stage of pregnancy, pathological disorders in the pregnancy are almost never seen; birth takes place normally and the fetus also looks normal. However, neonatal toxoplasmosis symptoms may manifest themselves some weeks or months later in a new-born baby, who was born apparently normal and healthy. Cerebral disorders appear, such as fever accompanied by convulsions, states of excitement or lethargy and other signs typical of encephalitis. The majority of the new-born babies with toxoplasmosis die in early infancy. In the autopsies, generalized lesions with parasites are found in the lungs, myocardium, liver and adrenal glands; these have provoked interstitial pneumonia, myocarditis, hepatitis and the formation of necrotic foci in the adrenals. The most significant lesions, however, are always found in the central nervous system. Here, extensive areas of softening, cyst-like lesions and often pronounced internal hydrocephalus are seen. These types of lesions had already been described by Virchow in the last century and were called 'brains resembling Swiss cheese'. Their aetiology, obviously, was unknown at that time (Figs. 11.38 and 11.39).

Under the microscope, areas of softening in different stages of evolution, cellular infiltrates of variable extensions, glial nodules, calcification, vasculitis and thrombotic processes can be found in the brain. Generally, parasites are scarce in this type of lesion. It has not yet been elucidated whether toxoplasmotic chorioretinitis has its origin in this sort of congenital infection or of some other. The percentage of infants with congenital, connatal or neonatal toxoplasmosis who survive is unknown. Also, it would be of interest to know what proportion of all cases of blindness, hydrocephalus, microcephalus, feeblemindedness, imbecility and other cerebral damage is caused by toxoplasmotic infection.

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12. BABESIOSIS

Introduction

Only isolated cases have been reported in Europe since 1957[1], but, today, human infections of this protozoan disease, which is transmitted by ticks, are increasingly observed and can no longer be considered rare. Cases have been found in Yugoslavia, France, USSR, Ireland, USA and Mexico[2–5], but, to our knowledge, this protozoan infection has, so far, not been found in Venezuela.

Natural *Babesia* infections are well known in cattle and rodents. In the former, *Babesia bovis* and *Babesia divergens* are noted and produce the so-called 'bovine malaria' or piroplasmosis[6–8] in tropical countries. *Theileria* species are similar to *Babesia* spp. and may cause the same disease. In rodents, *Babesia microti* leads to a latent localized infection.

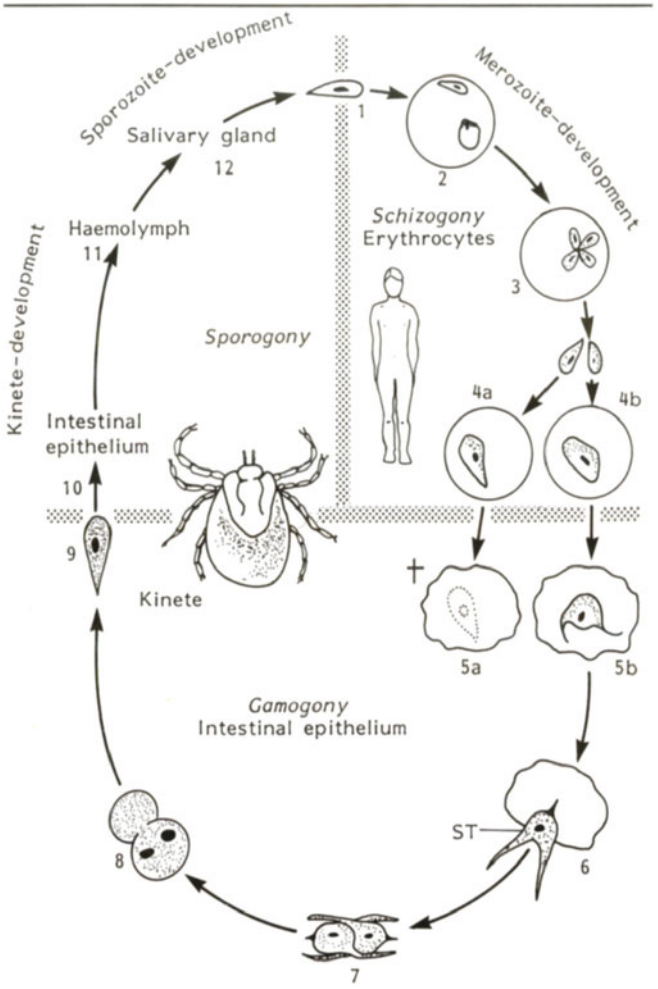
There are two clinical forms in man:

1. The first is mostly observed in Europe, generally takes a fatal course and is produced by *Babesia bovis* or *Babesia divergens*. It has been seen mostly in splenectomized patients.

2. The second form is found, predominantly, in the USA, mostly shows a latent form and is due to *Babesia microti*; the patients often have an intact spleen. Latent *Babesia* infection can lead to a severe, sometimes fatal, disease in the recipients of blood transfusions.

Clinical diagnosis is made by microscopic demonstration of the parasite in Giemsa-stained thin or thick blood films. Also intraperitoneal inoculation of hamsters with the blood of patients allows serological diagnosis of the disease to be made.

Table 4 Developmental cycle of *Babesia microti* (in part from Mehlhorn and Schein, 1984)



1. Sporozoite from tick saliva (*Ixodes* species)
2. Multiplication in erythrocyte by binary schizogony resulting in the formation of merozoites (also in lymphocytes?)
3. Erythrocyte containing characteristic Maltese cross stage
4a. Merozoite; disintegrating merozoite in tick intestine (5*a*)
5b–8. Gamogony with the formation of 'radiating' bodies (6) in the intestinal epithelium of the tick
9. A kinete develops from the zygote
10–12. Asexual multiplication of the kinetes in the tick; numerous sporozoites develop in the salivary gland

The parasite

Species of the genus *Babesia* belong to the Piroplasmia, a subclass of Sporozoa. Three are pathogenic for man: *Babesia bovis, Babesia divergens* and *Babesia microti*. The parasite reservoir hosts are small mammals, e.g. *Microtus* species.

Babesia spp. can easily be confused with the malaria parasite, *Plasmodium falciparum*. There are quite a few morphological similarities, but *Babesia* trophozoites in erythrocytes are more or less pear-shaped. On the other hand, they may also be ring-shaped, about 1 μm in diameter, as are the malaria trophozoites. After division, *Babesia* parasites present themselves inside erythrocytes in pairs or tetrades (also called 'Maltese cross') located marginally (*B. divergens*) or centrally (*B. microti*) within the erythrocyte. Since we do not possess human material we show babesiae in the blood of cattle (Fig. 12.1).

The developmental cycle of *Babesia microti* is seen in Table 4.

Pathogenesis

Vectors of these parasites are hard ticks, for example species of the genus *Ixodidae*, e.g. *Ixodes ricinus* or *Dermacentor reticulatus*. They ingest the intra-erythrocytic stages of *Babesia* when they feed on blood. Sexual development takes place in the tick, and later asexual multiplication and formation of sporozoites in the salivary glands. When the tick next feeds on blood, the parasites are transmitted to a new host[9]. Transmission of the parasites may occur, also, via blood transfusions[10].

The symptoms in the infected human host are similar to those of a recent malaria infection.

Pathology

There are no reports of gross or microscopic tissue lesions due to *Babesia* infections. Parasites of this sort can be found only in blood.

The *Babesia* organisms are similar to trophozoites of *Plasmodium falciparum* inside erythrocytes, as described in Section 16 on Malaria. In contrast to the malaria parasites, however, the *Babesia* organisms may be located in lymphocytes too. The following structural features have to be considered for the differential diagnosis. *Babesia* organisms do **not** have: schizonts, gamontes, the stippling of erythrocytes or pigment. In addition, malaria occurs in tropical or subtropical countries, while babesiosis is found worldwide and infection is acquired in regions where ticks live. Cases with symptoms of malaria resistant to therapy may be due to *Babesia* instead of *Plasmodium* infection. *Babesia* organisms may be confused with haemolysis-induced Pappenheimer bodies[11].

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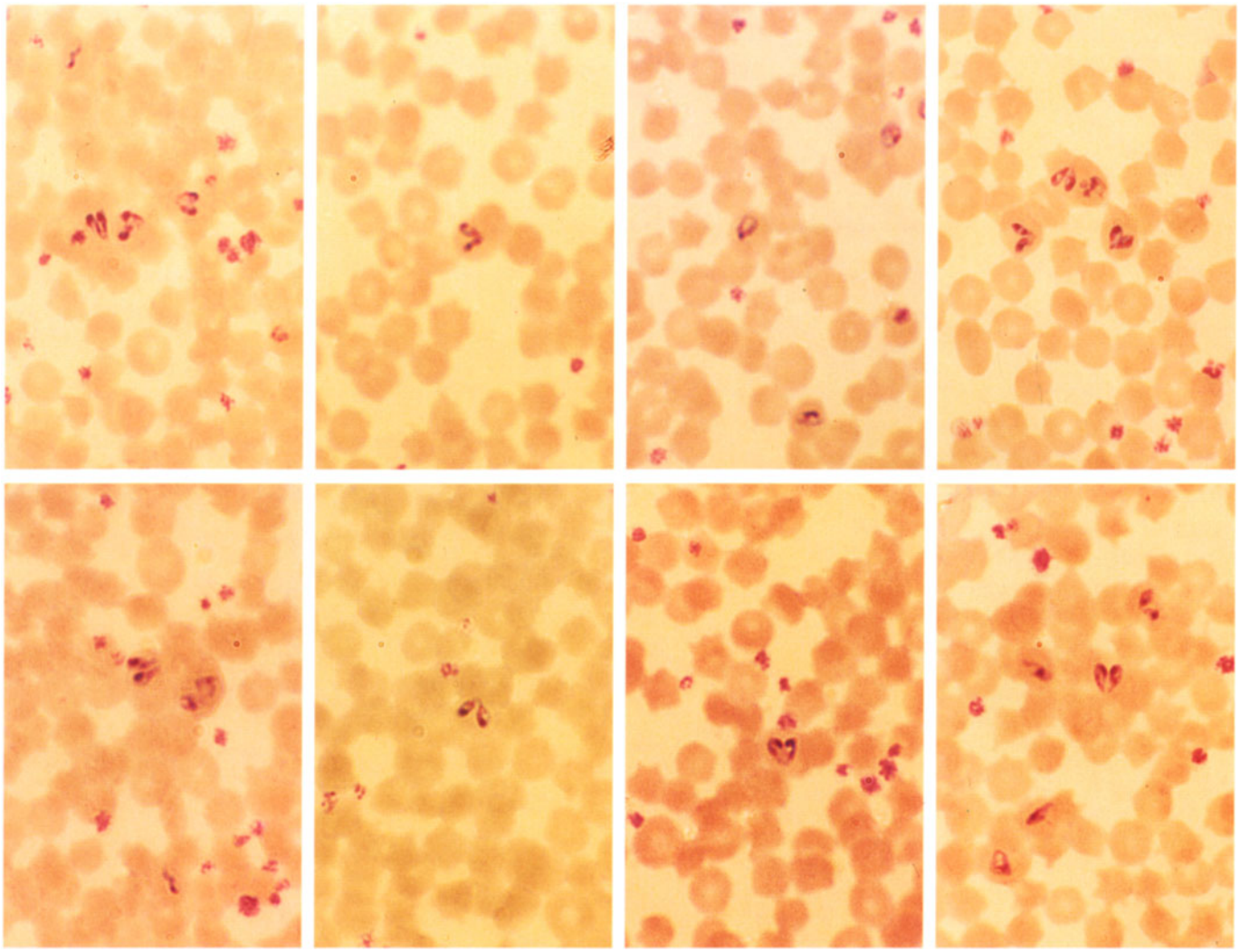

Fig. 12.1 Intraerythrocytic organisms of *Babesia bigemina* in thin blood smear of cattle. Giemsa

13. SARCOSPORIDIOSIS

Introduction

This parasitosis is one of the four important coccidian infections. The others are: **toxoplasmosis** (Section 11), the most significant from the clinical point of view; **isosporosis** (Section 14), today becoming more and more important in AIDS patients; and **coccidiosis**, of interest in veterinary medicine since the causal agents produce disease in poultry, rabbits and other lower animal species[1,2]. Practically, the latter does not cause disease in man and for this reason, it will be omitted here.

Lately, several species have been renamed, the nomenclature of parasites has been modified and details of the evolutionary cycle have been recognized[3,4], thus causing confusion for the non-parasitologists.

Sarcosporidiosis occurs worldwide wherever rare or insufficiently cooked beef or pork is consumed. It is common in North and Central Europe. In Venezuela, to our knowledge, infections of this sort have not been reported in man.

The infection of domestic animals and birds takes place via numerous differentiable species[5–8] (Figs. 13.1 and 13.2). All of these parasites go through an obligatory change of specific hosts. It is easy to infect rodents in the laboratory experimentally by giving them raw meat with cysts of *Sarcocystis* spp.

Clinically, it appears that infection with *Sarcocystis bovihominis* is mild. In contrast, infections with *Sarcocystis suihominis* may produce violent intestinal disorders. In Thailand, fatal intestinal infections have been reported[9].

Clinical diagnosis. The colourless and fragile sporocysts are not easy to detect during routine stool examinations for worms. Concentration procedures must be applied. In biopsies of the small bowel, sarcocysts should be looked for near the epithelium of the mucosa.

The parasite

Sarcocystis species belong to the group Coccidia which form with the *Plasmodia* the class Sporozoa. The species formerly known as *Isospora hominis* has now been named *Sarcocystis bovihominis* and *Sarcocystis suihominis*.

The species *Sarcocystis lindemanni* probably does not exist; it should not be considered a species specific for man. In a critical review by Beaver *et al.*[10], only a few human cases of this sort could be confirmed. The typical Miescher's tubules, known for almost 150 years, are observed frequently in the musculature of domestic animals and do not elicit an inflammatory reaction. *Sarcocystis bovicanis, S. suicanis* and *S. bovifelis* are not found in man.

Two *Sarcocystis* species can develop in man: *S. bovihominis* and *S. suihominis*. Their cycle of evolution is shown in Table 5.

Merozoites develop in the lamina propria of the intestinal mucosa into oocysts and sporocysts. Merozoites are banana-shaped intracellular parasites measuring 10–14 μm in length. Oocysts measure about 20 × 10 μm containing 4 sporozoites (about 14 × 8 μm) which later are released from the oocysts (Figs. 13.3–13.6).

Pathogenesis

About 5–10 days after eating parasite-containing raw meat, the first oocysts are excreted with the faeces. After perforation of the walls of the oocysts, sporocysts are released into the intestinal lumen. Oocysts and sporocysts are excreted over more than 6 weeks. The merozoites which derive from sporocysts penetrate into the lamina propria of the small intestine and cause an eosinophilic inflammation. This may be concluded from the reports of cases from Thailand and is inferable from the behaviour of the related species (*S. bovicanis* etc.) which infect only animals. In man, haematogenous dissemination of the parasites into organs other than the small intestine with formation of cysts has not been reported.

Pathology

In man, only involvement of the small intestine has been confirmed, although recently, involvement of skeletal muscles has also been reported in man[11,12]. In the animal host, beside the intestine, lesions have also been found in liver, kidney and brain with formation of whitish elongated foci, visible to the naked eye.

The enteritis found in man is present more often in the ileum than in the jejunum. It is characterized by a marked diffuse oedema of the submucosa and extensive infiltrates of eosinophilic granulocytes in mucosa and submucosa[9]. Oocysts with four sporozoites may be found in tissues. When more or fewer sporozoites are found in oocysts, this is due to cutting.

The cysts of sarcosporidians in lower animals are septate and show the elongated trophozoites of *Sarcocystis* spp.

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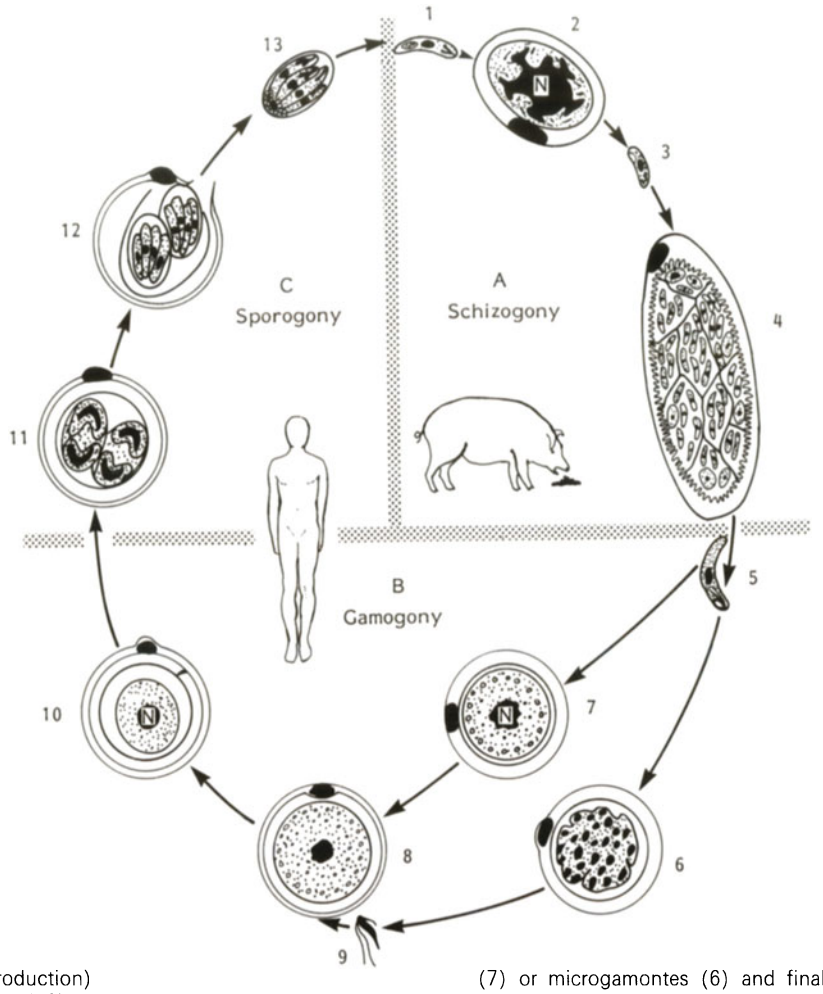
Table 5 Developmental cycle of *Sarcocystis suihominis* (Mehlhorn, 1980)



A. Schizogony (asexual reproduction)
Fully developed sporocysts (13) are ingested orally and reach the gastrointestinal canal; from them sporozoites are released (1), which multiply asexually in the endothelial cells of the liver, kidneys, lungs and other internal organs and form merozoites (2–5). This intracellular reproduction can repeat itself many times (5 → 2)
2. Schizont
3. Merozoite
4. Cysts in the musculature
} Stages of development in pigs
5. Merozoites from one cyst after consumption of infected muscle

B. Gamogony (sexual development)
Merozoites (5) develop in the lamina propria to macrogamontes (7) or microgamontes (6) and finally to macrogametes (8) or microgametes (9)

C. Sporogony
10. The oocyst develops from the zygote
11. Beginning of sporulation (still within the host cell)
12. Oocysts with two sporocysts: the oocyst wall splits open in the intestinal lumen
13. Free sporocysts containing four sporozoites (capable of infection)

14. ISOSPOROSIS

Introduction

Infection with *Isospora belli* does not generally produce severe disease except in those patients with AIDS. Isosporosis is found mostly in Asia and South America and also in the Mediterranean countries. It is rarely observed in regions with a temperate climate[1]. We do not know of any reports of this disease in Venezuela.

The reservoir hosts of *Isospora belli* are not well known at present. Experimentally, the gibbon may be infected with this parasite.

Frequently, infection with *Isospora belli* is symptomless, i.e. more than half of the infected persons are only carriers of this parasite. Symptoms of enterocolitis may be noted but severe and lasting damage is unlikely[2,3]. However, immunodeficient patients may present severe colitis.

In order to obtain a clinical diagnosis, concentration techniques of stools must be used. The uninucleate oocysts may be distinguished from sporocysts of Sarcosporidia.

The parasite

Isospora belli, today, is considered the sole causal agent of isosporosis. The species, *Isospora hominis*, has now been transferred to the Sarcosporidia, *S. bovihominis* and *S. suihominis*.

Only the oocysts of *Isospora belli* have been recognized until now. They are oval shaped, 20 × 30 μm in size and are pointed at one pole with a 'neck-like' constriction[4]. Soon, two sporocysts, each containing four sporozoites, develop from the oocysts (Figs. 14.1–14.3).

Pathogenesis

Transmission of *Isospora belli* to man occurs via the resistant oocysts or sporocysts without an intermediate host. The sporocysts liberate sporozoites which penetrate the intestinal mucosa. It is quite possible that *Isospora* in man behaves like the *Isospora* species in the dog and in

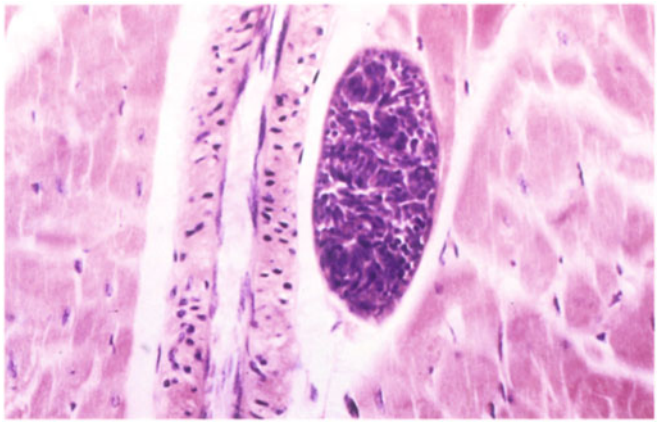


Fig. 13.1 Sarcosporidian cyst in myocardial fibre of cattle. H&E

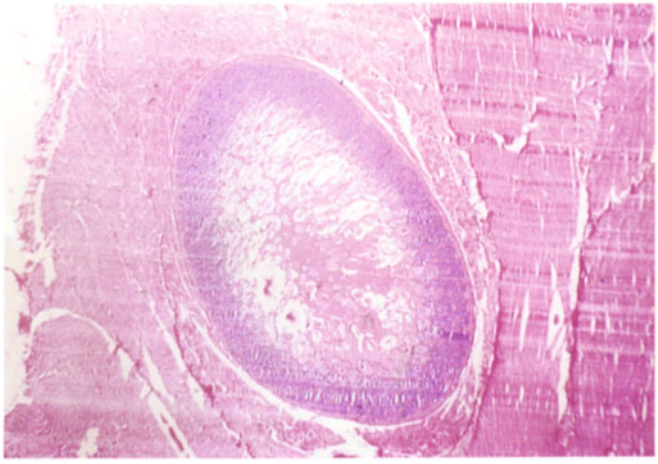


Fig. 13.2 *Sarcocystis* sp. cyst in the oesophageal musculature of buffalo from Thailand. H&E

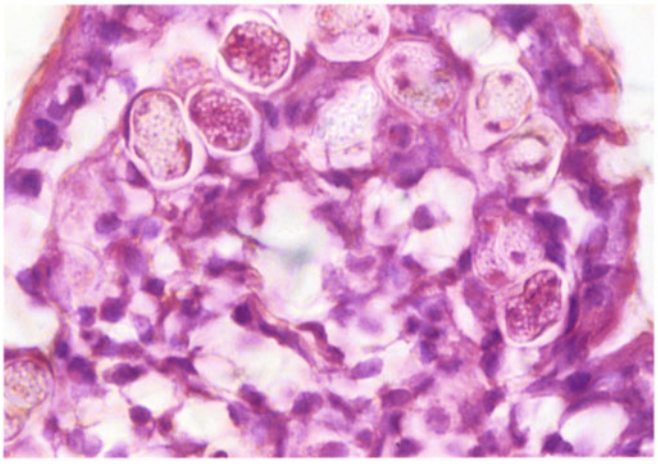


Fig. 13.3 Oocysts of *Sarcocystis* sp. in the small intestine of dog. H&E

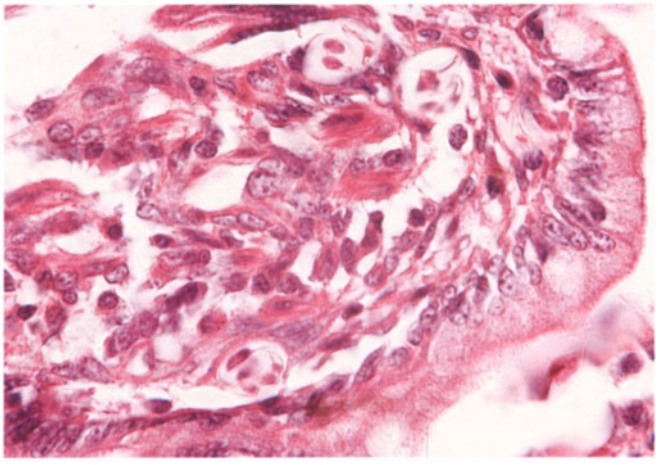


Fig. 13.4 Several oocysts of *Sarcocystis* sp. in the small intestine of a patient from Thailand. There was a marked eosinophilic enteritis in this case. H&E

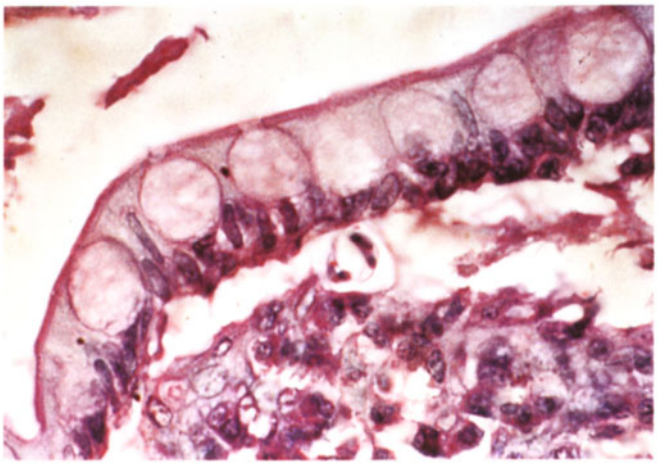


Fig. 13.5 Same case as in Fig. 13.4 with higher magnification. Oocysts contain 4 sporocysts which, however, do not show in every section. H&E

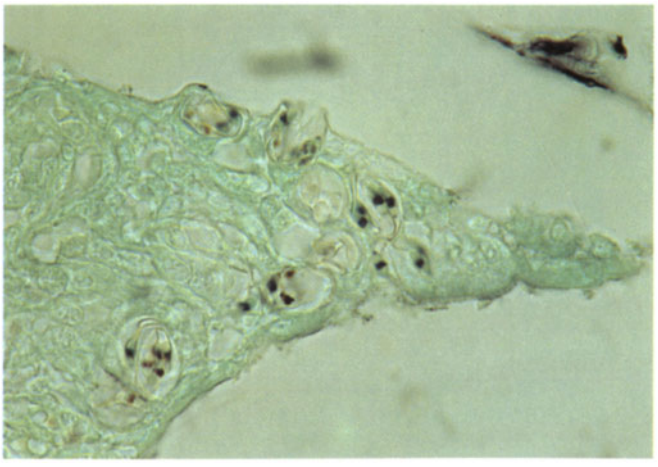


Fig. 13.6 Same case as Figs. 13.4 and 13.5. The oocysts with their sporocysts are marked also with this special staining method. Grocott

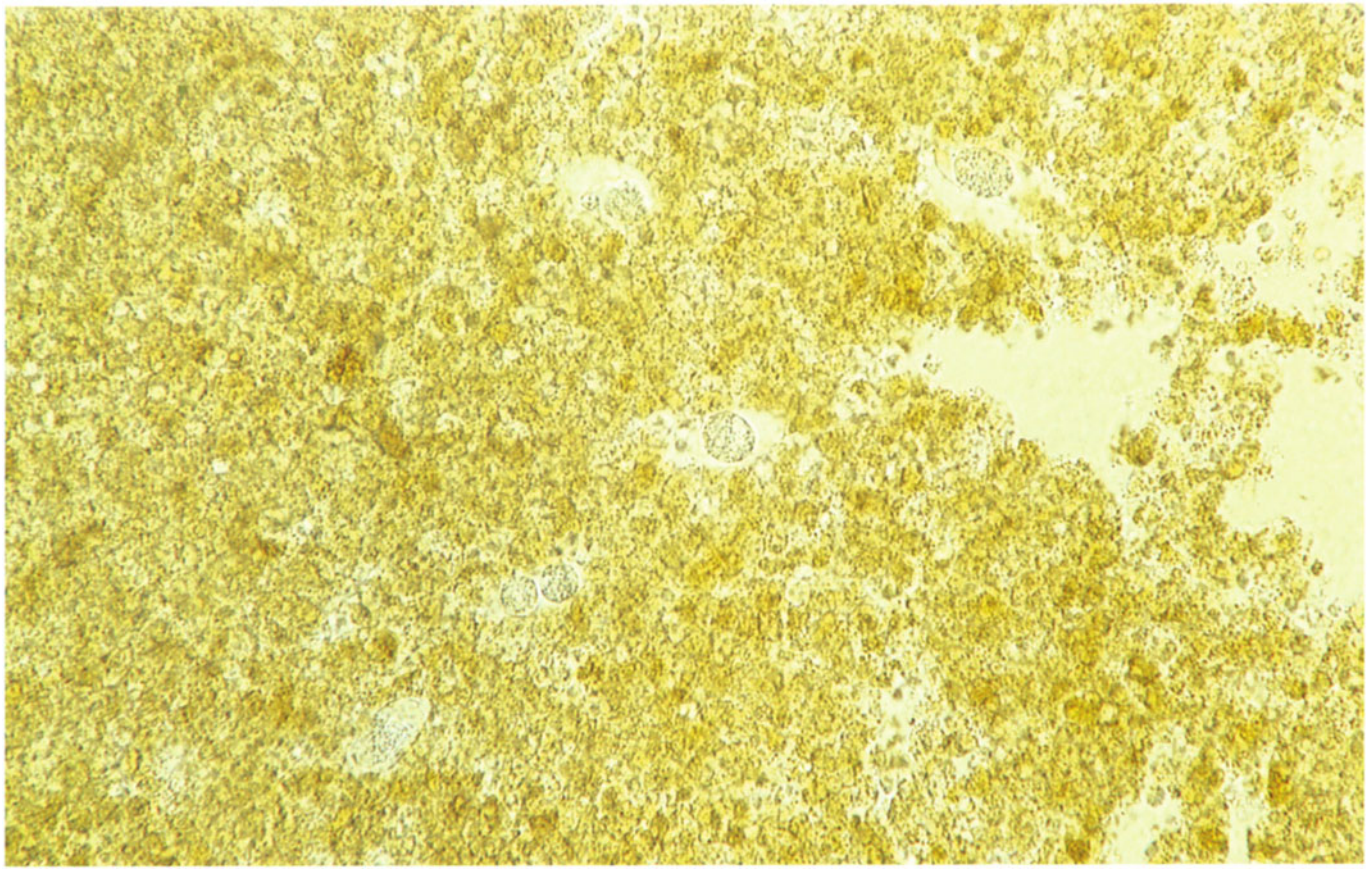


Fig. 14.1 Oocysts of *Isospora belli* in faeces at low power. Unstained fresh smear

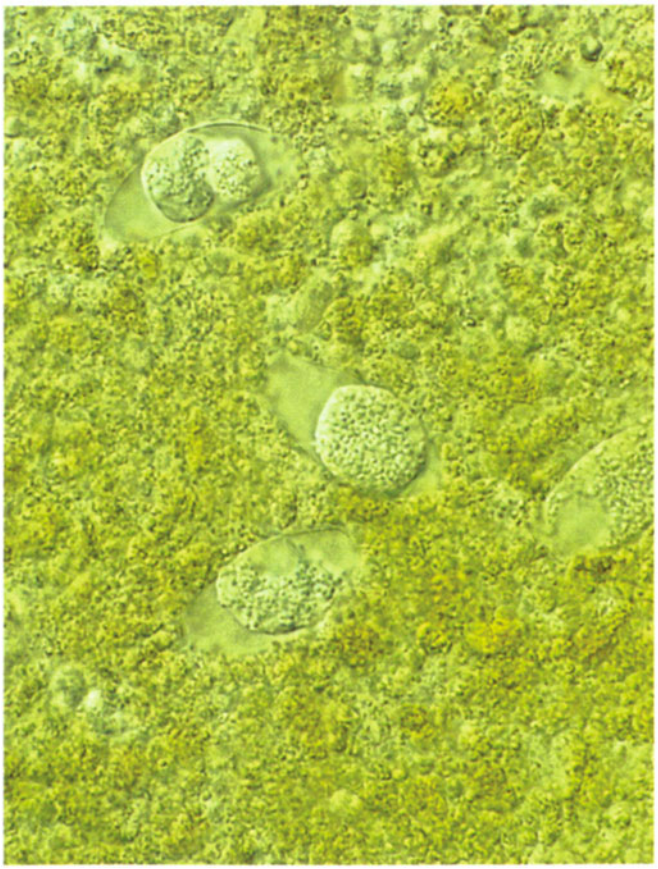


Fig. 14.2 The evolution starts with formation of 2 sporoblasts inside oocysts of *Isospora belli*. Unstained faecal smear

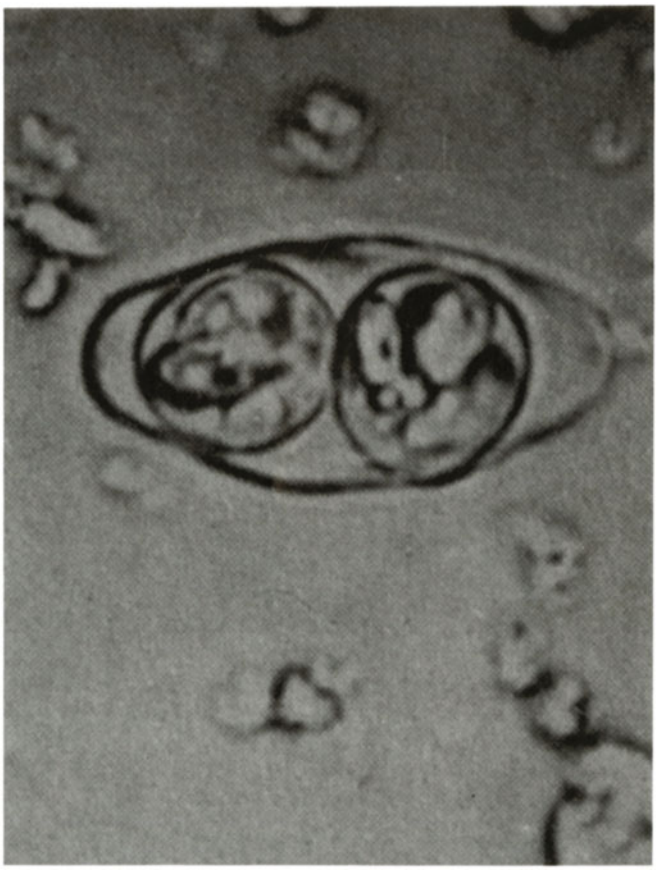


Fig. 14.3 Oocyst of *Isospora belli* with two sporoblasts. Unstained faecal smear

the cat. Minute microscopic superficial lesions in the intestinal mucosa may result[5,6].

Pathology

The ileum and caecum may be involved in infections with *Isospora belli*. Gross lesions and forms of *Isospora belli* in tissues have not yet been studied in man.

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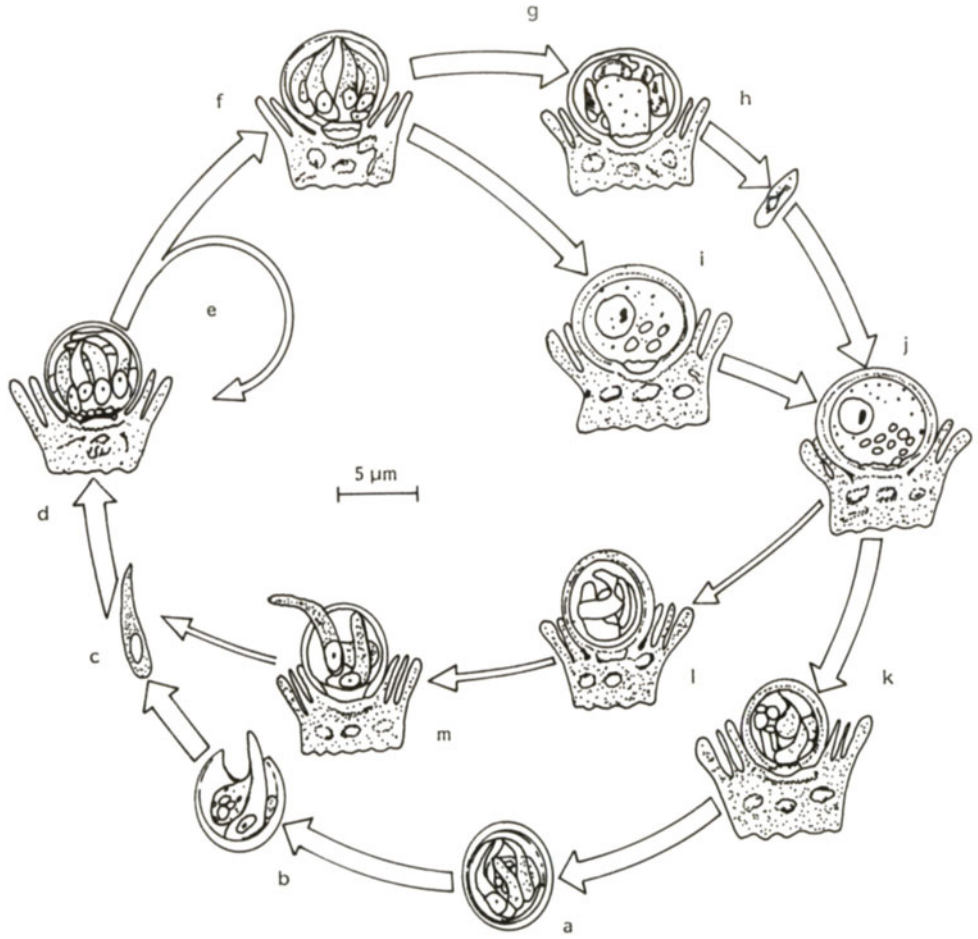
15. CRYPTOSPORIDIOSIS

Table 6 Developmental cycle of *Cryptosporidium* sp. (according to Current, 1985)



a. Sporozoite-containing oocysts from the faeces; b. excysting sporozoites; c. free sporozoite; d. schizont with 6–8 merozoites; e. new infection of intestinal cells; f. schizogony, which leads to micro- and macrogamontes (g, h, i) and a zygote (j) with a thick-walled oocyst (k). Thin-walled oocysts, which cause auto-infection can also be formed (l, m)

Introduction

Infection by cryptosporidians, a coccidian protozoan parasite, is a relatively 'new' human disease. The parasite was first described in mice at the beginning of this century, and has been reported in man only since 1976. Because of an increased veterinary interest and also the increasing importance of *Cryptosporidium* spp. as an opportunistic parasite, for instance in AIDS patients[1–3], there have been numerous publications on these parasites in the medical literature of recent years[4–6].

The distribution of this organism is worldwide. There is a high risk of infection for people in rural areas. The infection and symptomatic disease is well known in Venezuela[7].

The source of human infections are, in addition to pets, cattle, pigs, sheep and goats, also guinea pigs, mice, rats, as well as birds, snakes and fish, all of which may be carriers of cryptosporidians[8–17]. Several laboratory animal species may be used for experimental work[18–21].

In man, infection may be silent, but the symptomatic disease shows a picture of gastroenteritis with diarrhoea and watery stools which may continue for 3–14 days. The considerable loss of water and cramp-like abdominal pain may be followed by obstipation. Sometimes, weight

loss, vomiting and low fever may be associated. After about 3 weeks, the symptoms disappear or the condition becomes chronic. In AIDS patients, the course is more severe and can lead to death.

For clinical diagnosis, oocysts of *Cryptosporidium* sp. may be found in alcohol-fixed stool smears or in sections of cell blocks stained with Giemsa. The small oocysts are not easy to detect with this staining technique. We prefer to stain the oocysts with the Ziehl–Neelsen method.

The parasite

The parasite has been named *Cryptosporidium* sp. and belongs to the class of Sporozoa. It goes through a complicated cycle of evolution which cannot be followed using the light microscope; see Table 6. The thick-walled oocysts which contain four sporozoites are excreted and spread the infection transmitting it to a new host.

The oocysts we see in smears of watery stools are spherical in shape and measure 4–7 or 3–6 μm in diameter. They may remain unstained but, with the Ziehl–Neelsen method, they stain positive. Definite inner structures in these oocysts cannot be recognized in routinely examined material. The small banana-shaped sporozoites measure from 5–6 μm.

Pathogenesis

Infections occur by faecal contamination, i.e. by ingestion of oocysts with food, water etc. Transmission takes place directly from man, or domestic animal, to man without an intermediate host; predominantly, the parasites are passed from domestic animals to man. Incubation time is 3–12 days, the prepatent period about 1 week and excretion of oocysts occurs over 2–3 weeks. The sporozoites coming from the oocysts attach themselves to the surface of the gastrointestinal mucosa. This can be observed only with the electron microscope, and the damage produced cannot be confirmed with the light microscope. The sporozoites do not invade tissues.

Pathology

The stomach and the lower parts of jejunum or ileum are involved. Apparently, lung and biliary infections may occur, but only a few cases of this sort have been described[22]. Gross lesions of cryptosporidiosis are not known. The attachment of sporozoites to the surface of the mucosae and the different evolutionary phases of the parasite on the surface may be studied ultrastructurally only. The microvilli of the intestinal epithelium may be destroyed but penetration of the parasite into tissues does not occur.

The oocysts in the gastrointestinal lumen stain easily with the Giemsa and Ziehl–Neelsen methods (Figs. 15.1–15.3). They are Grocott-negative and may thus be differentiated from small yeasts. They are said to be PAS-positive. In histological sections, they are not always Ziehl–Neelsen-positive (Figs. 15.4–15.6).

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16. MALARIA

Introduction

Malaria is called also paludism, swamp or intermittent fever. It is still a very important and uncontrolled disease because the campaigns of eradication have not led to the desired results. It is estimated that still 200 million people are infected.

Circumscribed epidemic zones of malaria, situated between 40° latitude north and 30° latitude south, are found in Central America, South America, Southern Europe, Africa, Asia, the Philippines and many islands of the Pacific Ocean. The endemic zones in the South of the USA have practically disappeared since World War II. Today, malaria infections in the USA and Northern Europe are all 'imported' cases. Imported malaria, actually, is of great importance because symptomatology is often atypical and the diagnosis may not be made at the time. Each one of the four species of *Plasmodium* has, in

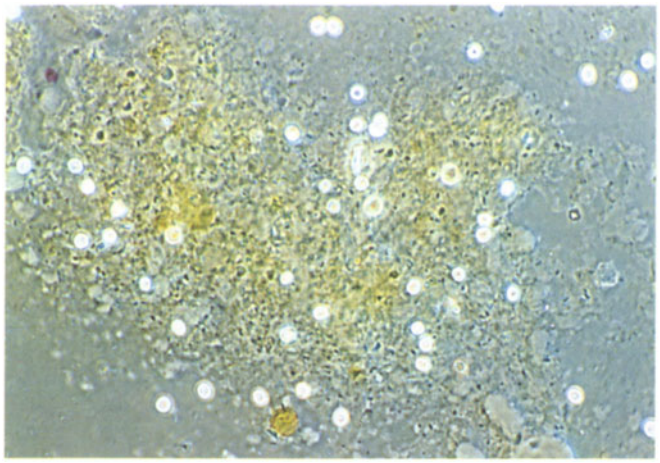


Fig. 15.1 Unstained smear of faecal material with numerous organisms of *Cryptosporidium* sp. Phase-contrast

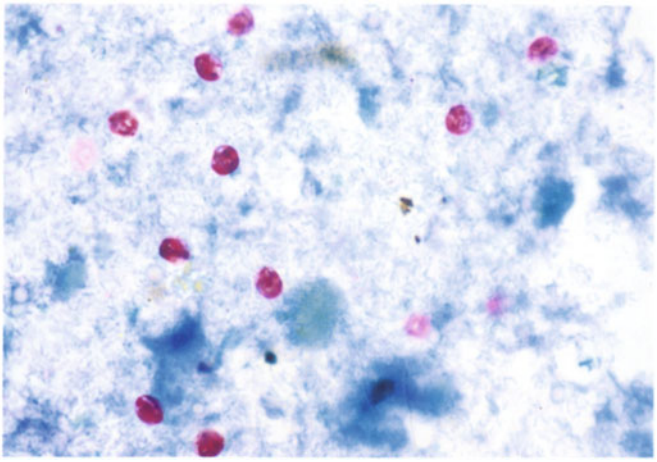


Fig. 15.2 Smear of faecal material with acid-fast organisms of *Cryptosporidium* sp. Ziehl–Neelsen

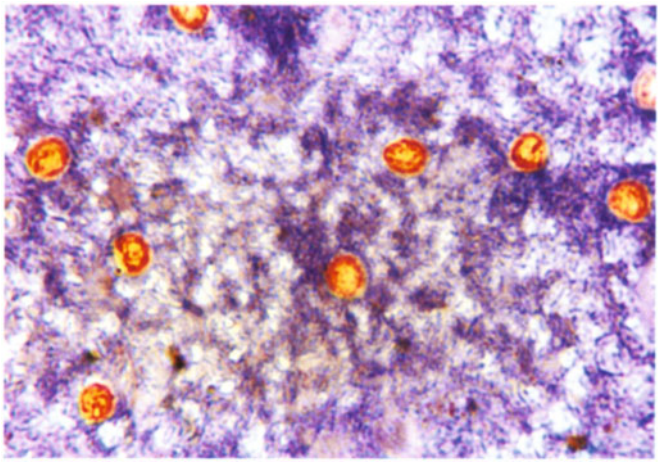


Fig. 15.3 Numerous acid-fast organisms of *Cryptosporidium* sp. in smear of faeces at high power. Ziehl–Neelsen

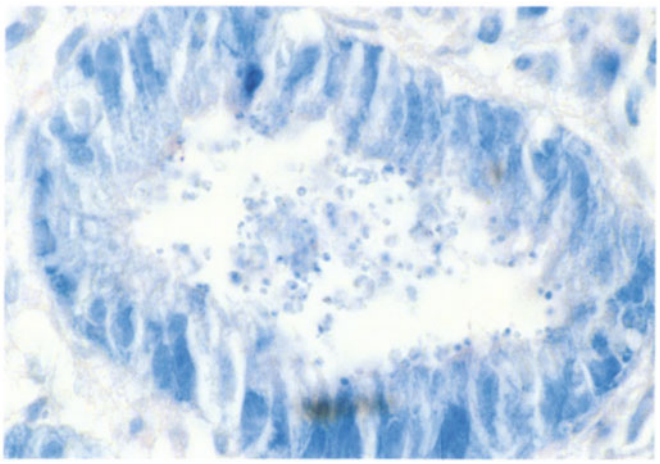


Fig. 15.4 Numerous cryptosporidians in the lumen of the intestine in case of AIDS (tissue section). Giemsa

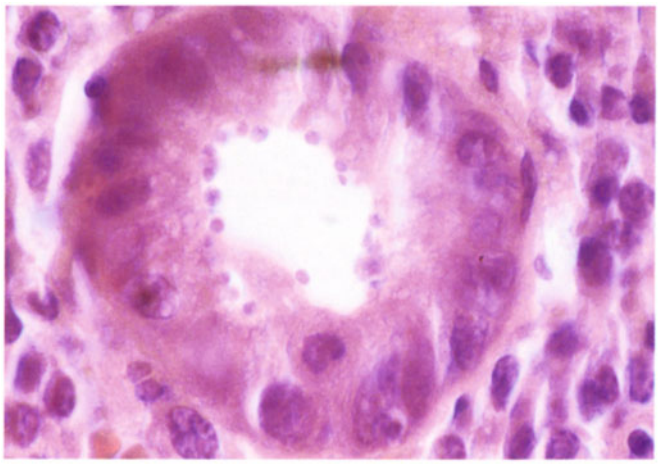


Fig. 15.5 Same case as Fig. 15.4 H&E

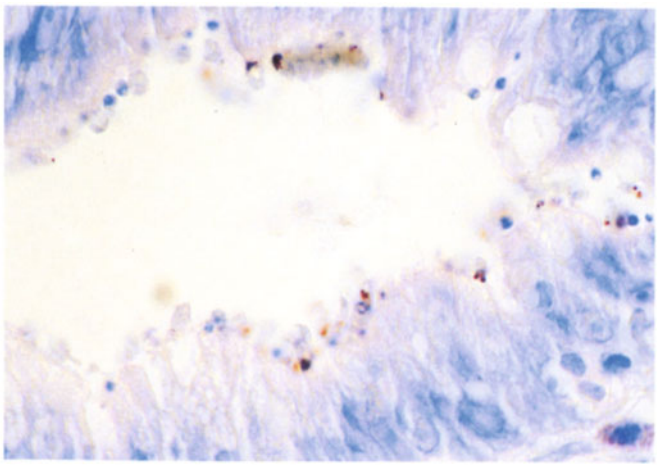
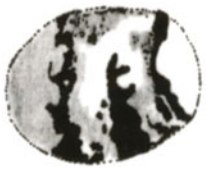
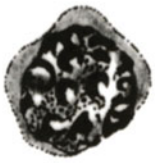


Fig. 15.6 Same case as Figs. 15.4 and 15.5. Cryptosporidians are partly impregnated with faecal material. Giemsa

Table 7 Intraerythrocytic structures of species of *Plasmodium*

P. vivax	*P. falciparum*	*P. malariae*	*P. ovale*
			
Middle aged trophozoite with Schueffner's punctuation	Young trophozoite with two dots	Young trophozoite	Young trophozoite annular form
			
Trophozoite with basophilic dots	Multiple trophozoites	Adult trophozoite, band-shaped	Trophozoite, double infection
			
Segmented schizont	Presegmented schizont	Segmented schizont	Schizont, progressive form
			
Young gametocyte	Adult macrogametocyte	Young microgametocyte	Mature gametocyte

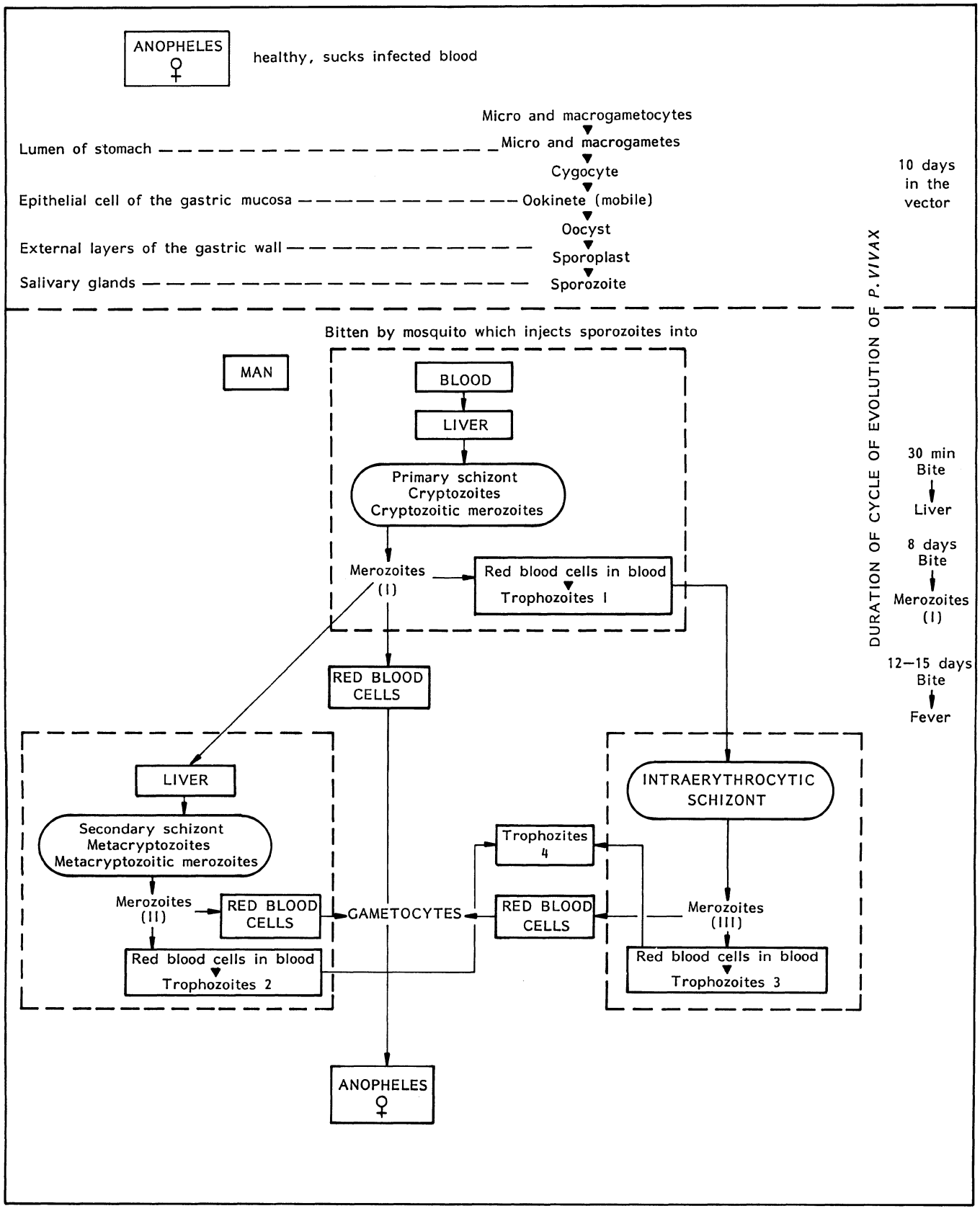
addition to diverse vectors, a different geographic distribution. Malaria is becoming endemic in Venezuela again, after having been completely eradicated in the fifties and sixties. In 1991 newspapers called it a 'national emergency'.

Hundreds of species of vertebrates and non-vertebrates may be infected by some 50 species of the genus *Plasmodium*. Monkeys may be affected naturally by three species of *Plasmodium* (*P. knowlesi, P. cynomolgi* and *P. brazilianum*). All of these may, rarely, infect man. *Plasmodium berghei* and other *Plasmodium* species are used for experimental work in various lower animal species[1–3].

The clinical course, at least in endemic regions, is relatively benign, possibly as a result of a relative immunity. Fatal outcome is observed practically only in infections with *Plasmodium falciparum*, and mainly among tourists. Almost one million deaths are estimated to occur per year.

The denominations of clinical forms of malaria do not depend only on the bouts of fever. Infections with *P. vivax* and *P. ovale* are called the 'tertiana type'; with *P. malariae* the 'quartana type'; and with *P. falciparum*

Table 8 Cycle of *Plasmodium* in man and vector



'tropical', 'pernicious' or 'malignant' malaria.

For clinical diagnosis, the plasmodians should be recognized in Giemsa-stained thin or thick blood films. Blood may be drawn at any time between or during the attacks of fever. The trophozoites of *Plasmodium falciparum* are one fifth the size of the diameter of a red blood cell.

The parasite

The four species of *Plasmodium* pathogenic for man (*P. vivax, P. falciparum, P. malariae* and *P. ovale*) belong to the group Haemosporidia of the class Sporozoa. The characteristic structures of the diverse species of *Plasmo-*

dium in red blood cells are shown schematically in Table 7. Trophozoites, schizonts and gametocytes are illustrated in Fig. 16.1. The red dot in the trophozoites represents the nucleus. The Schüffner dots in *Plasmodium vivax*-infected human erythrocytes are important in the identification of this malarial species[4].

Pathogenesis

Man acquires this disease mostly through the bite of an infected female *Anopheles* mosquito, which is haematophagous; the male mosquito does not suck blood. Exceptionally, malaria may be transmitted by blood transfusion. Infections do occur from mosquitos carried via airports from the tropics to countries with a temperate climate[5]. The genus *Anopheles* comprises more than 400 species; 65 of them are able to transmit human malaria. More than 300 species are considered potential vectors. The evolutionary cycle of *Plasmodium* is shown schematically in man and the vector in Table 8.

The three principal stages of evolution are:

1. Sexual reproduction of parasites in the female *Anopheles* mosquito, followed later by asexual development and sporozoites invading the salivary glands, through which humans are infected when bitten.
2. Asexual multiplication in the hepatic cells of the host (pre-erythrocytic stage) with formation of merozoites and subsequent invasion of red blood cells as trophozoites.
3. Asexual repeated multiplication in the erythrocytes, causing the clinical picture of the intermittent fever. The red blood cells may harbour multiple plasmodians[6].

The prepatent period (until the appearance of the parasite in blood) is 8 days for *P. falciparum*; the incubation period (until the first bout of fever) 12–15 days. The parasites destroy erythrocytes with the formation of iron-free malarial pigment, also called haemozoin. Symptomatology and the periodic bouts of fever are classical.

Pathology

The destruction of red blood cells leads to pathological alterations which may be summarized as: terminal circulatory disorders, storage phenomena in the PMS system and consequences of hypoxaemia. Ultrastructural lesions will not be discussed here[7–10]. With the light microscope, it may be possible to detect: thromboses, disseminated intravascular coagulation, micro-infarcts, necroses, haemorrhages and a slight inflammatory reaction.

In fatal cases, characteristics gross lesions are present in the liver and spleen; these organs show a diffuse greyish or black colour, while the other viscera and tissues show a less pronounced blackish colour. Kidneys, liver and spleen are slightly enlarged. In the chronic forms of malaria, the splenomegaly is more notable than in the acute fatal forms. Petechiae and oedema are observed in the brain (Figs. 16.3–16.8).

Regarding histological lesions, plasmodians are said to be seen in sections in red blood cells, situated in capillaries. Personally, we have been able to detect parasitized erythrocytes only in sections of a placenta (Fig. 16.9) and bone marrow in a *P. falciparum* infection. This case was kindly provided by Dr Francis W. Chandler from the CDC, Atlanta/Georgia, USA. Later, after a prolonged search, we could also see plasmodians in erythrocytes situated in cerebral capillaries (Fig. 16.10). However, these intra-erythrocytic structures, which may be trophozoites or schizonts of *Plasmodium*, were only faintly visible. The dark dots in red blood cells represent malaria pigment.

By contrast, malaria pigment is present in numerous organs (Figs. 16.11 and 16.12). It is called haematin or haemozoin and is a derivative of haemoglobin but is iron free and difficult to distinguish from formalin pigment. No histochemical methods are known by which it can be recognized as such. The malarial pigment is encountered as small black dots in erythrocytes and as blackish-brown irregular granules in circulating and fixed macrophages, above all in von Kupffer cells of the liver. The pigment is formed in erythrocytes and tissue cells each time an acute bout of fever, with destruction of red blood cells, occurs. In autopsies of individuals from endemic zones who suffered from acute malaria years ago, malaria pigment was found in viscera only exceptionally, i.e. the pigment disappears slowly from tissues. Double refraction of malarial pigment with polarization, as described in the literature, could not be detected in our material.

Renal lesions are frequent and typical in infections with *Plasmodium* species. Characteristic is blackwater fever or haemoglobinuric nephrosis, an intravasal haemolysis which leads to haemoglobinaemia and haemoglobinuria. In the renal tubules, destroyed red blood cells and haemosiderin are found in the haemoglobin casts. The dark colour of the urine, therefore, is not due to malarial pigment. The latter is not deposited in the spaces of Bowman nor in renal tubules. Renal insufficiency, azotaemia and uraemia are possible consequences of blackwater fever.

The classical malarial lesions in the brain are annular haemorrhages and granulomas of Dürck. Parasites are not found in the red blood cells situated in the annular haemorrhages around necrotic foci. The subcortical granulomas of Dürck consist of proliferated glial cells and appear only in patients who survive more than 12 days. The blockage of capillaries by *P. falciparum*-infected erythrocytes seems to be the principal cause of cerebral malaria[11–14].

The placenta is probably the organ where most parasitized red blood cells and pigment accumulate. *Plasmodium falciparum* matures in the placenta and, consequently, may cause miscarriages, or there may be transmission through the placenta, as described in the USA (congenital malaria)[15,16]. In the heart, thromboses of coronary arteries, infarcts and interstitial inflammatory reactions have been described, but only occasionally.

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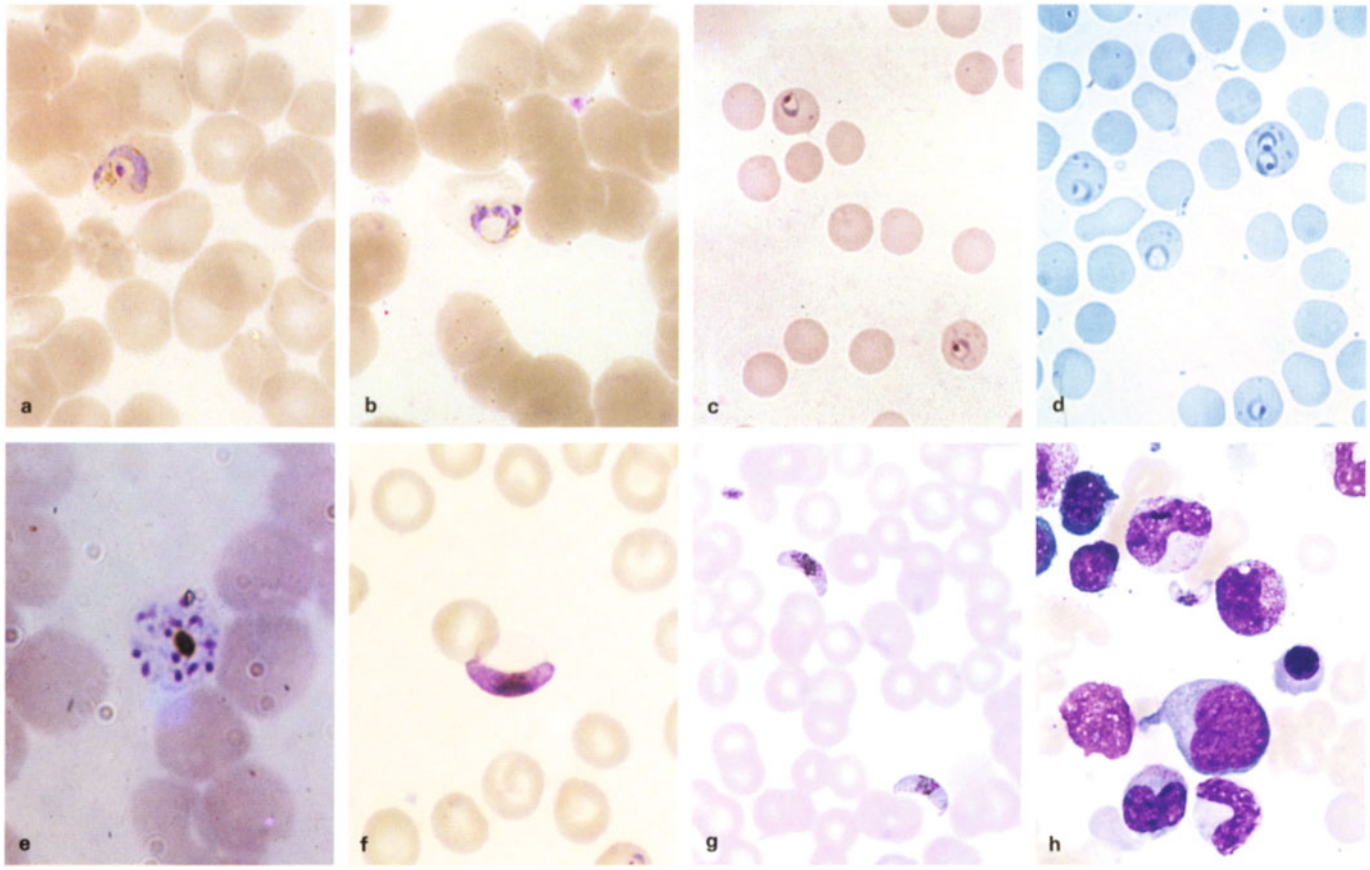

Fig. 16.1 Plasmodians: **a–d**. Trophozoites of *Plasmodium vivax*
e. Schizont of *Plasmodium vivax*
f, g. Gametocytes of *Plasmodium falciparum*
h. Gametocyte in bone marrow. Giemsa + May–Grünwald

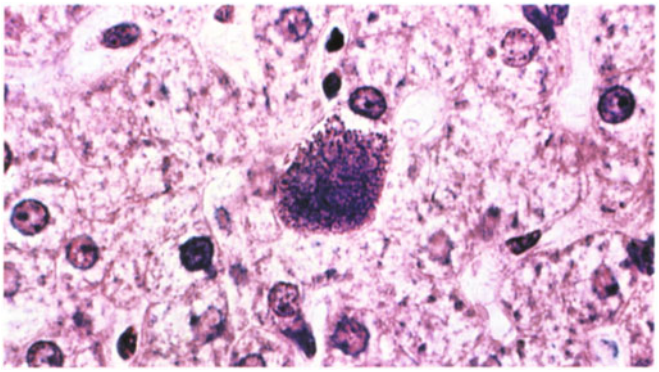

Fig. 16.2 Schizont in hepatocyte containing cryptozoites or metacryptozoites. Fortuitous finding at autopsy. H&E

Fig. 16.4 Cut surface of spleen in the same case as Fig. 16.3

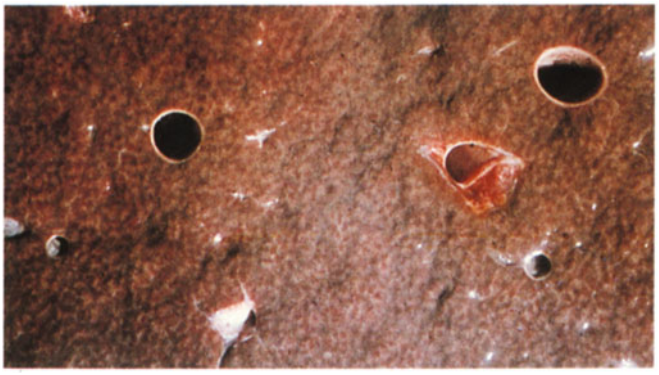

Fig. 16.3 Cut surface of liver in case of fatal tropical malaria (infection with *Plasmodium falciparum*)

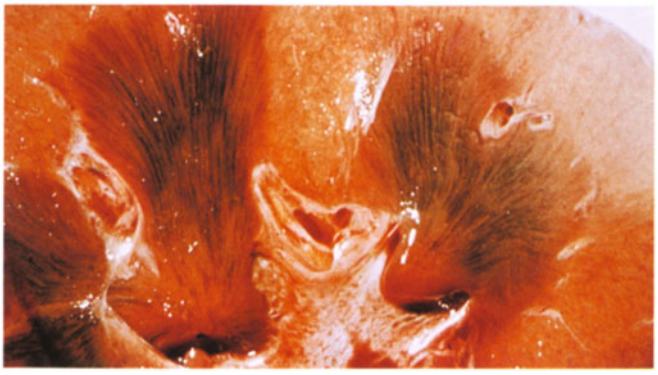

Fig. 16.5 Cut surface of kidney in the same case as Figs. 16.3 and 16.4

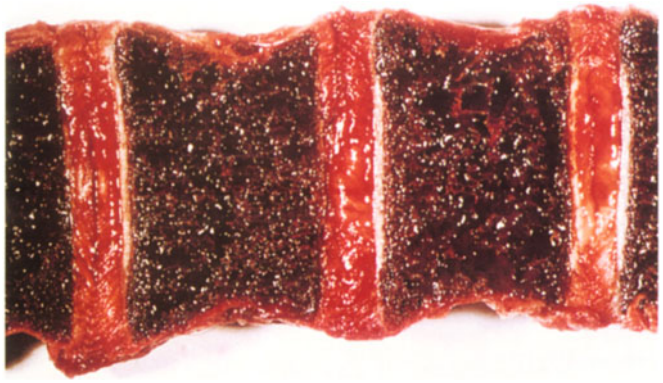

Fig. 16.6 Bone marrow in the same case as Figs. 16.3–16.5

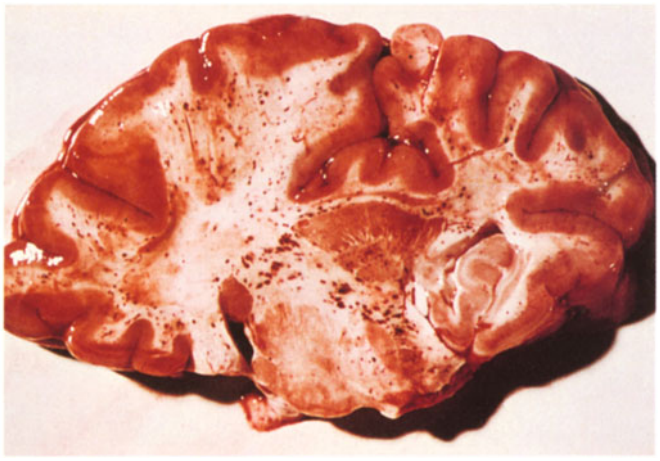

Fig. 16.7 Cut surface of brain with oedema and petechiae in the same infection as Figs. 16.3–16.6

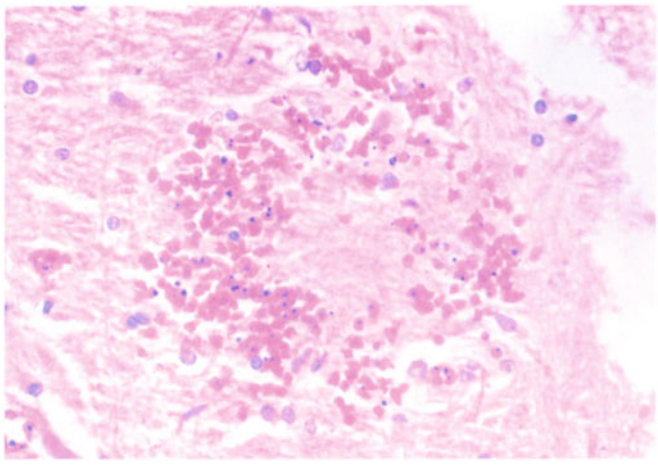

Fig. 16.8 Brain with annular haemorrhage in the same case as Fig. 16.7. The dark dots are granules of malaria pigment inside erythrocytes. H&E

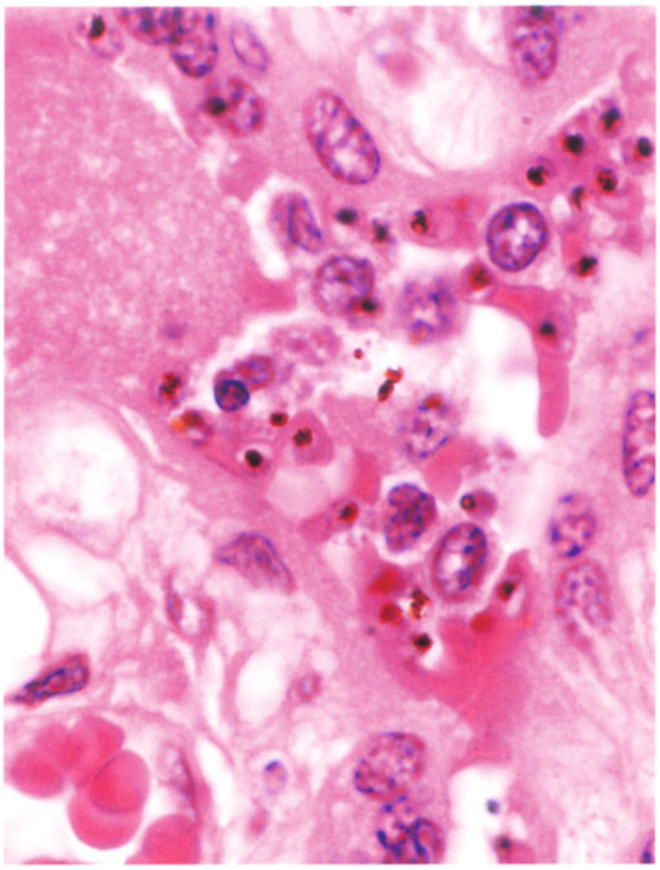

Fig. 16.9 Placenta with plasmodians and abundant deposits of malaria pigment. H&E

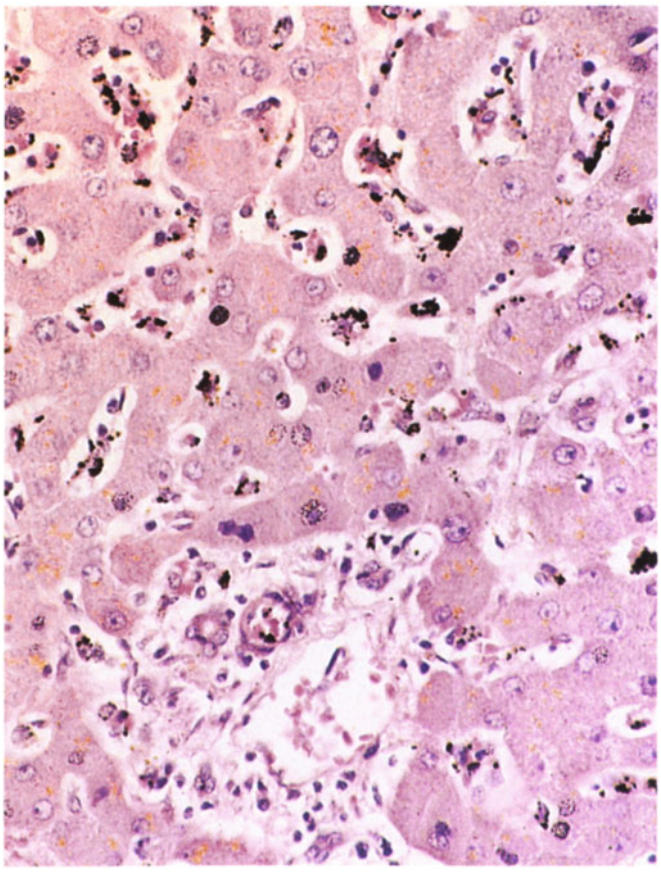

Fig. 16.10 Malaria pigment in Kupffer cells of the liver. H&E

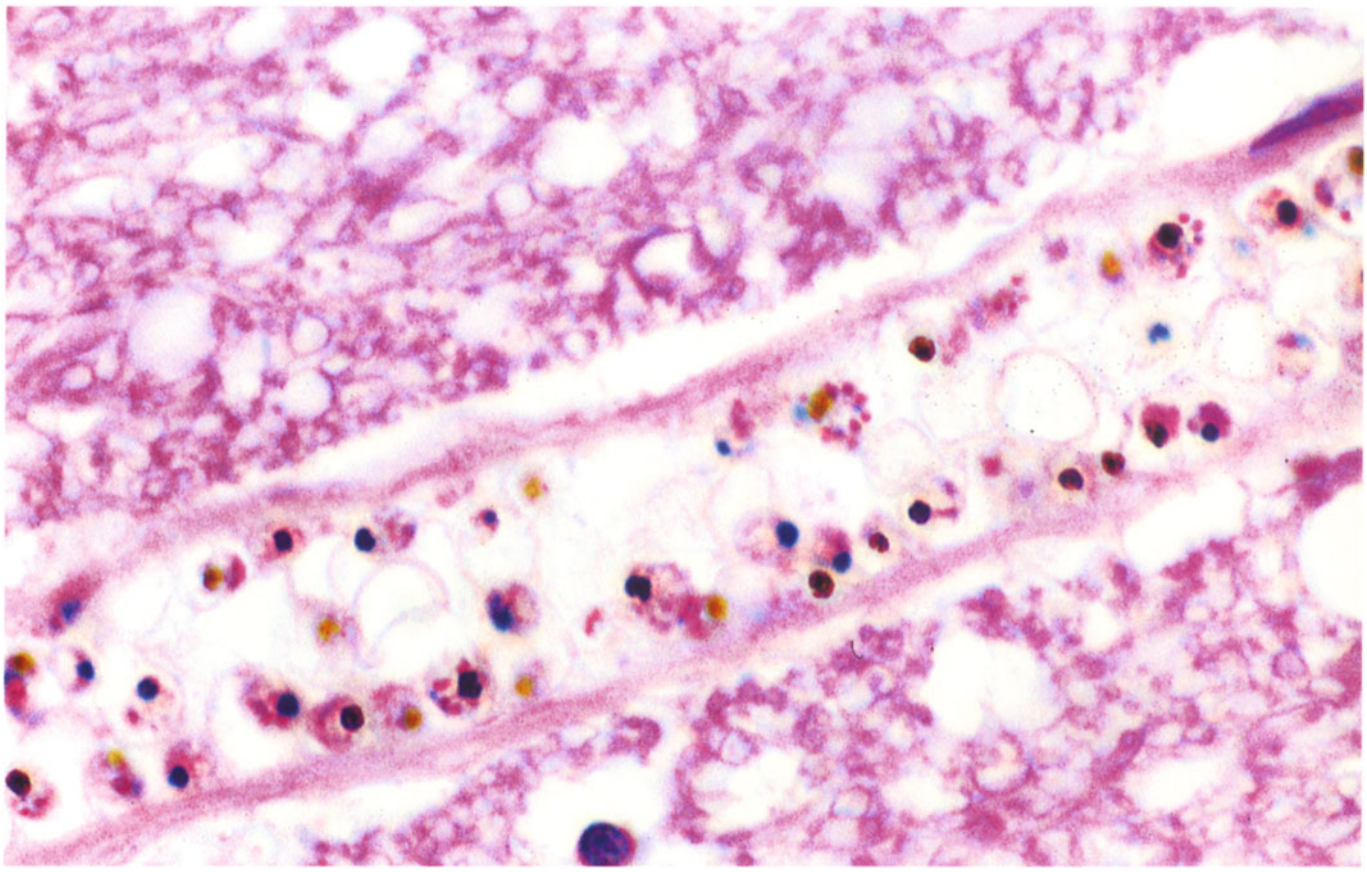

Fig. 16.11 Schizonts of *Plasmodium falciparum* seen faintly in a blood vessel of brain in addition to granules of malaria pigment. Plasmodians, as a rule, are not seen in tissue sections. H&E

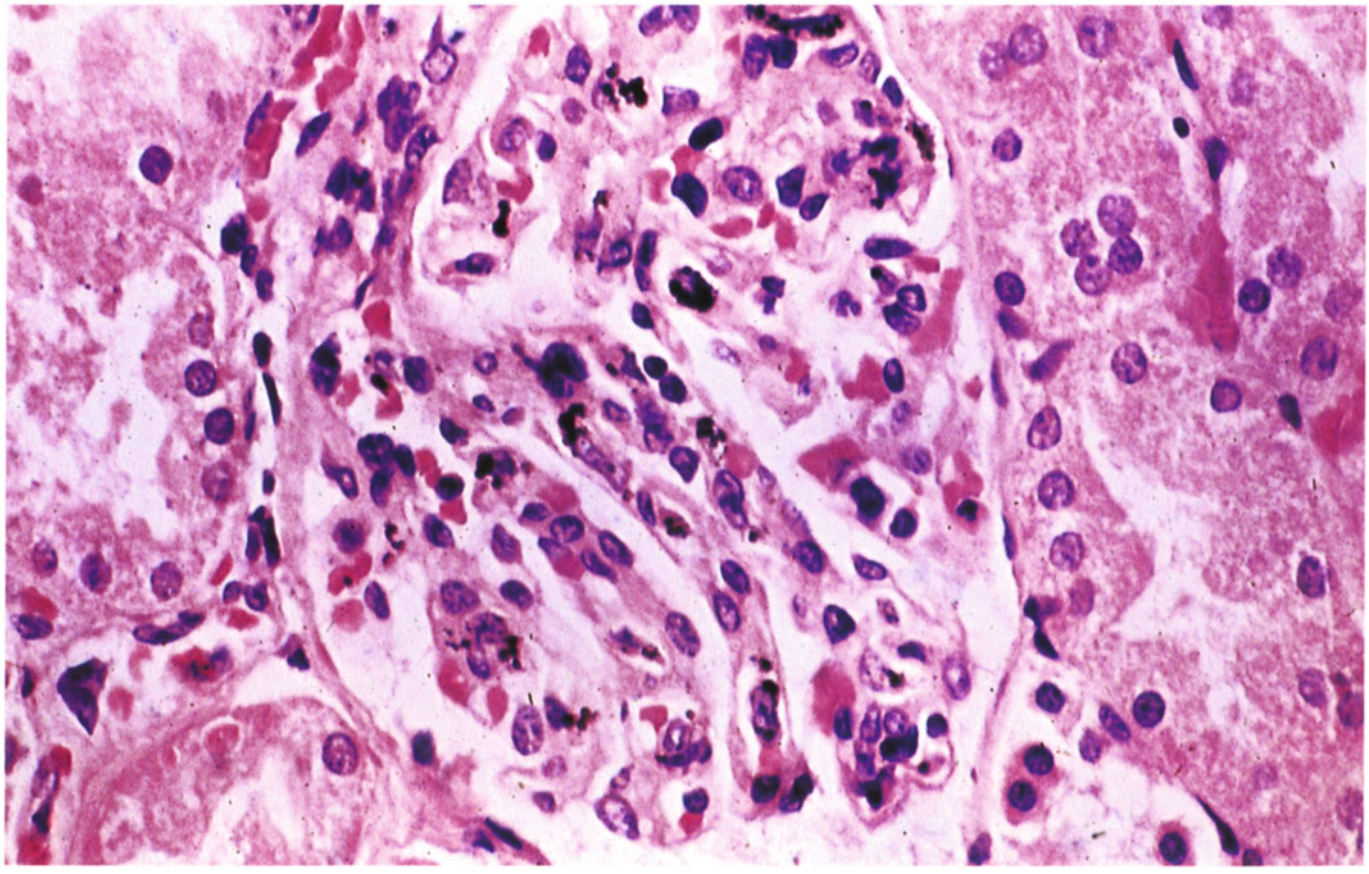

Fig. 16.12 Glomerulus in case of infection with *Plasmodium falciparum* showing malaria pigment. H&E

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17. PNEUMOCYSTOSIS

Introduction

This disease is also called Pneumocystis pneumonia or interstitial plasma cell pneumonia due to *Pneumocystis carinii* infection. It is an important and worldwide, mostly opportunistic, infection manifesting itself as a consequence of immunosuppressive treatment, primary immunodeficiency in premature newborns and AIDS. Here, often, disseminated forms and/or tumour-like manifestations with granulomatous tissue reaction are observed[1–8]. The number of individuals who become ill with Pneumocystis pneumonia is increasing constantly. In Venezuela, a tropical country, infections have been confirmed too[9,10].

Spontaneous infections have been found in numerous animal species: rats, mice, rabbits, dogs, cats, cattle and sheep. Experimentally, pneumocystosis may be produced easily by injecting rats for 10–14 days with corticosteroids.

The massive, epidemic and often fatal form of the disease, due to nosocomial infections in premature newborns and undernourished infants[11,12], hardly occurs any longer. This was a typical infection, with a high prevalence and a high mortality rate, in the years after World War II in Europe. Opportunistic infection in adults, as a consequence of immunodeficiencies of all sorts, is the most common form of this disease nowadays[13]. A latent, asymptomatic, pauci-parasitic and pauci-reactive form exists in immunocompromised and immunocompetent persons, above all infants. Connatal[14] and familial forms of the disease and cases with involvement of extrapulmonary sites are all rare. In pneumocystosis, commonly, the characteristic radiological picture of an interstitial pneumonia exists. Fever is generally present. The patients become cyanotic and death occurs due to pulmonary insufficiency and/or associated bacterial bronchopneumonia. The prognosis is bad, at least in untreated patients.

Clinical diagnosis is made by observing *Pneumocystis carinii* organisms. They are demonstrated, rarely, in smears of sputum or bronchial secretions. A special cytocentrifuge is recommended for processing sputa and fluids from broncho-alveolar lavage[15]. Examination of BAL sediments is the method of choice today.

In the books, the Giemsa method is recommended to stain the parasites. However, in our experience of examining routine material, the parasites are found only when stained by the Grocott or toluidine blue method. Immunobiological methods for diagnosis exist, as indirect fluorescent staining techniques. Not all are reliable.

The parasite

Pneumocystis carinii is the causal agent of the disease. It has not been encountered living free in nature, nor has it been cultured in artificial media. It seems to belong, on the basis of its appearance, to the Protozoa. The majority of scientists believe it belongs to the class Sporozoa and subclass Haplosporidia. Others assume it is probably related to the microsporidia. However, in all the classifications of Protozoa we know, it is not mentioned and some people consider it a fungus. This was stressed at the ISHAM Congress, June 1991, in Montreal, Canada.

Some scientists believe that the species of *Pneumocystis carinii* in man is different from that which is found in the rat, in spite of both being structurally the same.

The *Pneumocystis carinii* organisms in sections and smears are spherical, relatively thick-walled, cyst-like structures. Also, thin-walled parasites are said to be found. They measure about 3–8 μm in diameter and sometimes appear to be crescent-shaped or coffee bean-like when the cysts collapse. However, this happens too with the large yeast-like fungus cells in tissue sections. The Grocott and toluidine blue methods are the best techniques for bringing out these parasites. It must be emphasized, especially, that *P. carinii* does not stain with H&E. With the Giemsa method, the organisms are mostly difficult to recognize. We have seen them with this technique but only occasionally, in special cases and after a prolonged search. With the Giemsa and the Rhodamine stains, 'intracystic bodies' which measure about 1–2 μm, can be demonstrated. There may be up to 8 bodies (sporozoites?) in each parasite. Some investigators have observed PAS-positive membranes of the *P. carinii* organisms (Figs. 17.1–17.3). The cycle of evolution of *Pneumocystis carinii* in man and lower animals is not well understood (like its taxonomic position), and there are too many controversial concepts in this subject to venture a personal opinion.

Pathogenesis

Apparently, a latent infection occurs in humans, rodents and some domestic lower animals. The portal of entry of the parasites is the respiratory tract. Then, suddenly, the saprophyte-like inactive parasites multiply copiously, transforming into aggressive organisms, and produce pulmonary disease. They colonize the inferior airways and become attached to the surface of the bronchiolar mucosa and alveoli, obstructing the lumens of the cavities mechanically. The parasites reproduce rapidly, covering the remaining respiratory surface, and the patient practically suffocates. When, in addition, interstitial cell infiltrates form, the lung function is still more compromised. Extrapulmonary sites used to be involved only exceptionally, but such localizations are now seen with increasing frequency.

Pathology

The lungs are always involved in infections with *Pneumocystis carinii*. Additionally extrapulmonary lesions are found in lymph nodes, bone marrow, liver (Fig. 17.4) and spleen[16,17]. Recently, involvement of the small intestine, choroid and adrenal glands has been reported in AIDS patients[18–20]. Here, the granular and foamy masses are

Fig. 17.1 Cluster of *Pneumocystis carinii* organisms in smear of a cut lung surface in a case of massive pneumocystosis. Folds, 'sickle' and 'hat' forms as well as other deformations of the parasites, known in large yeast-like cells, are seen. *Pneumocystis carinii* does not stain with H&E. Grocott

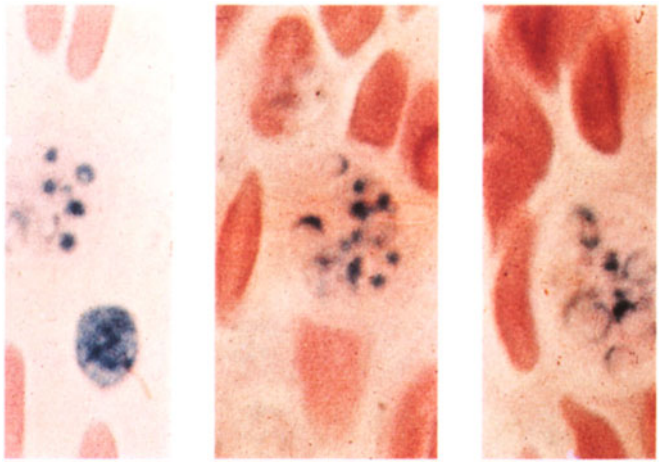

Fig. 17.2 Cystic parasites of *Pneumocystis carinii* showing internal corpuscles. This is from a smear of the cut surface of a rat lung. Giemsa

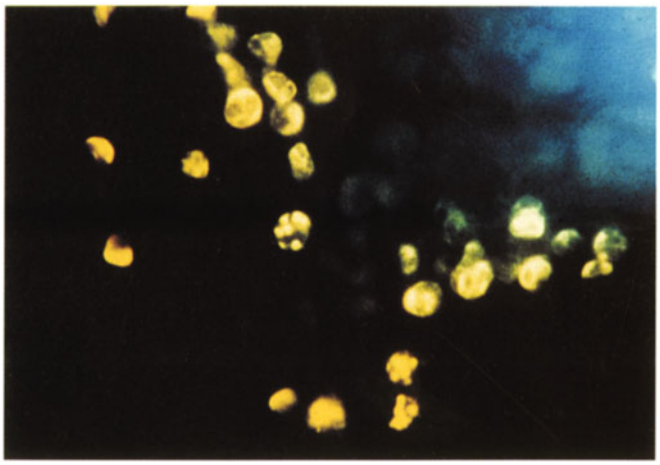

Fig. 17.3 Smear of the same case as Fig. 17.2 with the same internal corpuscles. Rhodamine; UV light

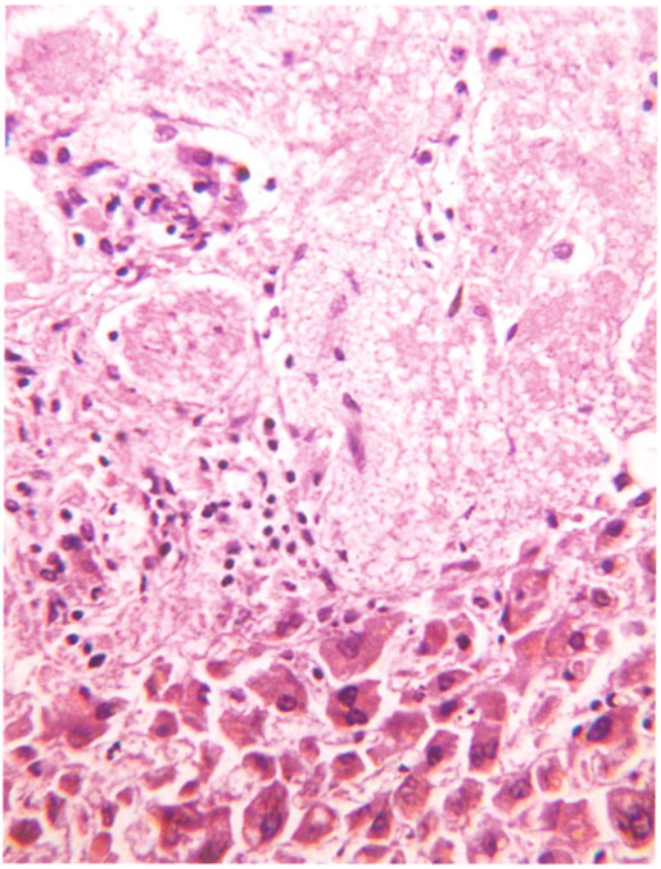

Fig. 17.4 In this human liver the parenchyma is replaced by the typical granular and foamy eosinophilic masses found inside pulmonary alveoli in cases of pneumocystosis. H&E

Fig. 17.5 Unfixed, resected pulmonary specimen with a tumorous mass (pneumocystoma) in an AIDS patient. (Case of Dr P. Deicke, Berlin)

the same as those present in the pulmonary alveoli. Additionally, necrobiotic lesions are found with replacement of the organic structure, together with parasites in these focal lesions.

Grossly, the pulmonary lesions resemble foci of bacterial bronchopneumonia. Irregularly delimited areas show increased consistency. Furthermore, large tumour-like nodules may be found in lungs and other organs[2,3,7] (Figs. 17.5 and 17.6).

Histologically, the organisms of *P. carinii* are found almost exclusively extracellularly and only inside the pulmonary alveoli. They cannot be detected in sections stained routinely with H&E. Often, they are arranged in clusters and attached to the alveolar or bronchiolar walls (Figs. 17.7–17.9). With the Giemsa method, it is very difficult and time-consuming to look for this species of parasites. Sometimes the internal bodies of the organisms can be seen with the Giemsa method (Fig. 17.2) or with Rhodamine stain (Fig. 17.3). The best method for staining pneumocysts is the Grocott technique, both in smears and in tissue sections. Stained in this way, they look like small yeast-like fungus cells, occasionally showing single internal dots (Fig. 17.9). Collapsed or squeezed protozoan cells may be seen, showing hat-, sickle- and pot-like forms. Another good stain for *P. carinii* organisms is toluidine blue.

In the lumens of the alveoli, it is possible to see single pneumocysts detached from the walls, together with typical inhomogeneous, granular and foamy substances. These are eosinophilic with H&E and stain faintly with the PAS method (Figs. 17.10–17.13). With the Grocott method, these intra-alveolar substances often stain blackish and may represent debris of destroyed *P. carinii* parasites. This foamy alveolar content is found mostly in infants who also show an interstitial pneumonia. The latter is said to be missing in immunocompromised patients. However, we found it recently in an AIDS patient in Mérida. In this case, additional bronchopneumonic foci were found, apparently due to an associated bacterial infection (Figs. 17.14 and 17.15). This type of alveolar content in cases of pneumocystosis can be distinguished from ordinary oedema or the lipoproteinaceous content of alveoli observed in histoplasmosis capsulati and other infections (see the *Atlas of Fungal Pathology* in this series). This was previously considered as typical in so-called lipoproteinosis. In cases of marked PAS-positivity of the alveolar content, an additional lipoproteinaceous reaction may be present.

In the interstitium, a cellular infiltrate consisting of mononuclear elements, lymphocytes and sometimes numerous plasma cells is found. These interstitial infiltrates are present mostly in cases of immunocompetent patients, while they are said to be absent in immunocompromised patients, as mentioned above.

Exceptionally, a granulomatous reaction has been noted in cases of pneumocystosis with formation of granulomas and giant cells[21] (Figs. 17.16–17.20). In the latter, parasites may be located. Granulomas of this sort cannot be distinguished from granulomas due to *Mycobacterium tuberculosis*. Granulomatous reaction is reported with increasing frequency in AIDS cases[2,4,7]. In the necrotic masses of the nodular (tumour-like) lesions, calcifications are often found (Fig. 17.21).

Frequently, in cases of infection with *Pneumocystis carinii*, another associated opportunistic infection may be present, e.g. cytomegaly or fungal infections.

The organisms of *P. carinii* must be differentiated from small yeast-like fungus cells, which are also Grocott-positive.

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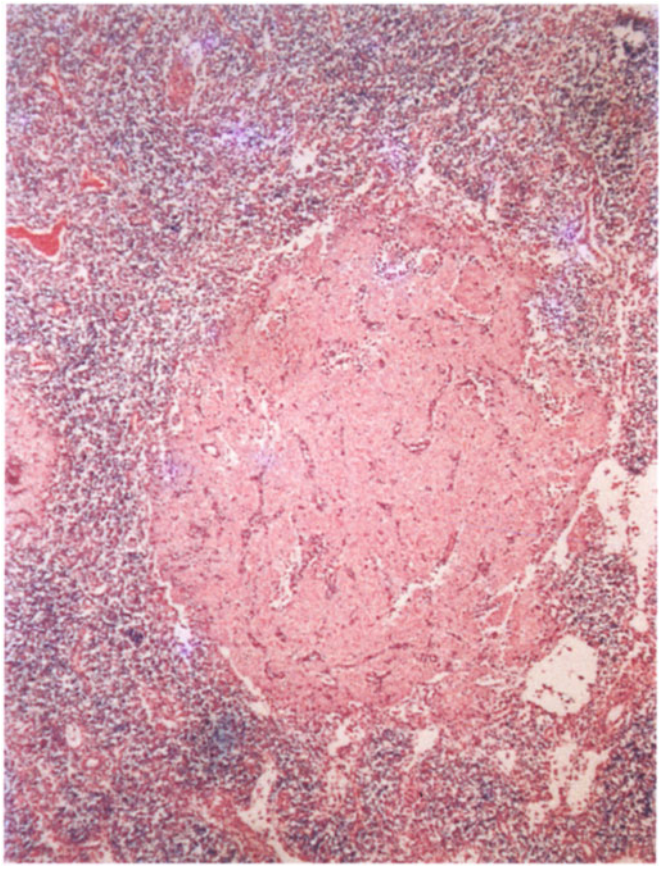

Fig. 17.6 Tumour-like focus of pneumocystosis in lymph node. H&E

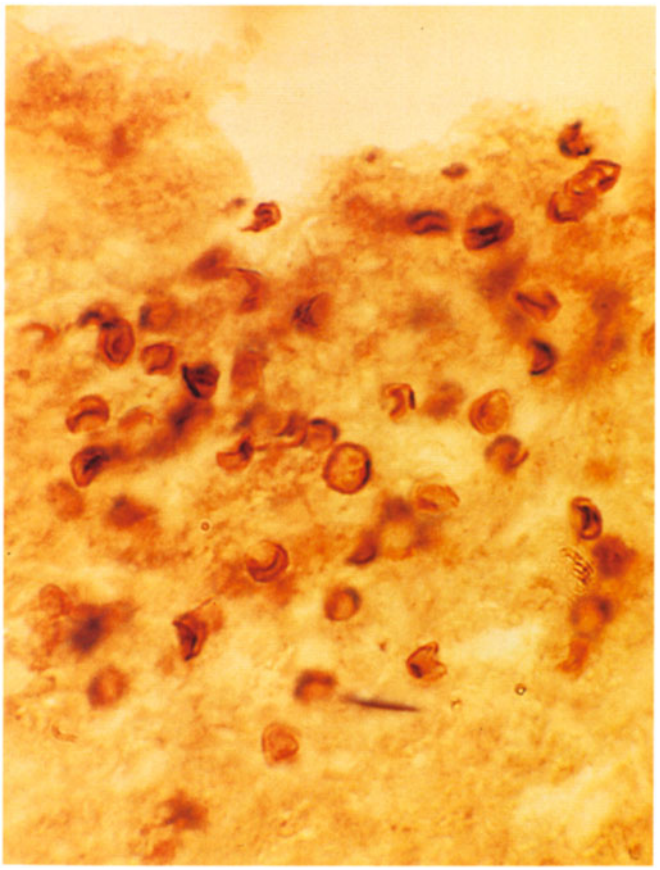

Fig. 17.7 Organisms of *Pneumocystis carinii* in lung tissue with deformation of parasites. A case of AIDS from Mérida. Grocott

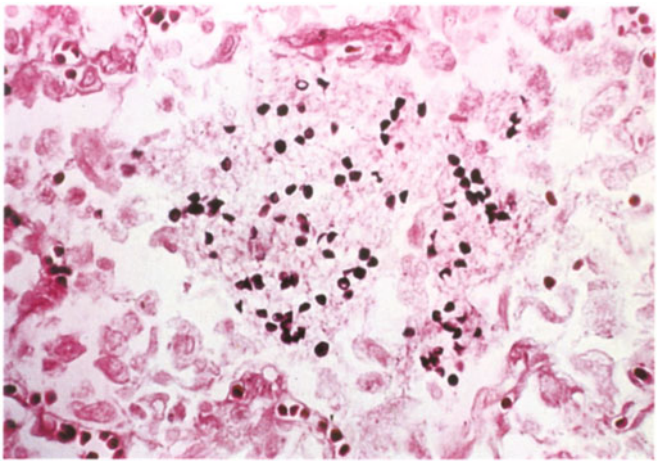

Fig. 17.8 Cluster of pneumocysts inside pulmonary alveoli in man. Grocott.

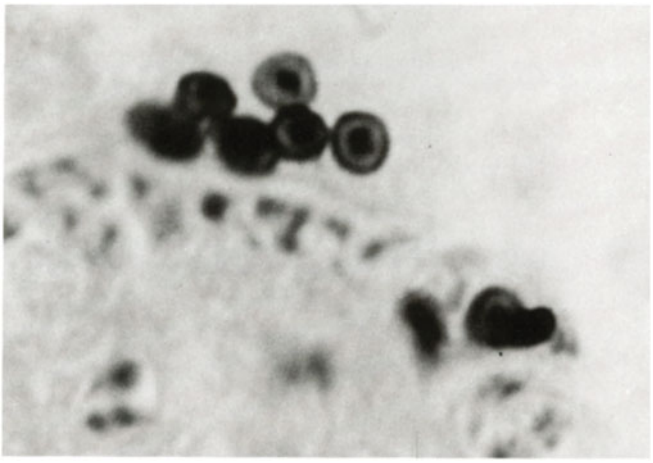

Fig. 17.9 Small cluster of organisms of *Pneumocystis carinii* attached to an alveolar wall in a case of pauciparasitic and paucireactive pneumocystosis in an infant from Mérida, observed many years ago. These organisms were first considered to be yeast-like fungus cells. Grocott

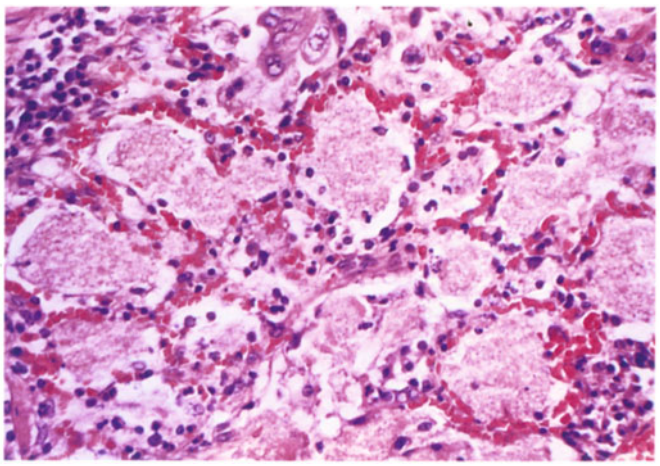

Fig. 17.10 The typical alveolar content in a human case of massive pneumocystosis. It is constituted, apparently, by necrotic parasites mixed with exudate. H&E

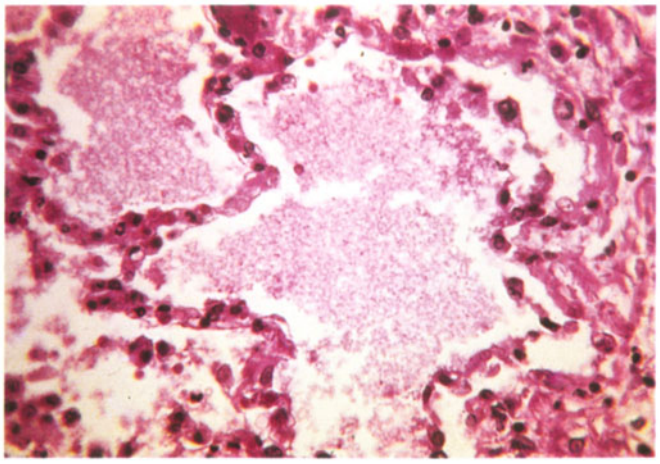

Fig. 17.11 The typical foamy alveolar content of pneumocystosis at higher power. H&E

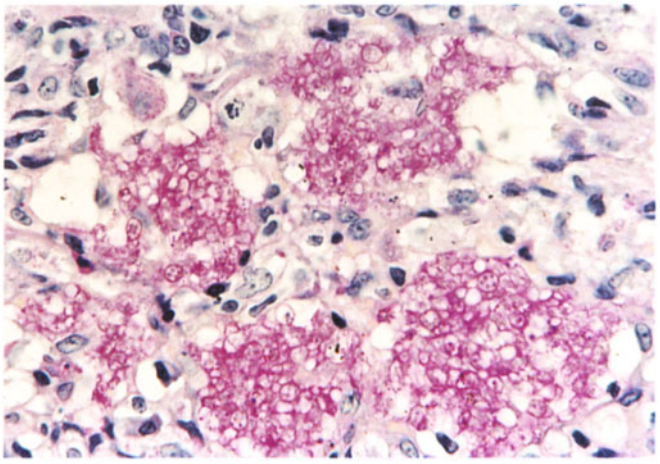

Fig. 17.12 The foamy structure of the alveolar content is clearly seen. Numerous unstained cyst-like forms may be discerned. Material from an AIDS patient. PAS

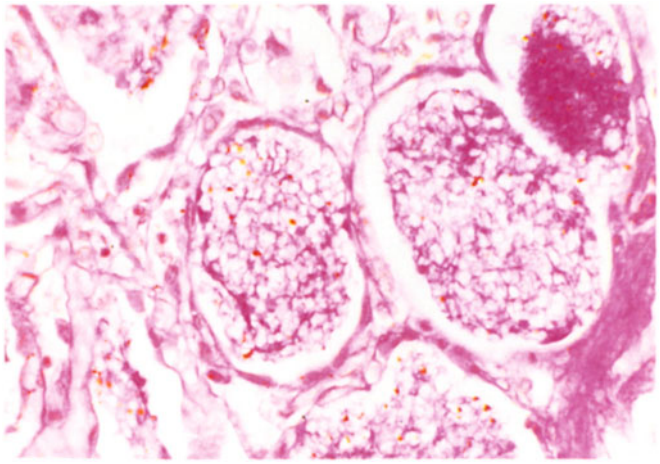

Fig. 17.13 The typical foamy alveolar content at higher magnification. An AIDS case from Mérida. PAS

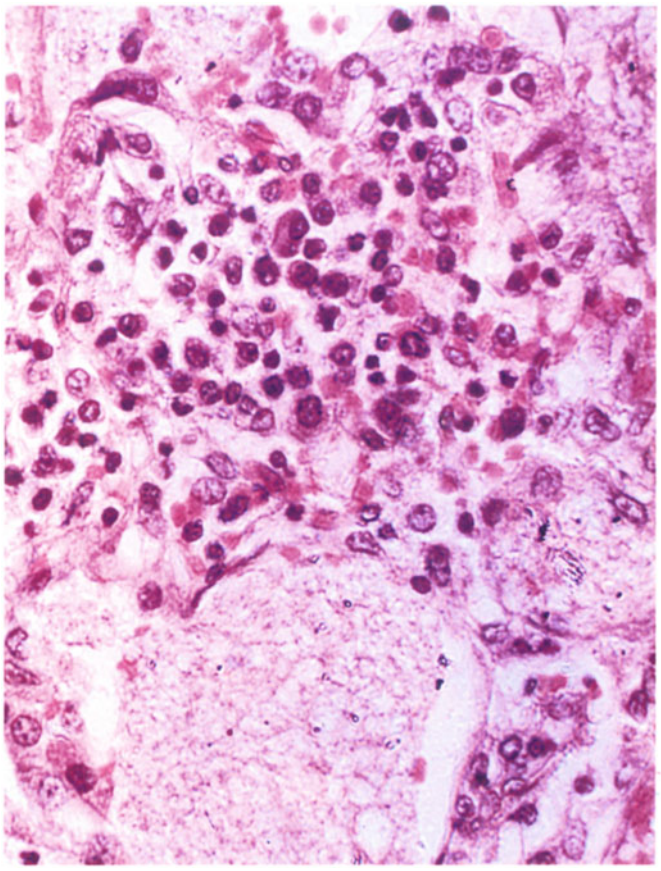

Fig. 17.14 Interstitial pneumonia with plasma cells in the AIDS case from Mérida. H&E

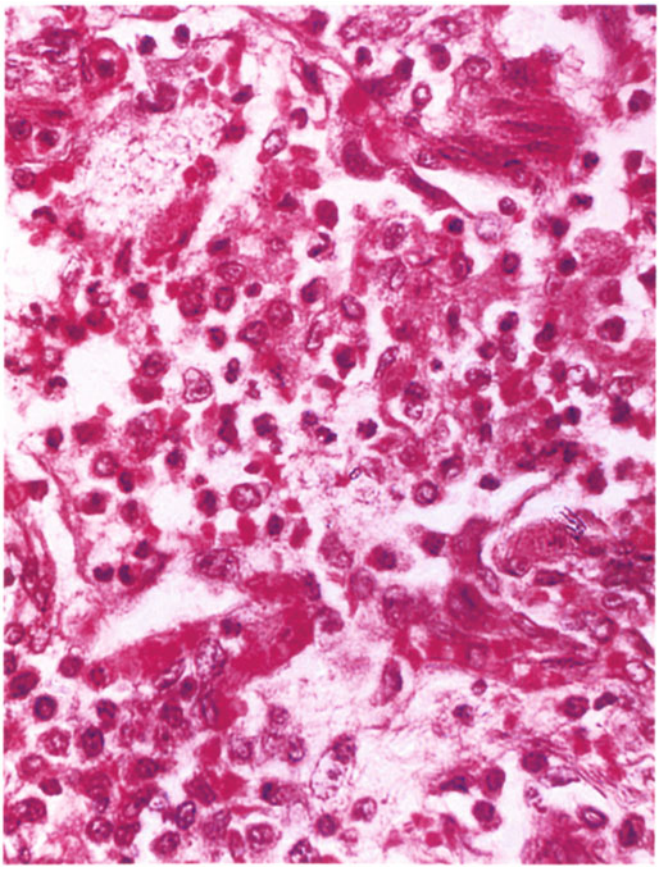

Fig. 17.15 Intra-alveolar exudate with granulocytes in the AIDS case from Mérida. H&E

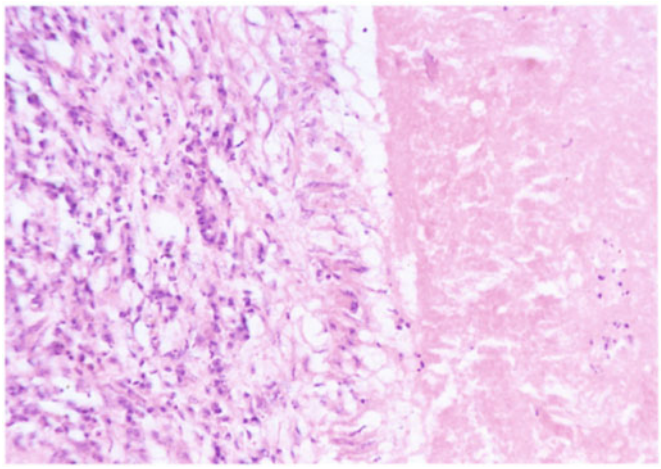

Fig. 17.16 Granulomatous reaction, i.e. epithelioid cells in a palisade-like fashion in the periphery of a pneumocystoma (AIDS patient). H&E

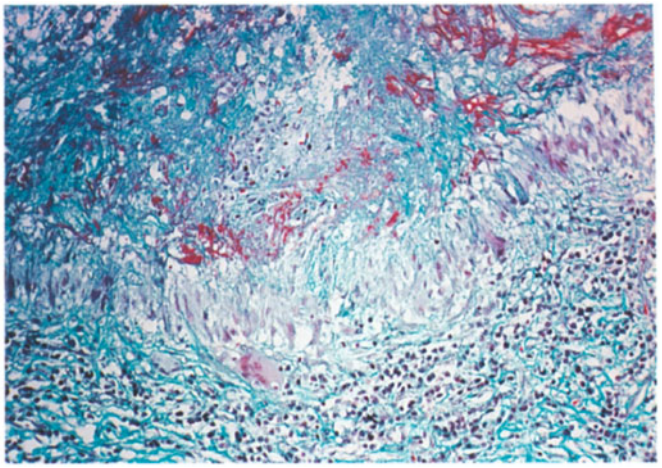

Fig. 17.17 The same pattern as in the case of Fig. 17.16. Trichrome (Goldner)

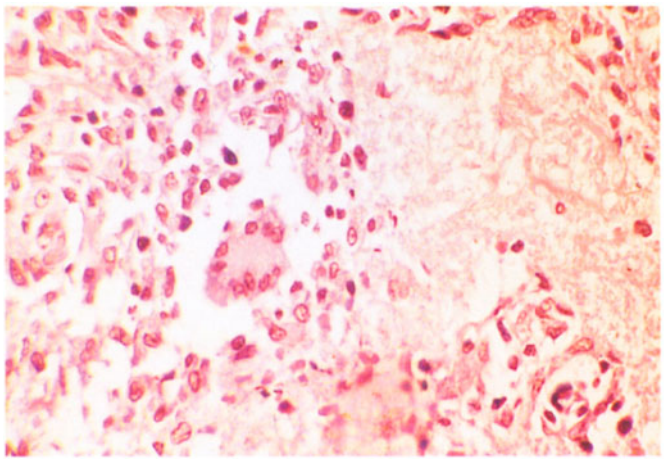

Fig. 17.18 Giant cell in the case of Figs. 17.16 and 17.17. H&E

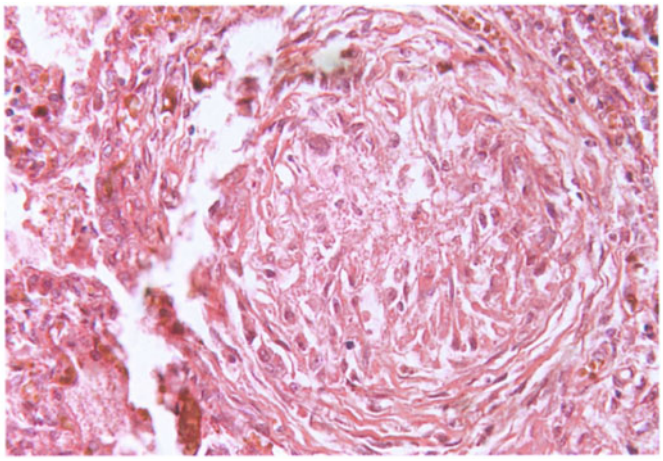

Fig. 17.19 Pulmonary granuloma in case of infection with *Pneumocystis carinii*. H&E

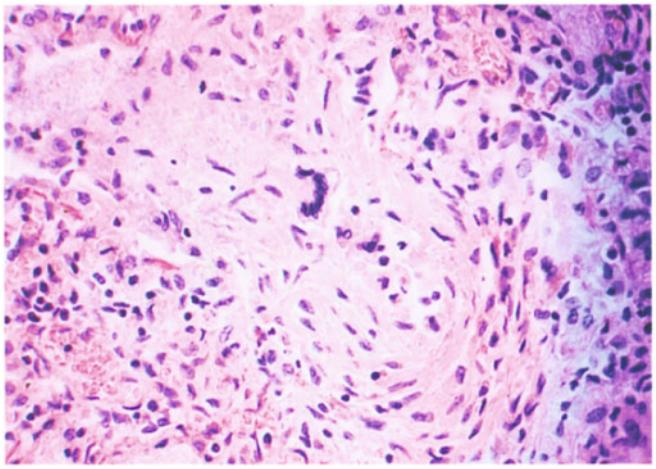

Fig. 17.20 Another case of pulmonary granuloma in a case of pneumocystosis. Organisms of *Pneumocystis carinii* may be found inside giant cells but are not seen in this field. H&E

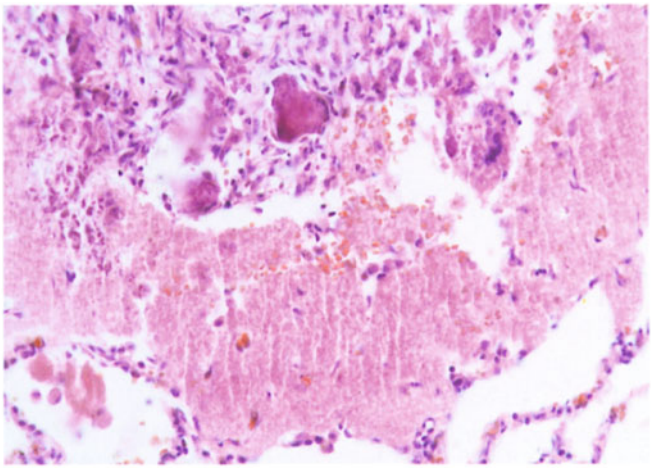

Fig. 17.21 Pulmonary pneumocystosis with granulomatous reaction and calcifications. H&E

18. BALANTIDIASIS

Introduction

This disease is also called balantidial dysentery or balantidial colitis. It is relatively rare in man because he is not the principal, but only the secondary or occasional host. The disease occurs worldwide, mostly in people dealing with pigs, such as pig breeders, veterinarians and butchers.

This infection is mostly observed in Eastern Europe, the former USSR, Asia and the Americas[1–3]. It is well known in Venezuela where its frequency has not increased over the last few years[4,5]. Fatal cases no longer exist (as they did some 30 years ago). There have been no reports on this protozoan infection in the medical literature for the past two years.

Almost all pigs are infected, but they rarely develop any disease. In addition, monkeys constitute a parasite reservoir, but, by contrast with pigs, they are sometimes severely ill. Cats, rabbits, rats and guinea pigs may be used for experimental infection[6].

In man, asymptomatic carriers are more common than cases of disease. Balantidiasis does not have characteristic symptoms, often takes a chronic course and may remain undiagnosed. Bloody stools and numerous leukocytes in faeces are typical of this infection. A fatal outcome may occur in cases with symptomatology for years, in cases with perforated ulcers and peritonitis and in cases not treated adequately[7].

Clinical diagnosis is made by examining fresh faeces. The trophozoites of *Balantidium coli* are easily recognized by their size and typical movement. Their cilia are not seen in permanent preparations and tissue sections. For diagnostic purposes, balantidians may also be cultured in artificial media, similar to amoebae.

The parasite

Balantidium coli is the largest protozoan pathogenic to man. It is ciliated and belongs to the class Ciliophora. Reproduction takes place asexually by transverse division. The parasite shows two forms:

1. The trophozoites (or vegetative forms) of *Balantidium coli* are spherical or oval shaped. Their maximum size is 70–150 μm. Cilia cover the entire surface of the trophozoites and make them mobile (Figs. 18.1 and 18.2).
2. The cysts are more roundish and a little bit smaller. The membrane is thicker than that of the trophozoites and there are no cilia. They do not develop in man.

At one pole of the balantidia, there is a funnel-shaped invagination (mouth) and, at the other pole, an anal orifice which is difficult to detect. Bacteria and other faecal material are the food for the balantidia. Typically, there are two nuclei, a kidney-shaped macronucleus and a smaller spherical micronucleus. Vacuoles are present in the cytoplasm. *Balantidium coli* may be cultured and grows like amoeba.

Pathogenesis

Balantidium coli is mostly an inoffensive commensal in the lumen of the large bowel of pigs. Seldom does the pig become ill. In nature, cysts derive from trophozoites of *B. coli* in faeces of pigs. They hardly ever form in man. Therefore, infection from man to man is rare. The resistant cysts are ingested with contaminated food or water and reach the large bowel of man. There they remain in the intestinal lumen; asymptomatic infection is more frequent than disease, as in pigs. In certain circumstances, however, the balantidia become virulent and aggressive and invade the intestinal wall, causing colitis. Balantidial dysentery is similar to amoebic dysentery in many respects.

Theories about the action of balantidia, i.e. how and why the parasites penetrate the intestinal wall, perhaps by production of proteolytic enzymes, and the following action of pathogenic bacteria, are still theories and need confirmation. Haematogenous and lymphogenous dissemination of parasites to extraintestinal sites occurs very rarely.

Pathology

The organ involved is almost exclusively the large bowel in its entire length (Fig. 18.3). Occasionally, the appendix is affected[4] and, exceptionally, the terminal ileum. Extraintestinal organs are involved very seldom: in single cases, involvement of mesenteric lymph nodes (Fig. 18.4), liver[5], lungs, ureter and bladder, vagina[8] and exocervix (Fig. 18.5) has been reported.

Grossly, intestinal lesions are very similar to those caused by amoebae. Also, ulcers of the mucosa with undermined borders may be found in the large bowel.

Microscopically, the balantidia in smears and tissue sections cannot be overlooked because they are so large and show characteristic structures. In fresh preparations, they are very mobile. With routine staining methods, like H&E (Figs. 18.6 and 18.7), iron haematoxylin and also the PAS method (Fig. 18.8), they come out well. Special staining techniques are not necessary for the detection of these large protozoans. Small balantidia may be confused with amoebae.

The tissue reaction consists of non-specific inflammation with exudation of leukocytes and abscess formation. Almost always, bacterial infection is associated.

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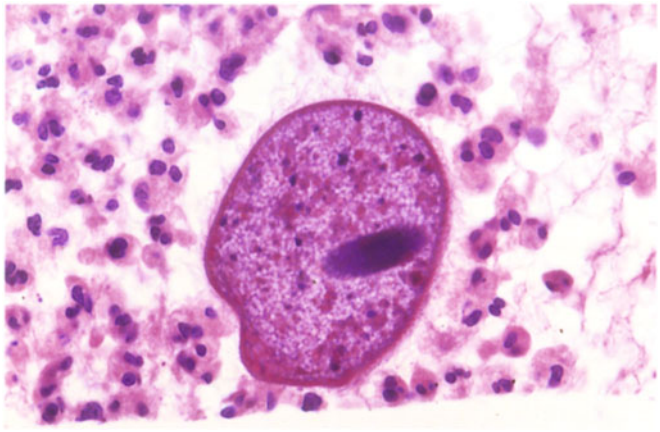

Fig. 18.1 Trophozoite of *Balantidium coli* in a faecal smear. H&E

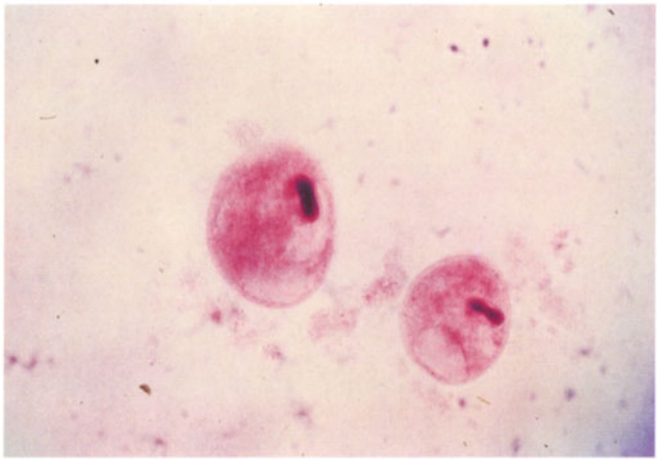

Fig. 18.2 Cysts of *Balantidium coli* in a faecal smear. H&E

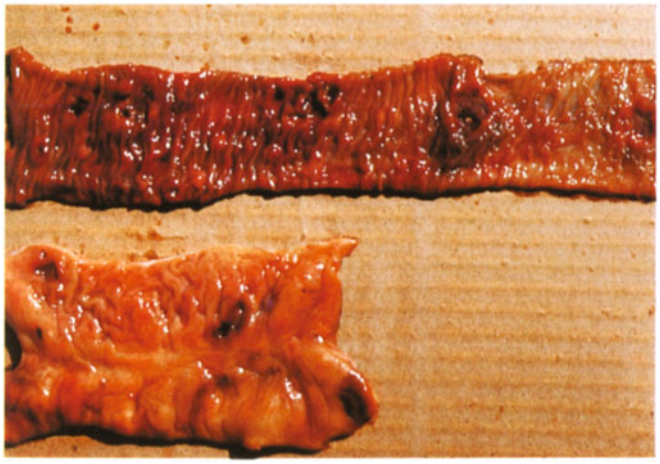

Fig. 18.3 Perforated ulcers in the ileum and colon due to balantidiasis. Autopsy material

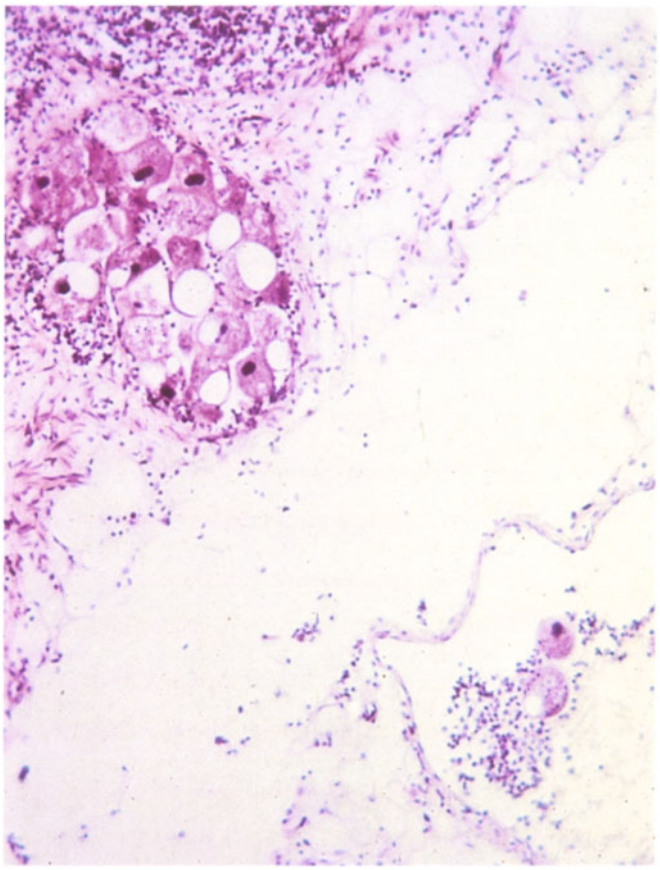

Fig. 18.4 Numerous balantidia inside a lymph vessel near a mesenteric lymph node and several parasites in a lymph vessel of the mesocolon. H&E

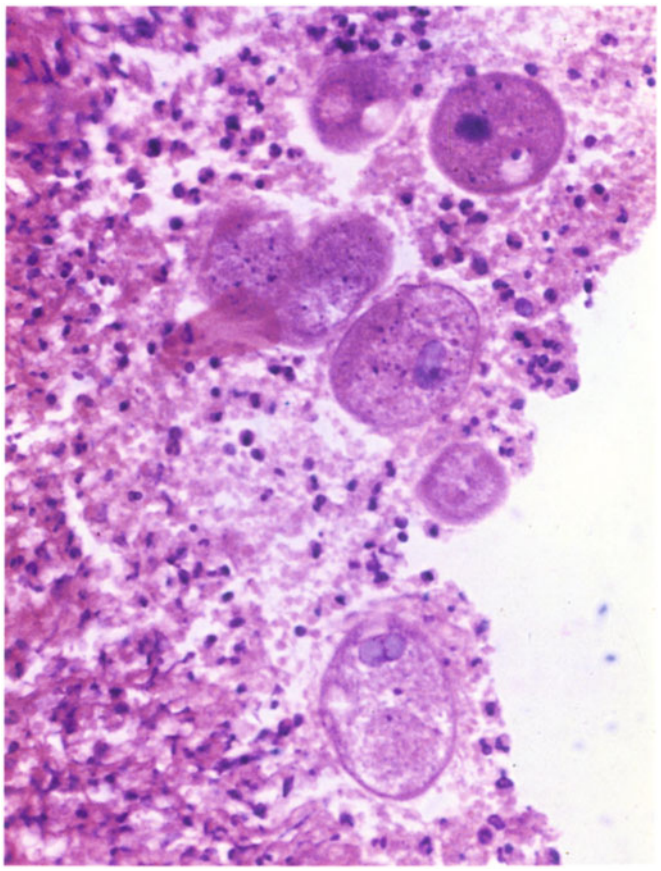

Fig. 18.5 Marked necrotizing inflammation at the exocervix with several balantidia. H&E

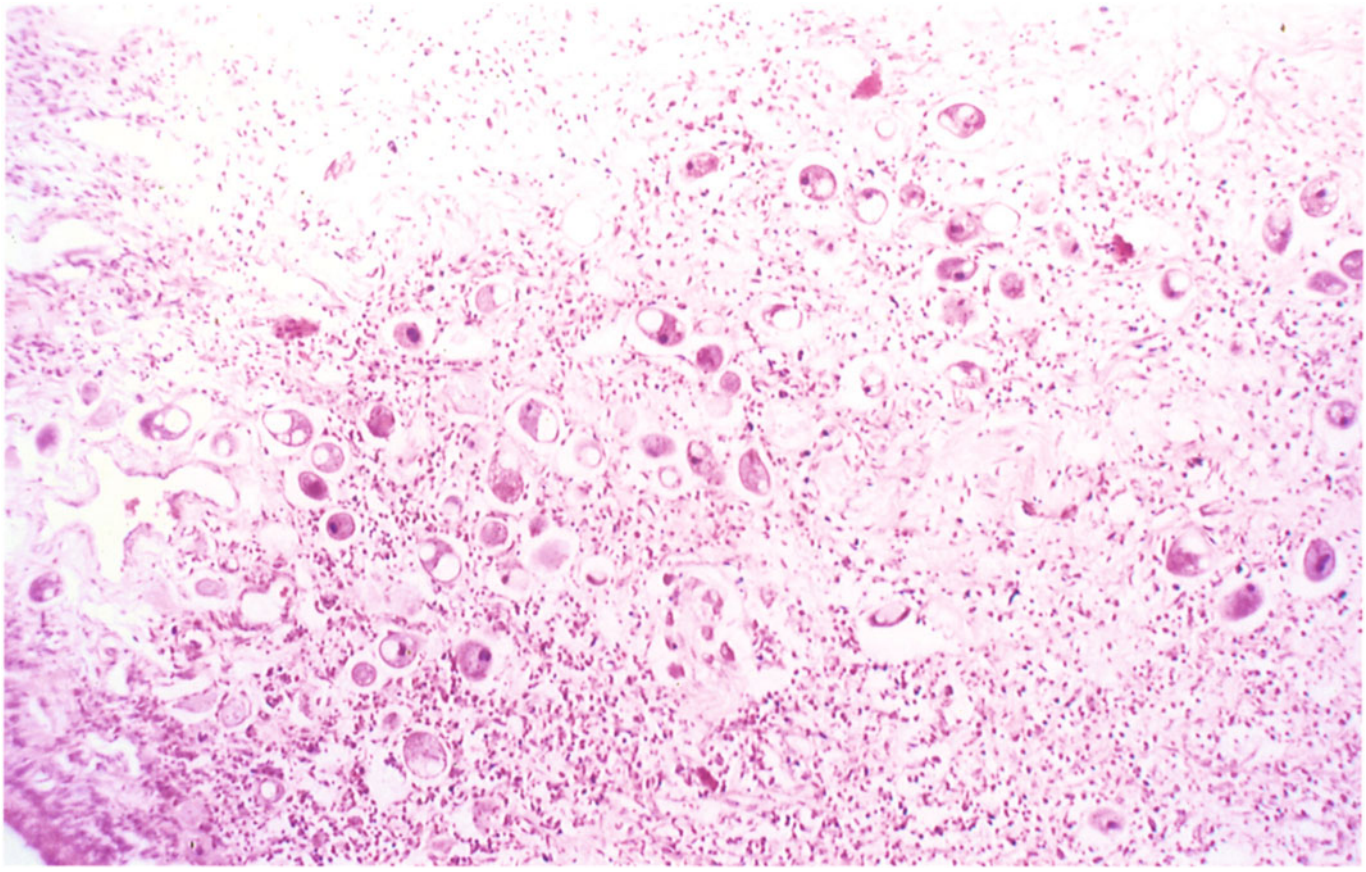

Fig. 18.6 Numerous balantidia in the wall of the large bowel (balantidial colitis) at low power. H&E

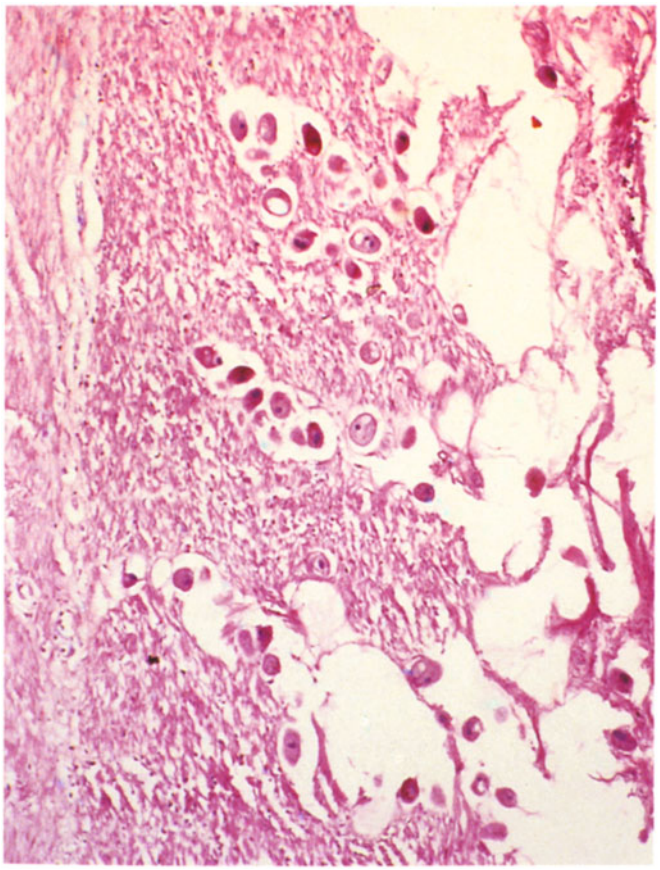

Fig. 18.7 Necrotizing colitis with groups of balantidia in the mucosa. H&E

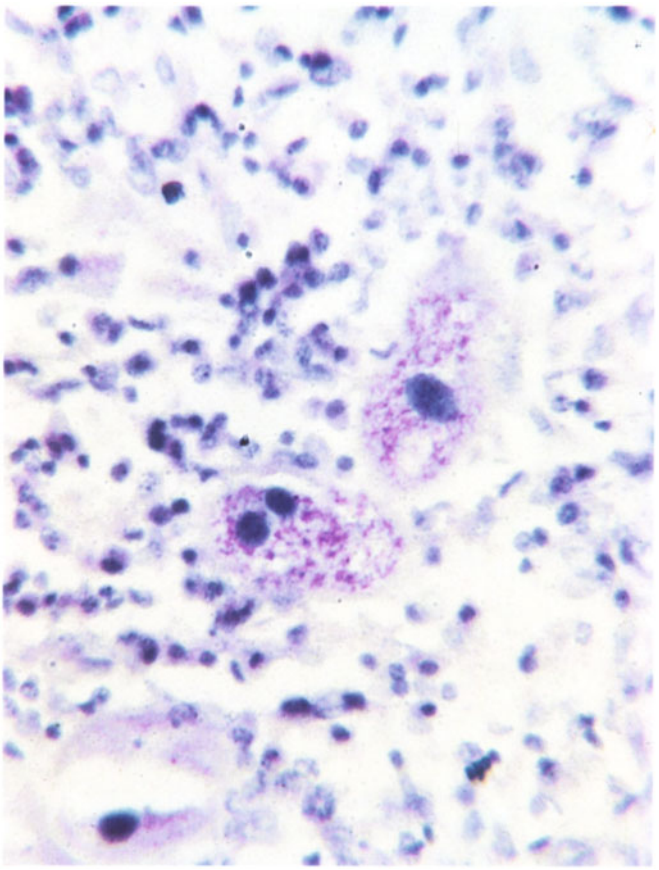

Fig. 18.8 Balantidia with red granulae in tissue section. PAS

19. MICROSPORIDIOSIS

Introduction

This protozoan disease is also named microsporidosis or nosematosis, and, lately, other names have been proposed since other microsporidians have been described. Microsporidiosis, to our knowledge, is not mentioned in European medial literature. The first case of human infection was reported in 1959[1].

Only a few cases of infection in humans have been reported, but, in recent years, their number has increased, although named differently. A few cases seem to have been opportunistic infections. The majority of cases have occurred in Asia and in children. This disease has not been reported in Venezuela.

The first natural infection in a lower animal species was described by Pasteur in 1870 in the silk worm (*Bombyx mori*). Later, microsporidians were found in numerous animal species (dogs, insects, fish and laboratory animals[2–7]). Vervet monkeys and rabbits may be infected experimentally[2,8].

Little is known about the clinical course or form. Only a few of the human cases have survived. Infections are known in immunocompromised persons and AIDS patients, with increasing frequency lately[6–17].

Clinical diagnosis is based on the observation of microsporidians in tissue sections of biopsies or in smears of fluids. Inoculation of laboratory animals cannot be used as a diagnostic tool because natural infection with microsporidians in these animals is normal.

The parasite

Microsporidia belong to the class Cnidosporidia and phylum Microspora. The species of the genera *Nosema, Encephalitozoon, Enterocytozoon* and *Thelohania* are the agents which produce the infection in mammals and, occasionally, man. The spores of *Nosema connori* are oval in shape and measure 2–4 μm. They stain weakly with H&E, are sometimes birefractive with polarized light[18] and their membranes stain weakly with the Grocott method. A characteristic element of the spores is a PAS-positive corpuscle. With a phase contrast microscope or an electron microscope, typical filaments arranged in a spiral form may be discerned.

Pathogenesis

Very little is known about transmission or infection. The portal of entry seems to be the digestive tract. The cycle of evolution of the microsporidians is not completely known at present.

Pathology

Mostly, musculature (Fig. 19.1) is involved, although several other tissues may be affected too. In some reported cases[17,19,20], the ocular cornea was invaded by this parasite, and, in another[21], tumour cells of a pancreatic carcinoma harboured microsporidians. Intestinal involvement has been reported mostly in AIDS patients[12–15]. Details of gross lesions are not known.

Microscopically, the spores of microsporidians are arranged in clusters, mostly intracellularly. Some microsporidians show a halo (Fig. 19.2), others a PAS-positive dot (Fig. 19.3) and others are slightly Gram-positive. The Grocott-positive membranes indicate fungal cells (Fig. 19.4).

Regarding differential diagnosis of the microsporidians, all small micro-organisms and inclusion bodies must be mentioned (Figs. 19.5 and 19.6). Muscle fibres may be invaded by the following protozoa: *Trypanosoma cruzi, Toxoplasma gondii, Sarcocystis* sp. and *Nosema connori*.

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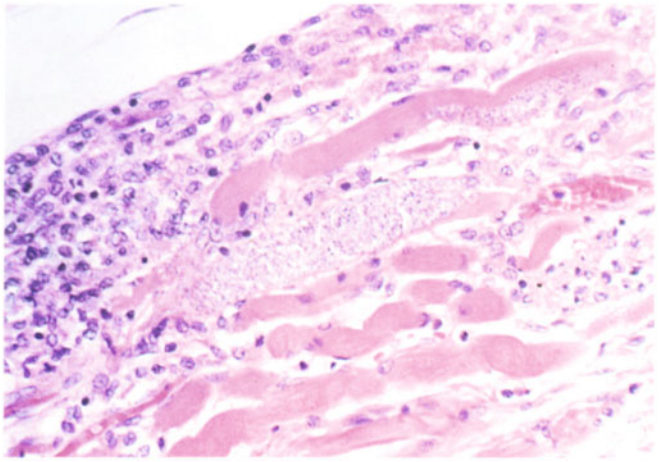

Fig. 19.1 Cluster of microsporidians in a muscle fibre of the diaphragm. with inflammation in the vicinity. H&E

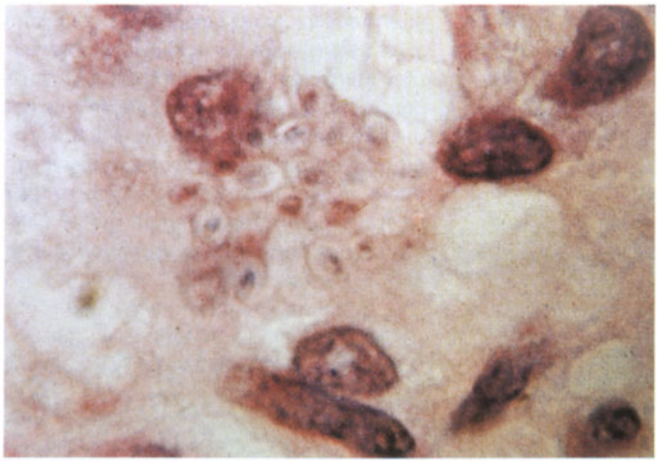

Fig. 19.2 Spores with a halo in a lesion caused by microsporidians. H&E

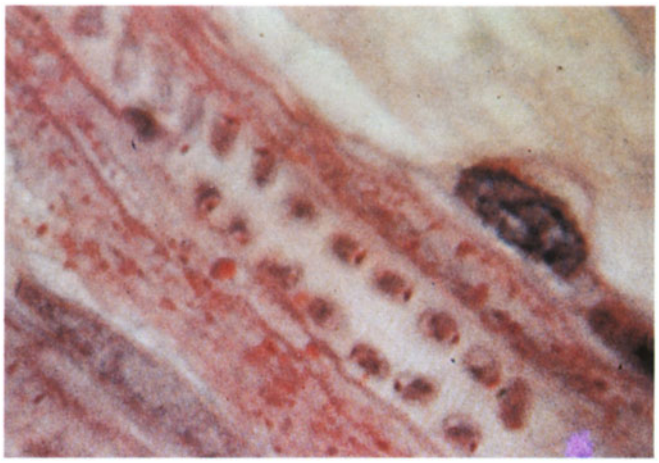

Fig. 19.3 Spores with PAS-positive dots inside a muscle fibre. PAS

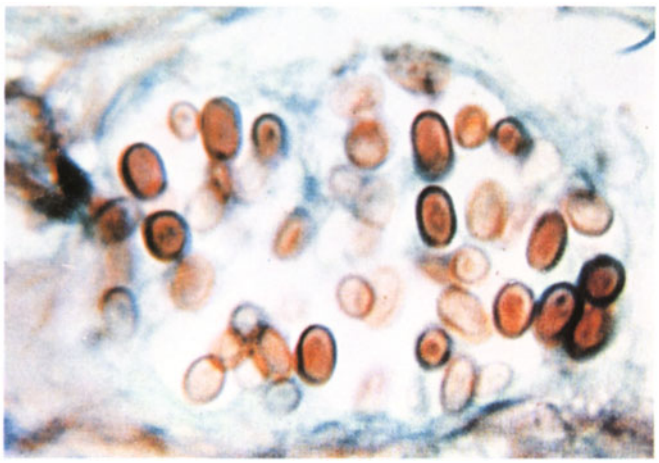

Fig. 19.4 These microsporidians at high power are partly Grocott-positive. Grocott

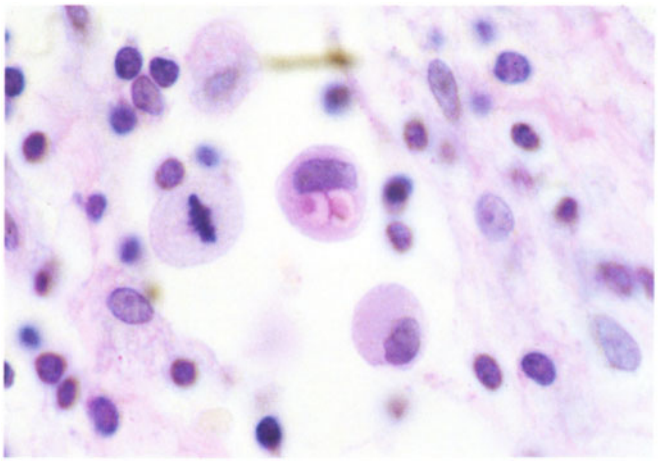

Fig. 19.5 Cytoplasmic inclusion bodies in carcinoma cells (metastasis of gastric carcinoma) are similar to microsporidians. PAS

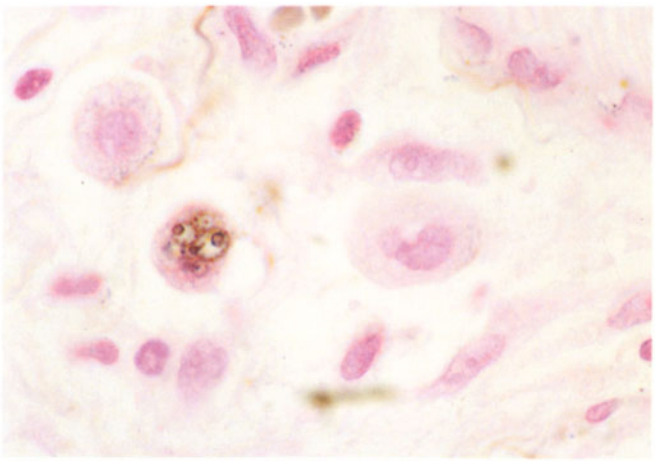

Fig. 19.6 Same case as Fig. 19.5. The inclusion bodies, variable in size, are positive with this special staining method. Grocott