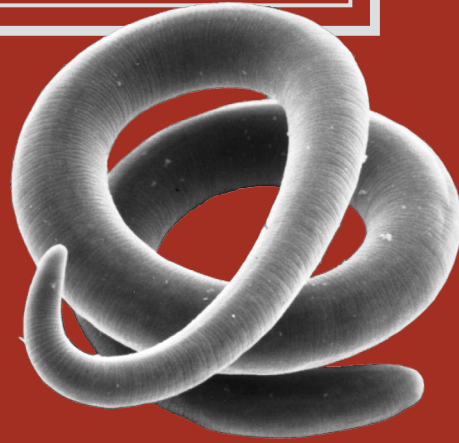This is an excerpt from **Parasitic Diseases 5th Edition**

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6. African trypanosomiasis:

Trypanosoma brucei gambiense
(Dutton 1902)
Trypanosoma brucei rhodesiense
(Stephens and Fantham 1910)

Introduction

Trypanosoma brucei gambiense and *T. b. rhodesiense* are vector-borne flagellated protozoans found only in Africa. They live in the bloodstream of mammals and cause a disease in humans referred to as African sleeping sickness. Some 60 million people are at risk from both varieties of trypanosomes, and the disease has recently been classified as a re-emerging infection on that continent. 450,000 cases occurred in 2005 throughout sub-Saharan Africa according to the World Health Organization.[1] This was due mainly to extensive forced migration caused by civil turmoil leading to the breakdown of control measures against the vector. *T. brucei* and related species are part of a larger group of organisms characterized by the presence of a kinetoplast (a primitive mitochondrion), and are termed the kinetoplastidae (e.g., *Leishmania spp., T. cruzi*).

Tsetse flies of the genus *Glossina* transmit trypanosomes throughout a broad region of equatorial Africa, ecologically restricted to the boundaries of the Sahara desert to the north and the dryer temperate regions south of the equator. *T. b. gambiense* is found mainly in the western and central African countries of Cameroon,

Figure 6.1. Metacyclic trypanomastigote from the tsetse fly. 20 μm. Courtesy I. Cunningham.

Figure 6.2. Tsetse fly taking a blood meal.

Benin, Central African Republic, Gabon, Ghana, Guinea, Ivory Coast, Liberia, Nigeria, Senegal, The Gambia, Uganda, and the Democratic Republic of Congo. *T. b. rhodesiense* occurs mainly in Burundi, Botswana, Congo, Ethiopia, Kenya, Mozambique, Rwanda, Sudan, Tanzania, Uganda, Zambia, and Zimbabwe.

In West Africa, the domestic pig is considered the only important reservoir host for *T. b. gambiense*.[2] In contrast, many species of wild animals and domestic cattle of East Africa are reservoirs for *T. b. rhodesiense*. Trypanosomiasis is also a serious problem in animal husbandry because many species of the trypanosomes related to those infecting humans cause severe disease in cattle.[3,4] Its genome is currently under intensive investigation and a complete sequence should be forthcoming in the near future.[5]
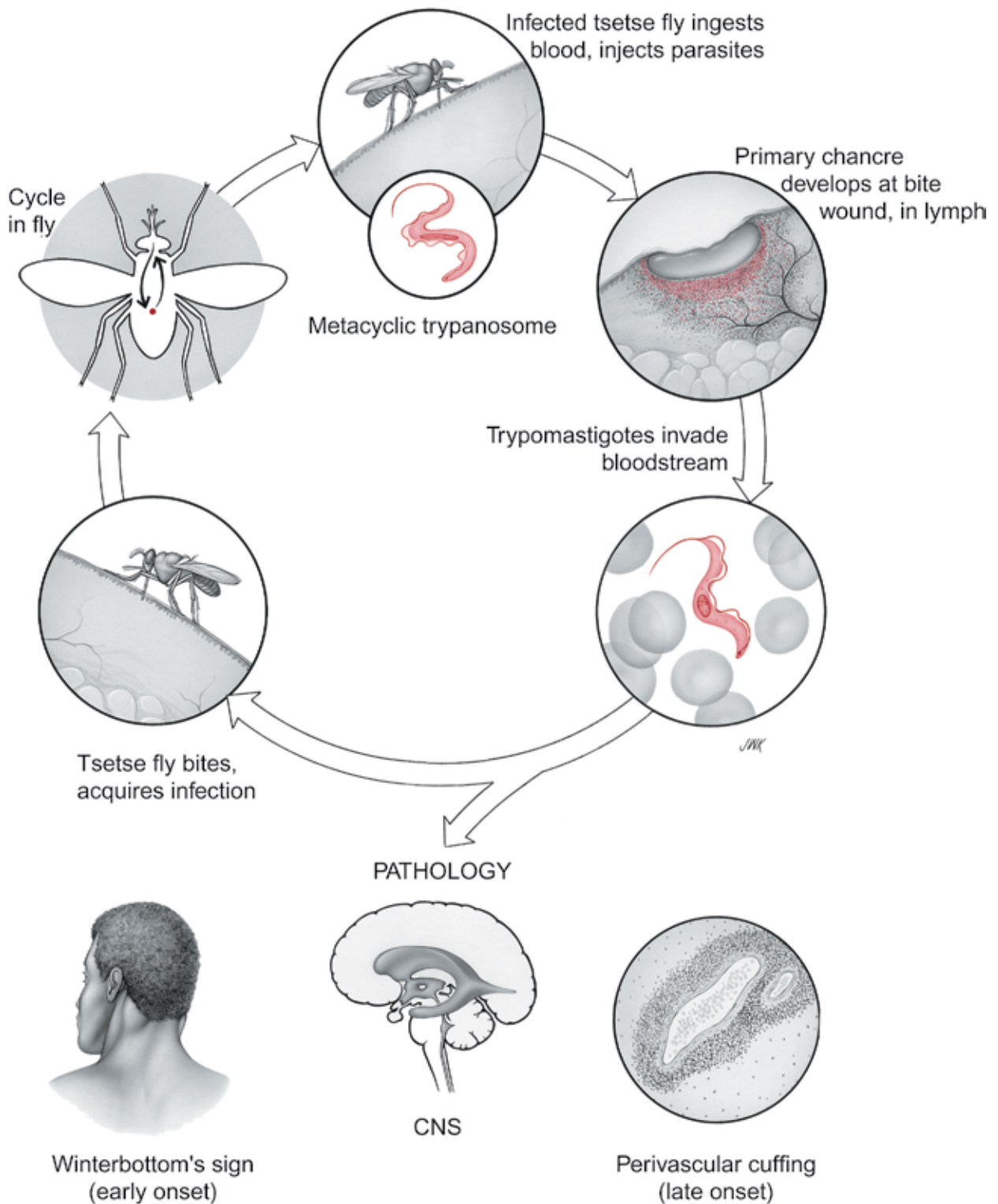
Historical Information

Sleeping sickness has been known in Europe since the 1700s, when Atkins published his observations of the disease.[6] In 1895, Bruce[7] described the disease and its causative agent by showing that *nagana*, a disease of cattle, was caused by trypanosomes, and that tsetse flies were the vectors. Ford, in 1902,[8] working in West Africa, described a clinical condition in humans similar to that in cattle caused by *T. b. gambiense*. Stephens and Fantham, in 1910,[9] isolated and described *T. b. rhodesiense* from human cases in East Africa. The two organisms are morphologically identical. Kinghorn and Yorke, in 1912,[10] demonstrated that *T. b. rhodesiense* could be transmitted from humans to animals by tsetse flies. They also concluded that game animals, such as water buck, hartebeest, impala, and warthog, could serve as reservoir hosts for the East African trypanosome.

Life Cycle

The biology of *Trypanosoma brucei* has been reviewed.[11] Biological characteristics of the two subspecies are so similar that what follows applies to both. African trypanosomes live extracellularly, both in the

Trypanosoma brucei gambiense and *T. b. rhodesiense*

Infected tsetse fly ingests
blood, injects parasites

Cycle
in fly

Metacyclic trypanosome

Primary chancre
develops at bite
wound, in lymph

Trypomastigotes invade
bloodstream

Tsetse fly bites,
acquires infection

PATHOLOGY

Winterbottom's sign
(early onset)

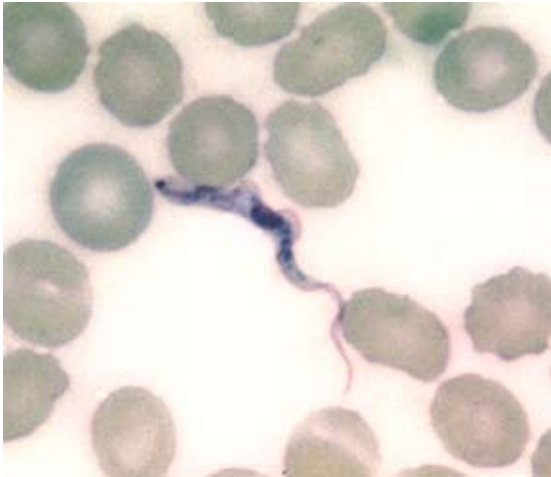CNS

Perivascular cuffing
(late onset)

Figure 6.3. Bloodstream trypanomastigotes. 15 μm.

mammalian and insect host. The bloodstream form measures 25-40 μm in length (Fig. 6.1).

Infection in the human host begins when the infective metacyclic stage is injected by the tsetse fly intradermally (Fig. 6.2). The organisms immediately transform into bloodstream form trypomastigotes (long, slender forms) (Fig. 6.3), and divide by binary fission in the interstitial spaces at the site of the bite. As the result of repeated replication cycles, buildup of metabolic wastes and cell debris occurs, leading to extensive necrosis and the formation of a soft, painless chancre. Replication continues in the blood, resulting in millions of new trypomastigotes. During this time, they behave like anaerobes, processing glucose. Trypanosomes have several intracellular inclusions; the kinetoplast-mitochondrion,[12] the glycosome,[13, 14, 53] and a multiprotein aggregate termed the editosome.[46] One of its unusual features is that all of the DNA of the mitochondrion, which can be up to 25% of the total cell DNA, is localized in the kinetoplast, adjacent to the flagellar pocket. Kinetoplast DNA or kDNA exists in two forms: mini circles and maxi circles.[15] Mini circle DNA encodes guide RNAs that direct extensive editing of RNA transcripts post transcriptionally.[16–18] Maxi circle DNA contains sequences that, when edited, direct the translation of mitochondrial proteins.[19, 20]

In the vertebrate host, trypanosomes depend entirely upon glucose for energy and are highly aerobic, despite the fact that the kinetoplast-mitochondrion completely lacks cytochromes. Instead, mitochondrial oxygen consumption is based on an alternative oxidase that does not produce ATP. The parasite develops a conventional cytochrome chain and TCA cycle in the vector.[21]

The surface of the trypanosome has numerous membrane-associated transport proteins[22, 23] for ob-

taining nucleic acid bases, glucose, and other small molecular weight nutrients.[24] None of these proteins react well with antibodies, because although they lie in exposed regions of membrane, they are shielded by allosteric interference provided by the variant surface glycoprotein (VSG) coat proteins.[25]

This flagellated stage enters the bloodstream through the lymphatics and divides further, producing a patent parasitemia. The number of parasites in the blood is generally so low that diagnosis by microscopic examination is often negative. At some point, trypanosomes enter the central nervous system, with serious pathological consequences for humans. Some parasites transform into the non-dividing short, stumpy form, which has a biochemistry similar to those of the long, slender form and the form found in the insect vector.[26]

The tsetse fly becomes infected by ingesting a blood meal from an infected host.[27] These short, stumpy forms are pre-adapted to the vector, having a well-developed mitochondrion with a partial TCA cycle. In the insect vector, the trypanosomes develop into procyclic trypomastigotes in the midgut of the fly, and continue to divide for approximately 10 days. Here they gain a fully functional cytochrome system and TCA cycle. When the division cycles are completed, the organisms migrate to the salivary glands, and transform into epimastigotes. These forms, in turn, divide and transform further into metacyclic trypanosomes, the infective stage for humans and reservoir hosts. The cycle in the insect takes 25-50 days, depending upon the species of the fly, the strain of the trypanosome, and the ambient temperature.[27] If tsetse flies ingest more than one strain of trypanosome, there is the possibility of genetic exchange between the two strains, generating an increase in genetic diversity in an organism that may not have a sexual cycle.[28]

The vector remains infected for life (2-3 months).

Figure 6.4. Impala, one of many reservoirs for *Trypanosoma brucei rhodesiense*.
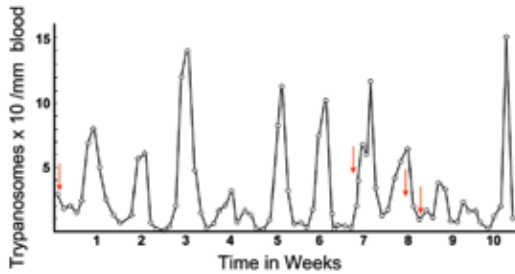
Figure 6.5. Parasitemia in a patient infected with *T. b. rhodesiense*. Each peak of parasitemia represents a new antigenic variant. Arrows indicate attempts at chemotherapy. Ultimately, the patient died of overwhelming infection.

Tsetse flies inject over 40,000 metacyclic trypanosomes when they feed. The minimum infective dose for most hosts is 300-500 organisms, although experimental animals have been infected with a single organism.

Infection can also be acquired by eating raw meat from an infected animal.[29] In East Africa, this mode of transmission may be important in maintaining the cycle in some reservoir hosts, such as lions, cheetahs, leopards, and scavengers (hyenas, dogs, etc.).

Cellular and Molecular Pathogenesis

African trypanosomes have evolved a balanced coexistence between themselves and their hosts, since none of the wild animals native to East Africa appear to be severely affected by this parasite (Fig. 6.4). In contrast, more recently evolved animals, such as humans, or the numerous mammalian species introduced into Africa from Europe, such as all non-African breeds of cattle, all suffer the pathological consequences of infection from this group of hemoflagellates.

Mechanisms of Escape from Host Immunity
African trypanosomes have evolved several molecular strategies enabling them to avoid elimination from the mammalian host: varying the antigenicity of its surface protein coat, destruction of compliment, and the ability to survive in elevated levels of interferon-γ.[30,][31] All infected mammals produce antibodies against a membrane-associated antigen of the trypanosome referred to as the variant surface glycoprotein (VSG).[32] Specific IgG antibodies destroy all clonal organisms sharing the same surface protein (e.g., VSG-1) by agglutination and lysis. However, a few trypanosomes can produce a second variety of surface protein (e.g., VSG-2), with a completely different antigenic signature, in addition to the original one. If some of these organisms shed VSG-1 prior to encountering antibody against it, and continue to synthesize VSG-2 exclusively, they es-

cape lysis,[33] and replace those that were destroyed.[34] A second IgG antibody with specificity to VSG-2 arises, killing all VSG-2 parasites but selecting for VSG-3 organisms, and so on. This antigen-antibody battle between parasite and host continues, until the infected individual is overcome by exhaustion due to glucose depletion and the buildup of metabolic wastes from the parasite (Fig. 6.5).

Antigenic variation depends upon trans-splicing of mRNAs encoded by genes that have been rearranged, duplicated,[35] and expressed at a unique site in the genome.[36] In experimental animal models, the repertoire of antigenic variants of the bloodstream trypomastigotes is large, numbering in the hundreds. In human disease, the maximum number of VSGs that can be produced remains unknown, although the genome codes for about 1000. Antigenic variation is the reason why vaccine development against this pathogen has not progressed.[37]

Neuropathology
Trypanosomes remain in the bloodstream and lymph nodes throughout the infection period, which can last weeks to years, depending upon the subspecies of parasite and the immune capabilities of the infected individual. All nodes become enlarged, but enlargement of the posterior cervical nodes is the most noticeable.[38] The invasion of the central nervous system induces a lethargic condition, leading eventually to coma and death.[39] Organisms enter the central nervous system much earlier in the infection with *T. b. rhodesiense* than with *T. b. gambiense*. Replication of the parasite in the CSF results in leptomeningitis, cerebral edema, and encephalopathy.[40] Dysregulated inflammation is the chief pathological correlate, with perivascular cuffing consisting of infiltrates of glial cells, lymphocytes, and plasma cells (Fig. 6.6). Astrocytes are induced to
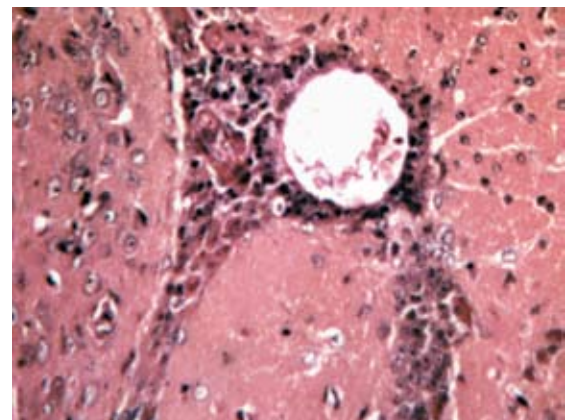

Figure 6.6. Perivascular cuffing around vein in brain of patient who died of sleeping sickness.
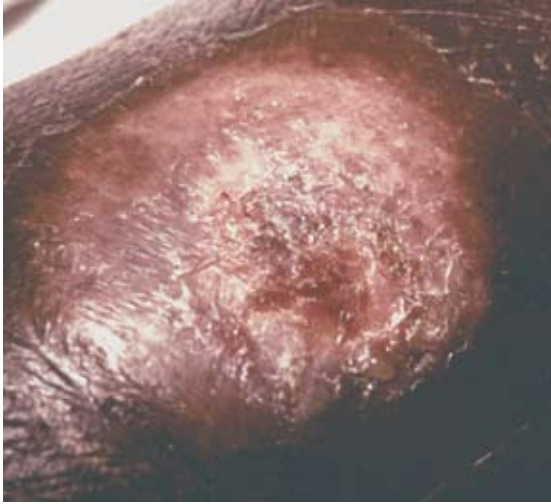
Figure 6.7. Chancre due to early infection with *T. b. gambiense*. Courtesy WHO.

release prostaglandin D2 (PGD2), a sleep regulating molecule.[41] Anti-inflammatory interleukins (IL-10 and TGF-β) are produced early on in the infection, but loose their effectiveness during the chronic and late phase.[42]

Clinical Disease

Both trypanosomes cause the same type of clinical disease; only the time scale of their evolution differs. Infection rapidly progresses on to disease with *T. b. rhodesiense*, with an incubation period of only 2-3 weeks, and a course of several weeks. Central nervous system involvement occurs some 3-4 weeks after infection. In contrast, *T. b. gambiense* has an incubation period of several weeks to months, and may not involve the brain for months or even years.

A painless chancre (Fig. 6.7) containing the dividing organisms develops at the site of the bite within 2-5 days and subsequently heals. Intermittent fever coincides with the organisms entering the bloodstream. Some patients develop rashes, particularly *erythema multiforme*. Lymphadenopathy of the posterior cervical nodes, referred to as Winterbottoms' sign,[38] is characteristic but not always present.

When trypanosomes invade the central nervous system, patients experience severe headache, stiff neck, periods of sleeplessness, and depression. Focal seizures, tremors, and palsies are also common. Coma eventually develops, and the patient dies, usually of associated causes such as pneumonia, inanition, or sepsis.

Anemia is a complication of infection with *T. b. rhodesiense*, but is not always seen due to the fulminating nature of this form of sleeping sickness.[43]

Diagnosis

Definitive diagnosis depends upon finding the organisms in blood smears stained with either Wright's stain or Giemsa stain (Fig. 6.3), or in the cerebrospinal fluid. Aspirates of lymph nodes may also contain organisms. Note that parasites are frequently very rare, even in a patient dying of the disease. Techniques to improve the sensitivity of diagnosis are to examine the buffy coat and the centrifuged sediment of the CSF and to make thick smears of samples stained without fixing, which loses erythrocytes but still reveals the characteristic morphology of the trypanosome.

Real time PCR will most likely become the laboratory method of choice for the rapid diagnosis of sleeping sickness,[44] since PCR has now replaced ELISA. In the field, testing cerebrospinal fluid for the presence of specific IgM antibodies shows promise.[45]

History of travel in an endemic area, recalling a painful fly bite, and the presence of a chancre can guide the clinician to the diagnosis. The differential diagnosis includes syphilis, Leishmaniasis, and malaria. Finding malarial parasites in the blood of a patient with trypanosomiasis is not unusual, and should not mislead the clinician, diverting their attention from the diagnosis of trypanosomiasis. Lymphoma must also be considered in any patients with protracted, unexplained fever.
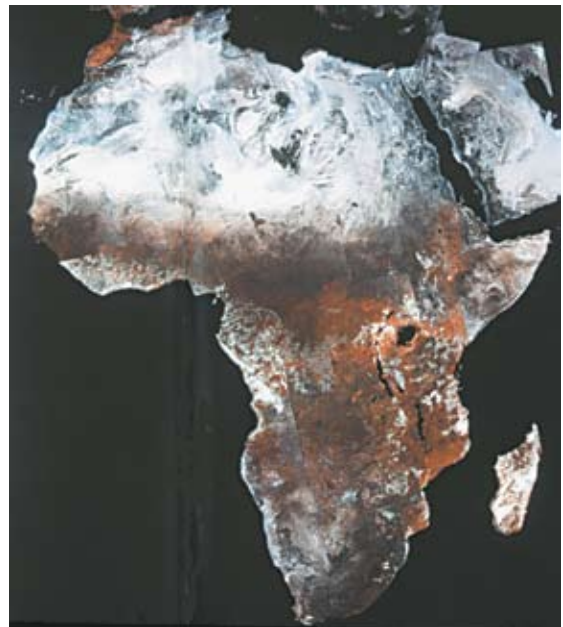

Figure 6.8. Landsat photograph of African continent, colorized to show vegetation (in brown). Photo, NASA.

Treatment

The treatment of sleeping sickness has been reviewed.[47] Suramin can be used only for the early stages of the infection with *T. b. rhodesiense*,[47] since it does not cross the blood-brain barrier. The mode of action of suramin is not known, and is associated with possibly severe kidney dysfunction. Penatmidine is used for the early phase of infection with *T. b. gambiense*. When available, difluoromethylornithine (DFMO, eflornithine) is the recommended drug for all stages of *T. brucei gambiense* infection, but it is far less effective against *T. brucei rhodesiense*.[48] DMFO is a relatively nontoxic drug that irreversibly inhibits the enzyme ornithine decarboxylase, an enzyme essential for polyamine biosynthesis. Since it attacks only a single point in the metabolic pathway for polyamine biosynthesis, resistance is likely to develop, and has already been induced in experimental animals and even in certain instances when humans were receiving treatment. For infection with *T. brucei gambiense*, the cure rates are above 99%. Melarsoprol is often used and is more toxic. It is the only effective drug for treatment of *T. brucei rhodesiense* with CNS involvement, although the drug is associated with encephalopathy in about 3% of cases, and a high rate (>10%) of failure to cure in some instances.[49] A new drug, DB289, with characteristics of pentamidine but with reduced side effects, is in stage II drug trials and may one day soon replace pentamidine.[47]

Prevention and Control

Widespread political upheaval in many parts of Africa (Fig. 6.8) over the last five years has resulted in a dramatic increase in human cases of sleeping sickness.[50] A 2005 WHO report indicated at least 450,000 new cases that year, alone,[1] while prior to 1995, the estimate was fewer than 70,000. Military action and civil unrest in the Sudan, Ethiopia, Sierra Leone, Congo, and Liberia are responsible for forced migration of millions of individuals, placing them at high risk from a number of opportunistic patristic infections. At the same time, control programs for tsetse fly have disappeared in these same regions, exacerbating an already intractable situation. On top of all this turmoil, HIV/AIDS, and malaria have also increased in prevalence, complicating the picture and adding new, unwanted dimensions to the general problem of disease control. Limited resources in countries bordering conflicted areas cannot keep up with the need for vector control, due to large influxes of refugees. Tsetse flies and mosquitoes do not obey political boundaries, and thrive in certain disturbed environments.[51]

Work on vaccines based on VSG antigens has essentially stopped. Other protein antigens, particularly transporters on the membrane of the flagellar pocket and tubulin offer promise.[52] To learn more about the ecology of tsetse flies and control programs that take advantage of their biology, see www.medicalecology.org/diseases/d_african_trypano.htm.

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