Report of the Scientific Working Group
meeting on African trypanosomiasis
Geneva, 4-8 June, 2001
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Message from the Executive Director, Communicable Diseases

Following the appointment of Dr Brundtland as Director-General of the World Health Organization (WHO) in 1998, functional restructuring placed both the control of infectious diseases and tropical diseases research under one cluster. This has permitted better identification of priorities and the linking of research with prevention and control activities, resulting in joint fundraising. The recent agreement with the drug industry on chemotherapy of African trypanosomiasis is a product of such joint activities. It is hoped that other agreements emphasizing sleeping sickness will also be signed.

In addition, there has been a concerted effort to move infectious diseases higher in the economic development agenda in various world economic fora, resulting in political commitment by governments of both developed and disease endemic countries, and in financial commitment by members of the G8, in particular France and the United States of America, to the Global Fund for AIDS and Health. Currently, the Global Fund is directed at the three major (based on morbidity and mortality data) infectious diseases – namely malaria, tuberculosis and AIDS. This will lead to improved health delivery systems that will be better placed to deal with other diseases such as sleeping sickness. It is envisaged that the health delivery systems will undergo diversification, allowing governments, NGOs and the private sector to work together to deliver health services more effectively. This will be of particular importance for diseases such as sleeping sickness, where NGOs have had to be depended on for continued support during periods of decreased resources.

This meeting aims to draw up a well thought out research agenda, provide data that can be used to convince policy-makers to place infectious diseases at the forefront of development activities, and show the world that the job on infectious diseases is not yet done.

David L. Heymann
World Health Organization
Geneva, June 2001
Message from the Director, TDR

In 1998, the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) underwent an external review. It was noted that, with the reorganization of the Programme into functional units, certain aspects of disease focus had been neglected. It was decided, therefore, that TDR should hold scientific working group (SWG) meetings to address each of the ten diseases handled by the Programme. Two of these SWG meetings, this SWG on African trypanosomiasis being the first, will be held each year in order that all ten diseases are covered in five years. It is expected that this first meeting will set a research agenda for African trypanosomiasis, closely linked to control needs and open to the opportunities that science and technology can provide, which will act as a guide not only to TDR but also to other parties interested in research on African trypanosomiasis.

Funding through TDR is on the increase, and a full-time disease research coordinator for African trypanosomiasis is to be recruited. This is a reflection of growing donor interest in the disease, for which a significant amount of funding has already been assured. However, the funding available is largely restricted to particular projects and limited in geographical coverage to a small proportion of the 250 known foci of sleeping sickness in Africa. Furthermore, the number and mix of donors is limited, as is the period covered by the current funding agreement, which is only for five years. Securing resources for the period following these five years is therefore a priority. Strategies to attract more funding will include creating a supportive environment, joining forces with others of like mind, and using the voices of affected countries. The current matrix approach to programme management in TDR lays great emphasis on accountability and gives a certain amount of funding security to diseases that are unlikely to receive additional funding from alternative sources.

Carlos M Morel
Director, TDR
Geneva, June 2001
Preface

During the last ten years, sleeping sickness has only been marginally recognized as a health problem and a research priority, but the recent re-emerging outbreaks and increasing drug resistance have painfully demonstrated the potential danger in any endemic area. Although sleeping sickness was brought to low endemic levels practically everywhere in Africa during the sixties, since then, most national sleeping sickness control programmes have gradually been sacrificed in favour of more prominent health needs. As a result, the core of national know-how simply disappeared while dependency on external support was rendered even greater. Recent outbreaks have opened eyes worldwide as to how serious the situation is, and increasing awareness is manifest in scientific publications, the layman’s press, and television programmes. The donor community, as well as industry, has been alerted, and is now providing unprecedented levels of support to both control and research. As this report states, it was an opportune time to convene a Scientific Working Group.

This TDR Scientific Working Group was asked to provide technical guidance to donors, responsible officers at national level, and research institutions as to the concrete directions research should take, in the Group’s opinion, and where resources should be made available. The relatively large group of approximately 30 participants permitted wide geographical representation and ample coverage in terms of scientific discipline. In order to guarantee that the recommendations issuing from the meeting were critical, the SWG decided to restrict these to the two of highest priority for each subsection of the report.

This report briefly reviews the recent emergence and re-emergence of trypanosomiasis, and elaborates on as yet unanswered questions and issues such as the role of animal reservoirs in T. b. gambiense transmission, the need for epidemiological indicators for monitoring control interventions, and the lack of a “gold standard” for new and existing diagnostic tests. One research area pertinent to the identification of the reservoir of infection and transmission patterns would be the improvement and validation of the current bloodmeal analysis technology. New tools for vector control per se are not considered a priority at this moment – “the tool box is full” – but research into the question of why relatively little use is being made of the currently available tools is indicated.

In the past, practically everywhere, trypanosomiasis control was in the hands of vertical programmes, which meant a great deal of adaptation was needed, both mentally and logistically, to integrate personnel and equipment into the general health services. Although in a few countries this took place gradually and more or less satisfactorily, in countries with a large number of endemic foci and where large numbers of personnel and facilities were involved, integration was, and remains, a complicated process. In such cases, local studies on new delivery pathways are urgently needed.
The disability adjusted life year (DALY) has finally found grace in socioeconomic reasoning and is now being applied in several economic analyses for trypanosomiasis control. However, the data collected so far indicate that further refinement is needed to be able to compare the cost-effectiveness of the different control approaches. Also, little is yet known of the personal costs a patient spends on seeking care, his/her participation in cost sharing, or the costs of death and incapacity for the family.

The arsenal of trypanocidal drugs, being scarce already, is becoming more and more limited due to increasing drug resistance. As an immediate relief measure, applied research to help develop standard protocols for optimal use of combinations of currently available compounds is needed. As a long-range objective, the highest priority should be given to laboratory research to develop new molecules.

When Mott published, in 1899, his classic report demonstrating perivascular infiltration to be the main histopathological characteristic of sleeping sickness, he probably would not have imagined how, a good hundred years later, specialists would still be busy following up the pathogenesis of these brain lesions. Although only a few research groups have been involved up till now, this field of interesting research is relevant to the possible development of non-invasive tests for determining the stage of disease. Pathogenesis research could also lead to prevention and treatment of the fatal reactive encephalopathies which occur during treatment.

Much attention has been paid in this report to genomics as a means of refining parasite identification, and to genetics for studies on the vectorial capacity of tsetse flies. The *T. brucei* genome network of collaborating centres, initiated by TDR jointly with other institutions, is expected to strengthen functional links at an international level. Emphasis is placed on strengthening laboratories in Africa for participation in the work on bioinformatics and genomics.

As a result of the lack of career openings, a high proportion of ex-trainees has left the trypanosomiasis field. One of the recommendations of the Group is to improve the position of local scientists in African research institutes by allowing for salary supplements. Making research careers more attractive and stable should eventually result in establishing an acceptable core of scientists in the endemic countries. Although the drain of specific knowledge and experience to elsewhere has been disappointing, the Group has no doubt that continued training is the only solution to filling the gap in availability of scientists and technicians in endemic countries.

Considering the improved funding perspectives for both control and research, it is now opportune to make efforts to enhance collaboration between the two. In order to be able to generate
valid disease data from each endemic country, it is recommended that a research nucleus group be identified and that inter-country collaboration and comparison of results between endemic countries be strongly advocated.

With this up-to-date review of the present epidemiological situation and the actual control needs, the Scientific Working Group calls for a response from the scientific community all over the world. The improved funding possibilities that exist at the present time provide the necessary momentum for introducing new research and control projects and for strengthening relevant ongoing programmes.

Peter de Raadt,
SWG Chairperson
1 Executive summary

The Scientific Working Group (SWG) meeting on African trypanosomiasis brought together a multidisciplinary group of scientists, partners and collaborators from academia, public and private sectors, sleeping sickness control programmes, and both disease endemic and disease non-endemic countries. The objectives of the meeting were to chart out a global research agenda on African trypanosomiasis, closely linked to control needs and open to opportunities arising from basic science, to guide TDR and other parties interested in research on African trypanosomiasis and provide data that can be used in advocacy to convince policy-makers and donor agencies to place control of this disease higher on their agenda.

The Group reviewed the current status of knowledge and made recommendations on what tools are needed for appropriate and effective management and control of sleeping sickness. Three broad areas were considered:

- Epidemiology, disease surveillance and control.
- Drug development, preclinical and clinical studies, drug resistance.
- Pathogenesis and applied genomics.

Considerable progress has been achieved in research on African trypanosomiasis in the following areas: diagnosis and development of diagnostic tests; epidemiology, host-parasite-vector relationships, animal reservoirs; development of tsetse traps and screens; understanding of the pathology of the disease; and, drug targeted biochemistry of trypanosomes. However, this progress has not been matched in control of the disease, due to lack of capacity to sustain improved interventions and to civil disorder in some endemic countries.

It was noted that recent scientific advances in applied genomics and bioinformatics provide opportunities that can be exploited to provide new tools for control of disease, and recent support from the pharmaceutical industry and a private foundation has given impetus to tackling the problem of African trypanosomiasis. However, the challenges of obtaining adequate donor support and commitment of governments of endemic countries, and of personnel recruitment and retention, are daunting. Research on African trypanosomiasis is an international effort and the need for partnerships cannot be overemphasized.

The meeting provided an opportunity to identify knowledge that could be exploited for developing new, and improving existing, tools for management of disease and vectors, and to determine the needs for research capability strengthening in basic sciences in disease endemic countries. TDR’s comparative advantage in enhancing existing, and developing new partnerships for maximal application of knowledge was highlighted.

The following are the highest priority recommendations made by the SWG. The SWG:

- noted with concern that sleeping sickness is a re-emerging disease which is not given due attention by governments of endemic countries or the international donor community until it attains epidemic proportions, and recommended that the disease burden and cost effectiveness of control strategies be calculated to show that the social and economic consequences of epidemics outweigh the cost of maintaining surveillance;
- noted that lack of appropriate field applicable diagnostic tools for disease detection, and stage of disease, critically affect the control of sleeping sickness, and recommended that simple non-invasive, single-format, field-applicable tests for diagnosis and determination of stage of disease be developed and validated;
• considered the small number of anti-trypanosomal drugs available, and recommended that synthetic and natural product libraries be integrated for use in drug development;

• acknowledged that the development of drugs for late-stage disease is hindered by the blood-brain barrier, which prevents the delivery of drugs to the central nervous system (CNS), and recommended that CNS-penetration models be used in drug development strategies for human African trypanosomiasis, and that strategies which facilitate the delivery of drugs across the blood brain barrier be developed;

• took into account the limited evidence suggesting that combination therapy with late-stage drugs has an additive effect, and, in view of the urgent need to have alternative treatments for melarsoprol refractory patients, recommended that combination chemotherapy using late-stage drugs be optimized;

• acknowledged the difficulties associated with the treatment of sleeping sickness patients, and the lengthy post-treatment follow-up, and recommended investigating and applying new information on immune parameters to (i) the determination of stage of disease, (ii) the prevention and/or amelioration of the encephalitis and encephalopathy associated with the disease and its treatment respectively, and (iii) the development and validation of a non-invasive protocol for determining cure and shortening the duration of after-treatment follow-up;

• expressed concern that the limited number of suitable centres in Africa created a situation where research was increasingly compartmentalized, and recommended that the capacity of laboratories/centres within Africa be strengthened in the basic sciences, including in bioinformatics, genomics and applied genomics, drug discovery and development;

• noted the possible co-existence of *T. b. rhodesiense* and *T. b. gambiense* sleeping sickness patients in foci where refugees are settled, the difficulties in differentiating the two trypanosome species, and the different treatment schedules for both forms of the disease, and recommended that genomics be applied to comparing *T. brucei* sub species, strains and life cycle stages for their differentiation and disease management;

• appreciated the important role that vector control plays in reducing the transmission of vector borne diseases, and recommended that tsetse-trypanosome interactions be investigated to determine the molecular basis of refractoriness for trypanosome transmission and mechanisms for driving desirable genes into vector populations;

• noted with concern the absence, within TDR, of a full-time staff member responsible for research activities on African trypanosomiasis, and recommended recruitment of such a person;

• recognized the inadequacy of infrastructure for research in different endemic countries, and recommended networking and cross country comparison of research progress to assist in capacity building and stimulate cross border interest and advocacy;

• noted with concern the low priority given to African trypanosomiasis by governments of endemic countries, and recommended strong advocacy to persuade disease endemic country governments to accord priority attention to research and control of African trypanosomiasis amidst their other health priorities.

Other high priority recommendations, with suggestions for studies and/or actions that should be undertaken to meet the objectives of the meeting, are listed in the text.
2 Overview and objectives

African trypanosomiasis is caused by protozoan parasites, trypanosomes, which are transmitted by tsetse flies (of the genus Glossina). The disease occurs in two forms: a chronic form caused by Trypanosoma brucei gambiense, which occurs in West and Central Africa; and an acute form, caused by T. b. rhodesiense, which occurs in Eastern and Southern Africa. The chronic infection lasts for years, whilst the acute disease may last for only weeks before death occurs, if treatment is not administered.

The epidemiology of sleeping sickness is complex and transmission cycles are subject to interactions between humans, tsetse flies and trypanosomes, and significantly, in T. b. rhodesiense sleeping sickness, domestic and wild animals. In T. b. gambiense disease, the classical human-fly-human transmission cycle occurs in both endemic and epidemic situations.

Sleeping sickness is a re-emergent disease, but does not get due attention, probably because its impact is regional. The disease occurs in 36 sub-Saharan countries, within the area of distribution of the tsetse fly. Over 60 million people living in some 250 foci within this region are at risk of contracting the disease (see figure 1).

Figure 1 - The focal distribution of human African trypanosomiasis

Left of dotted line: Trypanosoma brucei gambiense foci in West and Central Africa
Right of dotted line: T. b. rhodesiense foci in Eastern and South Africa
Source: WHO picture library
The number of cases reported annually is over 40,000, but this is highly underestimated, due to difficulties in diagnosis and remoteness of affected areas. It has been estimated that the actual number of cases is at least 300,000, the vast majority of whom are not diagnosed or treated (WHO 1998). These figures are relatively small compared to other tropical diseases, but African trypanosomiasis, without intervention, has the propensity to develop into epidemics, making it a major public health problem. Furthermore, the case fatality rate in untreated patients is 100%. This fact, combined with the focal nature of the disease, means that the disability adjusted life years (DALYs) averted per infection cured or prevented are very high. As a result, control of this disease in areas at risk is highly cost-effective, falling well below the accepted threshold value for money of US$25 per DALY averted (Dr A. Shaw, personal communication, see attached working document, Shaw and Cattand, Annex 4 section III).

At the beginning of the last century, huge sleeping sickness epidemics devastated large areas of the continent. In the 1960s, the prevalence of the disease had been successfully reduced to less than 0.1% in all endemic countries, through historic campaigns by the former colonial powers. Soon after independence, however, national governments failed to sustain such programmes due to lack or diversion of resources to other more pressing health problems. Breakdown of specialized mobile teams and health facilities in several countries, as a consequence of war and civil strife or change in health policy, resulted in dramatic resurgence of the disease, the distribution of which corresponds closely with that of major conflicts in sub-Saharan Africa.

The social and economic impact of sleeping sickness is often underestimated. During epidemics, large proportions of communities are affected, with loss of life and untold suffering. These have serious social and economic consequences, which far outweigh the cost of maintaining surveillance. The disease has been a major cause of depopulation of large tracts of Africa. The fear it causes has led to abandonment of fertile lands, and is an impediment to development. Some affected countries have agriculture-based economies, and workers in cocoa and coffee plantations are always at risk of contracting the disease, consequently decreasing the labour force. This is reinforced by the fact that the disease mainly strikes the active adult population.

Regular medical surveillance, involving accurate case detection and appropriate treatment, and tsetse control where applicable, is the backbone of the strategy for the control of sleeping sickness (WHO, 1998). With the available tools, control is a continuing effort rather than eradication. Experience has shown that where control is interrupted, for a variety of reasons, resurgence of the disease occurs sooner or later.

Over recent years, human trypanosomiasis has been the subject of renewed interest among the donor community and scientists. Substantial voluntary contributions have been made by Belgium and France for research and control in the endemic countries, as well as by the pharmaceutical industry, but these contributions only partly cover the current needs, and the number of donors is still very limited. Nongovernmental organizations (NGOs) have clearly committed efforts to participate in control. Special articles on the trypanosomiasis problem have appeared.

The objectives of the scientific working group (SWG) were to:

- Identify areas where there are gaps in knowledge, and studies that are necessary to fill these gaps.
- Identify research that is directly relevant to control programmes and treatment centres as a priority.
- Promote development of new tools for control and new methods for use of old tools, and effective approaches to disease control.
- Set objectives for research capability strengthening for basic science, genomics and applied genomics, drug discovery and development in disease endemic countries.

References

3 Epidemiology, disease surveillance and control

EMERGENCE AND RE-EMERGENCE

The persistence and re-emergence of sleeping sickness in Africa is attributable to various factors including lack of surveillance, shortage of drugs, and several other determinants which operate at different levels. Means for regular surveillance are often inadequate. At the individual and family levels, there may be inadequate knowledge about disease symptoms, transmission dynamics and treatment. Population movements, such as seasonal migration and refugees, may increase human-fly contact and hinder regular medical surveillance of the population at risk. In rhodesiense sleeping sickness, cattle movements also increase the risk of infection. Agro-ecological changes may alter tsetse habitat and increase human-fly contact. A significant resurgence of the disease has occurred, notably in Angola, the Democratic Republic of the Congo, Sudan and Uganda. New foci have also emerged in recent years.

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<td><em>T. b. gambiense</em></td>
<td>Angola</td>
<td>6 786</td>
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<td>287</td>
<td>299</td>
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Source: Compiled from the working documents of the various countries.

Ministries of health, research organizations and services often lack, or do not have, adequate economic resources for sleeping sickness control programmes due to competing health priorities. Recruitment of medium-level personnel is inhibited by lack of incentives and career prospects. Ministries may lack funds for the purchase of diagnostic tests and drugs, except as part of externally-funded programmes.

Central governments often accord sleeping sickness a low priority, until it assumes epidemic proportions. In addition, political upheavals, civil strife and wars lead to the breakdown of health services and of control programmes.

The international community is prepared to mobilize resources when epidemics occur but is often unable to provide long-term support for surveillance and preventive measures in endemic situations. Until recently, the continued production of anti-trypanosomal drugs was in question. This problem has been resolved for the next five years through generous donations.

Other factors that increase the risk of infection and human-fly contact include agricultural development such as coffee and cocoa plantations, and the tourism industry.
Reservoir hosts play an important part in sustaining endemicity and in the re-emergence of epidemics. In the case of *T. b. rhodesiense* sleeping sickness, several wild and domestic animal reservoir hosts have been identified and certain outbreaks brought under control by treating nearby cattle. Although natural infections with *T. b. gambiense* have been reported in domestic animals such as pigs, dogs, sheep and cattle, and may occur in wild animals as well, the role of an animal reservoir in the epidemiology of *T. b. gambiense* remains as yet undetermined. Another important group of reservoir hosts, especially in *T. b. gambiense* epidemiology, are the human carriers: infected individuals who remain undiagnosed due to inadequate surveillance and/or the limitations of current diagnostic tools in demonstrating low levels of trypanosome infections. The following areas for research were identified:

- Assessment of the epidemiological significance of an animal reservoir for gambiense sleeping sickness using new approaches such as the molecular approach.
- Assessment of the epidemiological and clinical significance of “unconfirmed cases”, defined as individuals with clinical signs, or as cases where indirect evidence of infection such as sero-positivity or polymerase chain reaction (PCR)-positivity exists but where the parasite cannot be demonstrated by microscopy.

by pharmaceutical companies. However, arrangements need to be made to ensure a sustained supply beyond this period.

In order to ensure that research results are comparable over a wide area, advantage should be taken of the potential of networking (for more general data collection) and using consortia of investigators, as appropriate, including public/private partnerships. Avenues should be explored as to how to make better use of existing networks, such as the Programme Against African Trypanosomiasis (PAAT) managed by the Food and Agriculture Organization (FAO), Organization of African Unity (OAU) and WHO, and new networks, such as the WHO surveillance network, should be supported.

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**EPIDEMIOLOGY**

**Reservoir Studies**

Reservoir hosts play an important part in sustaining endemicity and in the re-emergence of epidemics.
Incidence and Prevalence

To design optimal control strategies and estimate the burden of disease, knowledge of current prevalence and the impact of control measures is required. This could be obtained by:

- Standardizing and extending reporting systems using geographic information systems (GIS).
- Collating and analysing existing epidemiological data on the impact of past control schemes on prevalence.
- Introducing a standardized protocol for monitoring the impact of control measures on epidemiological indicators.
- Epidemiological modelling to better understand and predict the disease’s behaviour, e.g. to obtain better estimates of the ratio of unreported to reported cases, or of the basic reproductive number, Ro.

The validation of diagnostic tests is needed to obtain more accurate estimates of the real disease prevalence.

SURVEILLANCE AND INTERVENTION FOR CONTROL

Diagnostics

Currently, the screening tests used are only available in bulk presentation, which limits their use where small numbers of people or individuals need to be tested, e.g. in rural health centres, for surveillance on a small scale. There is a need for further development of simple, field applicable, single test formats (such as dipsticks), with high specificity. A multicentre validation of diagnostics based on molecular techniques (e.g. PCR) for epidemiological and clinical studies is strongly recommended. The need for field applicable, non-invasive diagnostic methods is recognized, especially to avoid lumbar punctures.

The main constraint to the development and validation of diagnostic tests is the lack of a gold
standard. Statistical methods exist for the evaluation of diagnostic tests in the absence of a gold standard, but so far these have not been applied to the diagnosis of human African trypanosomiasis. It is recommended that the use of these methods should be explored.

For stage determination and follow-up, the following investigations should be undertaken:

- Multicentre validation of latex/IgM and PCR tests on cerebrospinal fluid.
- Identification of new markers of neuropathogenesis, e.g. cytokines.
- Evaluation of the use of antigen detection tests for follow-up.

**Vectors**

A range of tools for the control of tsetse flies have been developed over the last twenty years, some of which can be applied by communities. However, their adoption and application by communities in endemic areas has not been sustainable. The exact role of the vector in relation to transmission of the disease from the various animal reservoir hosts is unclear. The following research areas were identified:

- The constraints affecting sustained use of vector control tools by affected communities.
- Comparison, validation and improvement of available tools for blood-meal analysis.

**Changing Institutional Environment**

Far-reaching institutional changes have occurred during the last decade which have had a major impact on the organization and implementation of sleeping sickness control. These include decentralization, integration of human African trypanosomiasis (HAT) control into the primary health care (PHC) system, cost recovery/sharing, and varying degrees of community involvement in surveillance and control. Capacity building in policy and health systems, to help identify and remedy the changes that have adversely affected disease control, is recommended. The following areas of research were identified:

- Evaluation of the impact of changes in delivery pathways for sleeping sickness control on the effectiveness of control measures.
- Investigation of the effects of changes in cost-sharing arrangements on disease detection rates, compliance, and effectiveness of control.

The monitoring protocols recommended above should be applied here.
Controlling sleeping sickness is a highly cost-effective intervention, with the cost per DALY comparing very favourably with other health interventions, and falling well below the accepted value of US$25 per DALY averted (Dr A. Shaw, personal communication). This reflects the focal nature of the disease, and the fact that the case fatality rate in untreated patients is 100%. Although the funding situation has improved somewhat, and greater awareness of sleeping sickness as a public health priority exists, it is vital to reinforce and extend this by generating appropriate socioeconomic information in order to:

- Determine the financial resources that are required for control.
- Choose the most appropriate and cost-effective control strategies.
- Promote advocacy through better understanding of the economic burden of the disease.
- Provide guidelines for allocating resources amongst competing health needs.

It is recommended that social science research address the following issues:

- Costing of the different control strategies to cover both endemic and epidemic situations, NGO and national programmes, and a range of countries.
- The direct and indirect costs to patients and their families of obtaining diagnosis, treatment, hospitalization, and follow-up examinations, as well as the costs of permanent disability.
- Refinement of the work done so far on calculation of DALYs, and its extension to other settings.
- Clarification of issues influencing community and individual support of, and involvement in, control measures including:
  - The development of approaches for enhancing and sustaining community participation in the control and surveillance of sleeping sickness in endemic areas.
  - The possible existence of gender issues in the diagnosis and treatment of sleeping sickness, focusing on knowledge, practice and health care seeking patterns.

When combined with the epidemiological information outlined in the section above (on epidemiology), such studies would enable the burden of disease to be estimated and the cost-effectiveness of different interventions to be calculated and compared.
RECOMMENDATIONS

The following are the highest and high priority recommendations from this section:

**Highest priority**
- Development and validation of non-invasive, field-applicable, single-test format diagnostics tests, including tests for disease-stage determination.
- Calculation of burden of disease and cost-effectiveness of control strategies.

**High priority**
- Assessment of the epidemiological and clinical significance of ‘unconfirmed suspects’.
- Systematic monitoring of disease incidence and prevalence, especially in relation to control measures.
- Identification of issues influencing individual and community participation in control measures.
4 Drug development, preclinical and clinical studies and drug resistance

PRECLINICAL STUDIES

The frequency and extent of use of the standard drugs against African trypanosomiasis, melarsoprol and pentamidine, is likely to lead to the development of resistance. Indeed, there has been an increase of late-stage cases refractory to melarsoprol treatment in the past decade (Legros et al, 1999). The availability of these agents, and of efornithine (DFMO), was not assured until recently. Even now, it is only assured for the next five years. With the exception of a new pentamidine-related drug (DB 289), which is due to enter phase II clinical trials in 2001, no new candidate agents are currently in the advanced stage of development. All the drugs are expensive and require hospitalization for administration by the parenteral route. In addition, melarsoprol is associated with a 10% incidence of reactive encephalopathy that is fatal in up to 5% of the victims. Therefore, there is an urgent need for novel, safe, rapidly-acting and inexpensive agents for the treatment of human African trypanosomiasis (HAT) in the 21st Century.

During the late stages of African trypanosomiasis, parasites lodge in privileged sites within the central nervous system, causing encephalitis. The biological nature of such parasites, the mechanisms by which they, and the drugs that cure late-stage infections, cross the blood-brain barrier, are unknown. There is evidence that efornithine acts additively with non-permeating drugs in late-stage infections and suppresses the encephalitis. The nature of these drug interactions is not known but understanding them is critical to the development of new approaches to chemotherapy.

In the past decade, drug discovery has proceeded along biochemical target-based approaches with little success. But with the advent of combinatorial chemistry and the sequencing of the trypanosome genome, new techniques can now be applied to the development of novel, specific and non-toxic agents for HAT. In addition, to foster continuation of research and development of new drugs after the end of the currently assured five-year period, capacity strengthening of laboratories and/or centres within Africa for drug discovery and development is recommended.

Resistance to Arsenicals and Diamidines

Laboratory studies have identified the P2 amino-purine transporter as one route of entry into trypanosomes for both melarsoprol and pentamidine. Loss or alterations to this transporter, caused by genetic modifications to the TbAT1 gene, can contribute to the development of resistance. However, other transporters have been implicated in the uptake of pentamidine, and possibly other analogues, since parasites lacking the P2 transporter have been shown to be sensitive to pentamidine. Moreover, genetically modified parasites, lacking the TbAT1 gene, are only marginally less sensitive to melarsoprol than wild type trypanosomes.

Treatment failure with melarsoprol may also be attributed to the level of melarsoprol permeating into the cerebrospinal fluid (CSF), which varies considerably between individuals. Concentrations of this drug within the central nervous system (CNS) are close to the minimum inhibitory concentration (MIC) of the drug against T. b. gambiense. Hence, modest increas-
es in MIC values in parasites can increase the proportion of late-stage patients showing refractoriness to treatment with melarsoprol. Therefore, the tripartite relationship between drug, host and parasite, and cross-resistance between veterinary trypanocides, e.g. diminazene aceturate, and HAT drugs, needs consideration in determining treatment failure in the field.

Three areas of research are recommended:

- Identification of modes of uptake, and of action and mechanisms of resistance to arsenicals and diamidines in veterinary and human use. The influence of both parasite and host factors on treatment failure requires consideration.
- Comparison of the mechanisms underlying treatment failure in field isolates of the parasite with the mechanisms underlying resistance in laboratory strains of the parasite in which resistance has been induced.
- Reliable diagnosis of drug resistance in the field.

**Blood-Brain Barrier**

The development of therapeutic drugs for the treatment of late stage HAT is limited by the blood-brain barrier (BBB) that prevents the free distribution of drugs into the CNS. An essential component in the development of drugs for the treatment of late stage HAT is to design or select compounds that can cross the BBB. This approach has been a priority in the development of drugs for the treatment of Alzheimer’s disease, brain tumours and neuro-psychiatric conditions, where novel in vitro methods to screen for compounds that cross the BBB or deliver drugs across the BBB have been utilized. It is recommended that the relevance of these approaches in the treatment of HAT be investigated and applied as the first approach in the drug screening pathway, followed by more focused animal model studies.

The following investigations are recommended:

- The use of models of CNS penetration (BBB models) for inclusion in the HAT drug development pathway, in particular:
  - In silico models that use the physico-chemical properties of compounds to determine which are most likely to distribute from the blood to the brain (primary screen).
  - In vitro cell culture models (e.g. MDCK and endothelial cells) that enable the study of permeation of drugs through a cell layer that has "tight" junctions (secondary screen).
- Strategies that facilitate the delivery of drugs across the blood-brain barrier in animal models using known trypanocides, including:
  - A bradykinin agonist (RMP-7, Cereport) that is on clinical trial for delivery of anticancer and antiviral drugs into the brain.
  - The role of drugs that modulate the CNS inflammatory response (for example, DFMO and azathioprine) on drug access and activity in animal models.
Role of CNS Trypanosomes

Drug action and efficacy depend on the physiological/metabolic state of the trypanosome, which may be different for stages in the CNS and CSF from those in the bloodstream. Metabolically inactive and non-dividing forms tend to be less sensitive to drugs, and, depending on the mode of action of the drug, can even be completely insensitive. Apart from parasites in the brain and CSF, trypanosomes in other tissue niches that are less accessible to the drugs, must be taken into account. Research therefore should be directed at:

- Development of suitable models for studies on CNS and CSF trypanosomes, including in vitro models and animal models.
- Studies on the biology of CNS and CSF parasites, their localization and means of entry from the vascular sites, and their inter-exchange.
- Studies on the metabolic state, and susceptibility to drugs, of CSF and CNS trypanosomes.

Drug Discovery and Drug Targets

While agencies funding scientific research devote considerable resources to carrying out research on the basic biology of trypanosomes, limited funding goes to drug discovery and development projects. Likewise, drug discovery efforts by the pharmaceutical industry, directed specifically towards new agents for the treatment of HAT, are nearly non-existent. A wealth of knowledge about the biology of the organism, and the promise of new drug targets resulting from genome research, will have little impact on the discovery of new drugs without support for the synthesis and testing of new molecules. The use of contemporary drug discovery methods should be encouraged to find new molecules for the treatment of HAT. These methods should follow current drug discovery practices utilized by the pharmaceutical industry. Building facilities and training scientists to carry out drug discovery and development research in endemic countries should be strongly encouraged.

The following areas of research are recommended:

- Drug discovery efforts which integrate synthetic and natural product libraries with structure-based modelling, computational chemistry and high-throughput screening (HTS) both for efficacy and toxicity. Target based libraries will initially require specific biochemical assays while diverse synthetic and natural product libraries will require whole cell assays.
- High-throughput screening of synthetic combinatorial libraries designed based on existing leads or obtained from existing combinatorial libraries in pharmaceutical companies.
- Investigation of target enzymes or pathways which, when inhibited, render parasites non-viable. Examples of such targets are S-adenosylmethionine decarboxylase, myristate metabolism and variable surface glycoprotein (VSG) synthesis, and farnesyltransferase.
- Identification, validation by genetic manipulation, and production of new targets based on exploitation of information from the genome project. Validated targets should enter HTS screens.
RECOMMENDATIONS

The following are the highest and high priority recommendations from this section:

Highest priority

• Drug discovery efforts which integrate synthetic and natural product libraries with structure-based modelling, computational chemistry and high-throughput screening, and whole cell assays, both for efficacy and toxicity.

• Investigation of the use of models of CNS penetration for inclusion in the HAT drug development pathway, and development of strategies that facilitate the delivery of drugs across the blood-brain barrier.

High priority

• Characterization of modes of uptake and action of melarsoprol and diamidines, and identification of mechanisms of treatment failure with these drugs in the field.

• Studies on the biology of CNS and CSF parasites, their localization and means of entry from the vascular site, and their inter-exchange.
CLINICAL STUDIES

Clinical Aspects of Treatment Failure and Monitoring

Treatment failures with melarsoprol have been observed in up to 30% of patients in some foci in north-western Uganda, southern Sudan and northern Angola (Legros, 1999). The WHO coordinated Sleeping Sickness Treatment and Drug Resistance Network is conducting sentinel surveillance for treatment failures. Reasons underlying treatment failures should be investigated.

Application of Existing Drugs

Early-stage drugs

1. Pentamidine
Recent data from pharmacokinetic studies suggest that the half-life of pentamidine is sufficiently long to allow shorter treatment regimens.

2. Suramin
The efficacy of a shorter course for the treatment of early stage T. b. rhodesiense should be explored.

Late-stage drugs

1. Melarsoprol
Currently, the 10-day (conceise) melarsoprol regimen is being reviewed in 17 centres in 7 countries. A full report is expected at the end of 2002.

Preclinical and clinical evaluation of the concise melarsoprol regimen for treatment of T. b. rhodesiense infections is recommended.

The possibilities of a new formulation for melarsoprol should be explored in order to avoid the adverse extravascular effects at the site of administration caused by the currently used solvent propylene glycol. CNS penetration of new formulations should be equivalent or superior to the current formulation.

2. Eflornithine
The ongoing pharmacokinetic study of oral eflornithine monotherapy should be completed, and the results used in planning further development. In addition, development of a new route of synthesis should be considered in view of the technical difficulties with the current route.

3. Nifurtimox
Nifurtimox is being used on a compassionate basis for the treatment of melarsoprol refractory cases. However, it is not registered for treatment of sleeping sickness. Complete development of the drug up to registration is recommended. Currently available data suggest that nifurtimox may be more suitable for use in combination than as monotherapy.

Other potential drugs

1. Diminazene aceturate
Diminazene aceturate has been used by sleeping sickness control programmes in several countries, but has not been registered for human use. Development for human use as well as an oral preparation should be considered.

2. Benznidazole
Limited experimental data suggest that benznidazole has some activity against strains of T. brucei. It has an established safety record in humans in the treatment of American trypanosomiasis (Chagas disease). It should be considered as a possible alternative compound for use in combination therapy of sleeping sickness.

New Compounds

1. DB 289
An international consortium is conducting clinical research to develop DB 289 for oral use against first-stage sleeping sickness. Phase I trials have been concluded and a Phase IIa (proof-of-principle) trial is planned. Results of the investigations will be made available to TDR.
2. Megazol
Limited studies in animal models suggest that megazol is effective as monotherapy in first-stage sleeping sickness and in combination with suramin in second-stage *T. gambiense* infections. Toxicity studies should be completed. Should the data from these studies be favourable, further investigations on the efficacy of monotherapy in first- and second-stage disease, using appropriate animal models, should be carried out.

Combination Therapy

There is experimental and limited clinical evidence suggesting that combinations of presently available late-stage drugs, melarsoprol, eflornithine and/or nifurtimox, act additively (Jennings, 1988, 1993). The current simultaneous availability of these drugs gives an unprecedented opportunity to establish optimal combination treatment regimens. Although there are indications that the early-stage drug suramin, in combination with several other compounds, can cure sleeping sickness, priority should be given to studies of combinations of late-stage drugs.

In view of the urgent need to have available alternative treatments for melarsoprol refractory patients, short-term as well as longer-term solutions are needed. To identify alternative treatment for melarsoprol-refractory patients, the following clinical trials are currently being conducted:

- Combination of melarsoprol and nifurtimox vs. monotherapy with each of the drugs. Preliminary results show that the combination therapy with low-dose consecutive melarsoprol combined with short-duration nifurtimox was superior to monotherapy with either melarsoprol or nifurtimox.
- Trials comparing melarsoprol and nifurtimox, melarsoprol and intravenous (iv) eflornithine, iv eflornithine and nifurtimox, have recently started.

In the longer term, combination treatment regimens should be optimized (i.e. maximum efficacy, minimum toxicity, shortest possible duration, simplicity, with preferably oral administration, and minimal cost). Further pharmacological and preclinical investigations are necessary, including experimental studies on the optimal proportions of drugs in combination treatment regimens. Those investigations should be followed by appropriate clinical trials, preferably including pharmacokinetic data collection. Oral eflornithine would be preferred for combination therapy as the current complicated iv regimen of eflornithine is not suitable for large-scale treatment in rural areas. Drug combinations with melarsoprol should be based on the concise melarsoprol treatment regimen.
Follow-up of Treatment

One of the major problems of clinical trials and case management is the long two-year follow-up period, requiring multiple lumbar punctures. Appropriately planned studies to determine the interval between treatment and relapses, which may allow a reduction of the follow-up period, are recommended. Research on less invasive markers for stage determination and cure should be given high priority, with emphasis on their applicability in the field.

Prevention and Management of Encephalopathy Syndromes

Encephalopathy syndromes, which occur during administration of late-stage drugs, are a major problem in the treatment of sleeping sickness. Research into the underlying mechanisms is critical for the development of improved strategies for prevention and management of the complications of treatment.

Coordination of Clinical Trials

The current number of suitable centres (with adequate equipment, trained staff, accessibility and security) to conduct clinical trials in the field of sleeping sickness is very limited. In view of the anticipated number of clinical trials, coordination will be necessary. It is recommended that a clinical trial group be created within the WHO sleeping sickness treatment and drug resistance network. This group should coordinate clinical trials and provide appropriate training (good clinical practice and ethics).
RECOMMENDATIONS

The following are the highest and high priority recommendations from this section:

**Highest priority**

- Development and optimization of a protocol for combination therapy using late-stage drugs.
- Development of tools for shortening the duration of after-treatment follow-up and for disease-stage determination.

**High priority**

- Development of nifurtimox up to registration for use against African trypanosomiasis.
- Preclinical evaluation of the 10-day concise melarsoprol regimen for treatment of *T. b. rhodesiense* (evaluation in an appropriate monkey model).
- If the toxicological data are favourable, appropriate preclinical studies on the efficacy of megazol in first- and second-stage infections.
- Elucidation of the underlying mechanisms of encephalopathy syndromes in view of their prevention and management.
- Exploration of the possibility of better formulations of melarsoprol.

**References**


5 Pathogenesis and applied genomics

PATHOGENESIS

Background

Proper and safe chemotherapy of trypanosomiasis is acutely dependent upon accurately identifying the clinical stage of infection (staging). Staging has been difficult in the past because of a lack of accurate, sensitive and relatively non-invasive clinical or parasitological tools for diagnosis. However, recent advances in the immunology of trypanosomiasis and in parasite cell biology suggest new avenues for accurate staging. Immunological results from both experimental and clinical trypanosomiasis studies have shown that infection may be broken down into three relatively well-defined stages: early innate response; early acquired immune response; late acquired immune response. Each of these responses exhibits distinguishing prognostic characteristics.

The early innate response is triggered by the presence of trypanosomes within tissues, in sufficiently high numbers, leading to the release of pro-inflammmatory mediators including the cytokines interleukin-1 (IL-1), IL-6, IL-12 and tumour necrosis factor alpha (TNFα) as well as nitric oxide (NO). The early release of these pro-inflammatory factors helps set the stage for early acquired immune responses to the parasite variant surface glycoprotein (VSG). The early release of IL-12 promotes T helper (Th) to release cytokines, particularly interferon-gamma (IFNγ), which is responsible for host resistance against parasites spreading throughout host tissues. This may be more important than anti-VSG antibodies which are only found within blood vessels. This early resistance is, however, superseded by later immune responses that are associated with the spread of trypanosomes to the CNS. The late-stage acquired immune response is typified by a loss of Th1 cells secreting IFNγ and the production of very high levels of the anti-inflammatory cytokine IL-10. The high IL-10 levels are associated with loss of tissue resistance and spread of trypanosomes to the CNS.

Early- and late-stage trypanosomiasis can therefore be characterized by the levels of specific cytokines in patients. High IFNγ but low IL-10 levels are associated with the early non-invasive disease, and high IL-10 but low IFNγ levels are associated with late-stage invasive disease. Since preliminary clinical measurements show that there is concordance with respect to serum and CSF levels of these cytokines, the prospects for determining early- or late-stage disease by testing serum for IFNγ or IL-10 levels, respectively, is clear. Furthermore, examination for cytokine levels, which are very short-lived in serum and CSF, may help clarify whether a seropositive individual has had a recurrence of disease. Moreover, information concerning immune status and trypanosome-derived inflammatory factors (e.g. glycosyl phosphatidylinositol) may impact on how patients are treated for late-stage disease. However, the picture is clouded by new information about the trypanosome itself. The following areas of study were identified:

- Validation of experimental and preliminary clinical results suggesting that stage of disease can be determined by the presence of specific cytokines including IFNγ, IL-10 and TNF.
- Definition of the pathogenesis of the encephalitis associated with late-stage sleeping sickness and the encephalopathy associated with treatment, and
investigation of ways to prevent or ameliorate these phenomena using in vitro and in vivo disease models.

- Utilization of the data and technical approaches of the human genome project to unravel the composite immune response to trypanosomes at each stage of the disease process.

### Trypanosome Biological Phenotype

Trypanosomes have evolved mechanisms to regulate their biological virulence phenotype at the clonal level. There is evidence that clonally derived “virulent variants” are capable of entering the CNS at an accelerated rate in non-human primates, suggesting the possibility for development of discrete potential for CNS invasion within the host. The molecular mechanisms behind such clonal changes are unknown. This information might be useful in choosing the appropriate clinical treatment at any stage of disease. For example, the presence of trypanosomes expressing “CNS invasion markers” at any stage of disease might be used to initiate late-stage chemotherapy. In addition, such information has the potential to detect drug resistant variants in patients and aid the choice of drugs used to treat such patients.

### RECOMMENDATIONS

The following are the highest and high priority recommendations from this section:

**Highest priority**

- Identification of parameters within the host immune system for disease-stage determination and improved management of post treatment encephalopathy.

**High priority**

- Determination of the biological phenotypes of trypanosomes lodging in different tissue compartments to identify tissue tropism and drug-resistance characteristics.
APPLIED GENOMICS

Trypanosoma brucei Genomics

The T. brucei genome network was formed by TDR with a brief to coordinate the analysis and sequencing of the nuclear genome. This huge task can progress most efficiently if the community works together with open sharing of data and resources. The network has sufficient funds (from the Wellcome Trust UK and the US National Institute of Allergy and Infectious Diseases/National Institutes of Health [NIAID/NIH] USA) to complete the sequencing of the megabase chromosomes, where the majority of genes are located, which will take until 2004. Approximately 85-90% of the genome is currently available as fragmentary sequences in databases in the sequencing centre websites, and will ultimately be available in public web-based databases.

The Wellcome Trust has provided funds to establish a genome database, at the Sanger Centre, that will contain all data related to sequencing and functional analysis of genes. Two resource centres have been established: DNA-based resources in Cambridge, and derivative and mutant lines of trypanosomes in Glasgow. These centres are funded to the end of the sequencing phase, at which time a re-appraisal of resource provision will be required.

The TDR planning meeting for the Parasite Genome Initiative assigned some activities to be undertaken in Africa, but these activities were not sustained for various reasons which included reduced funding for African trypanosomiasis by TDR and changes in available technologies and priorities within the genome networks. The involvement of African scientists and TDR is crucial in ensuring the application of information from genomics into disease control. African institutions must develop the capacity to fully exploit the resources from the genome projects. It is anticipated that capacity strengthening in African institutions will impact on the applications from both the parasite and the vector genome projects.

RECOMMENDATIONS

The following are the highest and high priority recommendations from this section:

Highest priority
- Strengthening of capacity of research laboratories/centres in Africa in bioinformatics, genomics and applied genomics.
- Application of genomics to comparison of Trypanosoma brucei subspecies, strains, and life cycle stages.

High priority
- Identification, application and database collation of DNA-based polymorphic markers for species differentiation, reservoir identification and determination of drug resistance.
- Application of bioinformatics and experimental methods of determination of gene function to identify novel drug targets.
- Use of resources available from the human genome project to investigate host response to infection.
Recent advances in molecular technologies, and their application to insects, are being widely explored because they have the potential to result in the development of novel strategies for control of vector borne diseases. The technologies needed to undertake such studies have been developed for other insect vectors and thus can facilitate rapid application to tsetse biology.

Limited field data indicate extensive genetic sub-structuring in populations, which display differences in vectorial capacity. Information on population genetics would facilitate more effective and targeted disease management strategies by identifying sub-populations of flies responsible for disease transmission. There exists a mid-gut symbiont-based genetic transformation system which allows expression of gene products that confer trypanosome refractory phenotypes. Candidate genes with either anti-trypanosomal traits or which, when expressed, can block parasite transformation and/or differentiation, should be identified for expression in the tsetse mid-gut. The eventual genetic engineering of anti-trypanosomal traits into tsetse populations would result in new vector control strategies. The laboratory and field-based research recommended below is necessary to develop the applied approaches that can lead to novel vector-based disease-control strategies.

TSETSE GENETICS

Tsetse-trypanosome interactions
- Determination of the molecular basis of refractoriness for trypanosome transmission.
- Engineering of trypanosome refractory traits into strains of tsetse fly.
- Investigation of gene spreading mechanisms that can be used for population replacement studies, e.g. cytoplasm incompatibility mediated by Wolbachia symbionts of tsetse.

Population genetics of tsetse
- Development of molecular polymorphic DNA markers and their application to field flies to understand the extent of genetic sub-structuring existing in tsetse populations.
- Investigation of mating incompatibilities existing among field populations.
- Investigation of differences in vector competence abilities of genetically isolated sub-populations of flies.
RECOMMENDATIONS

The following are the highest and high priority recommendations from this section:

**Highest priority**
- Determination of the molecular basis of refractoriness for trypanosome transmission and development of mechanisms for driving the desired genes/trait into tsetse populations.

**High priority**
- Development and application of molecular markers to determine genetic sub-structuring and mating incompatibilities in tsetse populations, and vector competence of genetically isolated sub-populations.
- Development of a tsetse-parasite genome network to obtain and coordinate information on expressed sequence tags (EST), genomic sequences, physical map locations of selected genes, and eventual proteomics approaches.
- Coordination of the network by TDR, whose comparative advantage is evidenced by the successful management of the mosquito-parasite genome networks.
6 Cross-cutting issues

RESOURCE FLOW

Funding support for control, and for research and development, requires long-term commitment. Over the past 20 years, many countries have provided support for control of, and research on, African trypanosomiasis; in particular the governments of Belgium, France and UK are major supporters. The European Union, USA, Canada, the Organization of Petroleum Exporting Countries (OPEC) Fund and China are also partners. A list of countries and institutions (by no means complete) that provide financial support is attached (Annex 1).

Considerable sums of money are invested in basic research on trypanosomes by institutions in USA, and EU countries, because the trypanosome is a good model for basic research on cell biology. Consequently, there is probably more information on the cellular structure, biochemistry and molecular biology of trypanosomes than any other non-mammalian cell type, and a great deal is known about the differences between trypanosomes and mammalian cells. However, only a small amount of this knowledge is being applied directly in the management and control of the disease. Some of the knowledge is exploitable for development of new tools for disease and vector control as well as for improved patient management. The SWG meeting provided an opportunity to identify the knowledge that could be exploited for development of new tools and improvement of existing ones for disease and vector management, as well as to determine the needs for research capability strengthening in disease endemic countries for basic sciences. TDR’s comparative advantage in enhancing existing, and developing new, partnerships for maximal application of the available knowledge was highlighted.

The SWG was held at an opportune time when the funding level through TDR is increasing and when African trypanosomiasis is re-surfacing in the global health agenda. The funding and assurance of a five-year drug supply by the private sector provides a unique opportunity to deal with the current epidemic in Africa as well as to develop protocols for combination chemotherapy in the face of increased melarsoprol refractoriness. In addition, the five years provides a period of grace during which TDR and the scientific community can lay the foundations for ensuring continued availability of medicaments beyond the five years by investing in research and capability strengthening within Africa for drug discovery and development as well as for genomics, applied genomics and proteomics. The SWG acknowledged the comparative advantage that TDR has in coordinating and networking the activities of various scientific groups, laboratories and centres in different parts of the world. Consequently, the group recommended recruitment of a full-time professional staff member within TDR to coordinate the various activities on African trypanosomiasis.
ADVOCACY AND MARKETING FOR SLEEPING SICKNESS

The SWG noted with appreciation the increase in funding coming to TDR for African trypanosomiasis. However, there was concern that, in the previous six years or so, there had been little interest from the donor community to fund activities on African trypanosomiasis. A need to present the disease in a way that changes donor perception of the problem was identified. The Group was informed that one way of changing is to present projects that are convincing, focused and concise, emphasizing donor/researcher partnerships in project management and follow-up and the benefits to the target communities. Networking appears to be more attractive to donors than single, isolated projects.

The Group indicated a number of networks already existed, both formal and informal (with NGOs and national systems). A need to enhance collaboration between the groups working in research and those working in control, and also between groups working in disease and non-disease endemic countries, was identified. This would increase the much needed credibility when requesting donor support. The SWG noted that scientists working in disease endemic countries often work in isolation and have difficulties developing the required credibility for donor support, and identified a need to support good ideas from young scientists, enabling them to seek independent funding, i.e. to support them to a point where donors have confidence in them. In addition, a mechanism to assist young scientists to develop their ideas into fundable proposals, where their ideas are likely to provide vital information, should be put in place.

INSTITUTIONAL DEVELOPMENT AND CAPACITY BUILDING

Given the re-emergent nature of African trypanosomiasis, it is necessary to identify and strengthen a nucleus of research groups and institutions in different endemic countries to generate data, on disease burden and surveillance, for advocacy. Where whole institutions are difficult to maintain as disease specific research institutes, their disease mandates could be expanded to enable them to add on other disease research activities while retaining core activities in the area of sleeping sickness.

The SWG commended TDR for the great number of scientists it has supported for post-graduate training. However, continued training of research personnel in African trypanosomiasis, and linking of this training to specific technological needs, which vary from country to country, was recommended. A need to train technical staff for specific needs in research, disease surveillance and management was also identified. The Group further noted the difficulties of sustaining trained staff within the field of African trypanosomiasis in disease endemic countries, and suggested lack of career development opportunities as one of the factors contributing to this shortage.

Staff retention is a global problem, not peculiar to the field of African trypanosomiasis. Those working in this field are in the public sector, where salaries are not attractive and career advancement is slow. Lack of resources to carry out research, and to attend short-term training courses, is a disincentive to remain in the sector. Funding for research, on a more sustainable
basis and for longer periods, is a necessity; while linking of national institutions with overseas ones, with donor support, is an area for possible expansion.

The SWG welcomed the recent TDR initiative of institutional strengthening grants; a call for letters of intent was already issued. TDR is also working on strategies for strengthening whole national health research systems in countries that do not yet have a strong research culture, and a mechanism is being worked out to give priority to funding of research-strengthening proposals.

The commitment of African governments to support trypanosomiasis research and control, with improved terms of service for those working in the sector, is important. Very few countries in the disease endemic areas have identified scientists or technicians in scientific institutions as a national resource with unique operational needs. It was agreed that advocacy would be crucial, and international forums, such as the Organization of African Unity (OAU) International Scientific Council for Trypanosomiasis Research and Control (ISC-TRC), would be used to send the message. The presence of TDR at such forums, and in other African national systems, would also be useful. At the same time, there is a need to ensure appropriate networking in order to avail the necessary assistance in writing good proposals. The possibility of providing salary support with research grants should also be given consideration by TDR.

SOUTH-SOUTH COLLABORATION

In March 2001, WHO held a meeting in Harare, Zimbabwe, to discuss an initiative aimed at increasing discussion and scientific interaction between investigators in disease endemic countries. The meeting was attended by scientists from several countries in Africa, Asia and Latin America, who discussed ways of increasing the collaboration that already exists between investigators in the South and collaborators in more advanced laboratories of the North. The need to develop more sustainable pathways for training, that will make more efficient use of available resources by exploiting opportunities for the exchange of complementary expertise present within laboratories in Africa, Asia and Latin America, was emphasized. This networking will enhance the application of molecular biology techniques and genomics (functional and applied) to developing solutions to public health problems. Initiatives for multicentre projects, which incorporate capacity building and training, will be developed. Additionally, a number of short-term training courses in basic and applied biology, that incorporate the application of the latest technologies, are planned. It is anticipated that the results of this network will include process indicators (academic qualifications, highest quality publications), products (e.g. tools, chemotherapeutic agents), and an impact on alleviating disease.
RECOMMENDATIONS

The following are the highest and high priority recommendations from this section:

- Recruitment by TDR of a full-time professional staff member to coordinate African trypanosomiasis activities.
- Identification and strengthening of a nucleus of research groups and institutions in different endemic countries to generate data on disease surveillance and advocacy.
- Networking and cross-country comparison of research progress to assist in capacity building and stimulate cross border interest and advocacy.
- Strong advocacy to persuade DEC governments to give priority to research and control of African trypanosomiasis amidst other health priorities.
- Payment of a salary component within TDR funded grants to enhance retention of disease endemic country scientists in the field.
Annex 1

RESOURCE FLOW FOR AFRICAN TRYPANOSOMIASIS
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Annex 2
POSITION PAPER
A POSITION PAPER ON AFRICAN TRYPANOSOMIASIS

Felix A.S. Kuzoe
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MEETING THE CHALLENGE
1. New tools are needed for the control of African trypanosomiasis (sleeping sickness); however, these on their own will not improve the trypanosomiasis situation. Factors that militate against the effective use of existing tools, such as continued worsening economies and structural adjustment programmes in the affected countries, will remain.

- Operational research should be conducted on the effective use of available tools with existing capacities, such as the health services, strong control programmes in other diseases, NGOs, etc.
- Development of simple surveillance systems with available tools that can be integrated into available capacities to improve case detection and active screening of populations at risk will permit early diagnosis of cases and increase the number of people under medical surveillance.
- Long-term commitment by the international community to national sleeping sickness control programmes, rather than support for crisis management, will ensure sustainability.
- Social, economic and cultural factors enhance or hinder efforts to control sleeping sickness. There is need for studies on social, socio-cultural and anthropological aspects of endemicity of sleeping sickness. The effects of decentralization of health services on African trypanosomiasis need to be studied for improved management of control programmes.

3. The private sector has the expertise for drug development. However, for diseases such as African trypanosomiasis that have no market for drugs, development must be based on public sector financing.

- The recent award of US$ 15 million by the Bill & Melinda Gates Foundation to a consortium of scientists towards the development of drugs for African trypanosomiasis and Leishmaniasis is a welcome development that has no precedence in the history of African trypanosomiasis. Furthermore, the World Health Organization (WHO) and Aventis Pharma have announced a major initiative to control African trypanosomiasis, whereby Aventis Pharma has committed US$25 million to support WHO’s activities in this field for a period of five years.
- TDR has links with the outside world with special reference to product development. Over its lifetime, it has generated multiple partnerships for product development and has gained considerable experience. Thus, it has been successful and taken some products up to registration in collaboration with the private sector, for example, mefloquine, eflornithine, Ambisome and artemether. More partners are needed in drug discovery research and drug development for African trypanosomiasis.
- Basic research on trypanosomes continues to be funded with considerable sums of money in the North. No drugs have yet come out of basic research by rational drug design. Functional genomics, bioinformatics and proteomics provide new opportunities that should be explored for drug discovery and development of tools for trypanosomiasis control and patient management.
- Institutions in the endemic countries should be strengthened to enable implementation research. Clinical studies should be conducted according to the concept of good clinical practice (GCP). A few clinical research centres or treatment centre networks should be identified and strengthened and clinical investigators given appropriate training.

SUMMARY
This position paper provides an overview of the main features of African trypanosomiasis and the problems relating to its control. It gives a historical account of the disease, its epidemiology and socioeconomic impact. It describes the strategy for control, namely case detection, chemotherapy and vector control, and the limitations. It discusses global funding of basic research on African trypanosomes, and the pivotal role of WHO/TDR in facilitating the evaluation of any spin-offs from the research towards development of public health tools. Considerable progress has been achieved in research on African trypanosomiasis, but this progress has not been reflected in the field due to lack of interest in development of new tools and lack of sustainability of control methods, resulting largely from inadequate resources for public health and wars and civil strife in some endemic countries. With the decrease in resources for African trypanosomiasis in TDR, which started in 1994, there have been grave consequences in endemic countries, both in terms of reduction in human resources and degrading of facilities for research and evaluation of new tools for African trypanosomiasis. To meet the challenge of African trypanosomiasis in the 21st century, this position paper makes a number of proposals.

AFRICAN TRYPANOSOMIASIS AS A PUBLIC HEALTH PROBLEM
At the beginning of the last century, sleeping sickness was perceived by the colonial powers as by far the most important public health problem in Africa. Huge epidemics devastated large areas of the continent. In the 1960s, the prevalence of sleeping sickness was successfully reduced in all endemic countries to less than 0.1%, through historic campaigns by the former colonial powers. Soon after independence, however, national governments were either lacking in resources or had diverted resources to other pressing health problems. Breakdown of specialized mobile teams and health facilities in several countries, as a consequence of war and civil strife or change in health policy, resulted in dramatic resurgence of African try-
panosomiasis, the distribution of which corresponds closely with that of major conflicts in sub-Saharan Africa. In the Democratic Republic of the Congo (DRC), the number of cases being reported yearly has now reached levels comparable to the 1930s, and may result in as many deaths in adults as HIV/AIDS (Ekwanzala et al 1996). And yet African trypanosomiasis is curable. Other countries affected by the resurgence/epidemics are Angola, the DRC, Central African Republic, Sudan and Uganda. Sleeping sickness due to *T. b. rhodesiense* seems to be quiescent at present and less widespread, with active foci occurring in Tanzania and Uganda. African trypanosomiasis is a re-emergent disease, but does not get due attention, probably because its impact is regional.

The disease occurs in some 36 sub-Saharan countries, within the area of distribution of the tsetse fly. Over 60 million people living in some 250 foci within this region are at risk of contracting the disease. The number of cases reported annually is over 40,000, but this is highly underestimated due to the difficulty of diagnosis and remoteness of affected areas. It has been estimated that the actual number of cases is about 300,000 (WHO 1998). Though these figures are relatively small compared to other tropical diseases, African trypanosomiasis, without intervention, has the propensity to develop into epidemics. This characteristic makes it a major public health problem. During epidemics, large proportions of communities are affected with great loss of life and untold human suffering. Epidemics have serious social and economic consequences, which far outweigh the cost of maintaining surveillance. Sleeping sickness has perhaps been a major cause of depopulation of large tracts of Africa, and fear of the disease has led to abandonment of fertile lands and is an impediment to development. The World Bank Report (1993) estimated that, in 1990, there were 55,000 deaths due to African trypanosomiasis and 1.8 million disability adjusted life years (DALYs) lost due to the disease. More recent estimates are rather similar, with 2.1 million DALYs lost and 66,000 deaths in 1999. As a comparison, the number of millions of DALYs lost is estimated at 45.0 for malaria, 4.9 for lymphatic filariasis, 2.0 for leishmaniasis, 1.9 for schistosomiasis, 1.1 for onchocerciasis and 0.7 for Chagas disease (WHO 2000).

THE DISEASE

African trypanosomiasis is caused by protozoan haemoflagellates, trypanosomes that are transmitted by tsetse flies (*Glossina* spp). The disease occurs in two forms: the chronic form caused by *Trypanosoma brucei gambiense*, which occurs in West and Central Africa; and the acute form, caused by *T. b. rhodesiense*, which occurs in Central and Southern Africa. The chronic infection lasts for years, whilst the acute infection may last only for weeks.

Other forms of trypanosomiasis, called nagana, affect livestock, and are considered the most important infectious disease holding back the development of livestock production in much of Africa. The annual losses in meat production attributed to trypanosomiasis are estimated to be US$5 billion (ILRAD 1993). No new drug has entered the market for over 30 years because the disease does not affect livestock in Western developed countries.

Epidemiology

The epidemiology of sleeping sickness is complex and the transmission cycles are subject to interactions between humans, tsetse flies, trypanosomes and, significantly in *T. b. rhodesiense* sleeping sickness, domestic and wild animals. In *T. b. gambiense* disease, the classical human-fly-human transmission cycle occurs in both endemic and epidemic situations. Humans are the important reservoir and hence it is possible to reduce transmission through diagnosis and treatment of the infected population. Although it has been demonstrated by biochemical and DNA techniques that trypanosomes identical to those which cause *T. b. gambiense* disease in humans occur in domestic animals and some game, the significance of these potential reservoir hosts on transmission is not clear. In *T. b. rhodesiense* disease, on the other hand, it has been recognized that infections in humans in endemic situations are acquired from savannah species of tsetse flies, all of which feed preferentially on a wide variety of game. The game-fly-human cycle is typical. Endemic *T. b. rhodesiense* situations are sporadic in nature and patchy in distribution, and adult men tend to be predominantly infected. In epidemic *T. b. rhodesiense*, however, human-fly-human or domestic animal-fly-human cycles predominate. There is equal distribution of infection among men, women, and children. For *T. b. rhodesiense* disease, chemoprophylaxis of the domestic animal reservoir has been recommended as a new approach to control. Available evidence suggests that HIV infection has had little impact on the epidemiology of *T. b. gambiense* African trypanosomiasis (Louis et al 1991; Pepin et al 1992; Meda et al 1995).

Medical Surveillance

Regular medical surveillance, involving case detection and treatment, and tsetse fly control, where applicable, is the backbone of the strategy for control of sleeping sickness (WHO, 1998). With available tools, control is a continuing effort rather than...
eradication. Experience over the last 50 years has shown that, where control efforts are interrupted, e.g. due to civil strife, political upheavals, economic constraints, or out of complacency, sooner or later, there will be resurgence of the disease.

**Diagnosis**

For unequivocal diagnosis in humans, it is essential to demonstrate trypanosomes in lymph node aspirate, blood or cerebrospinal fluid (CSF). A number of tools exist for the diagnosis of patients. In *T. b. gambiense* areas, the Card Agglutination Test for Trypanosomiasis (CATT), a serological test detecting antibodies, is used for mass screening. Serologically positive cases are then confirmed using parasitological tests. Since parasitemia varies between foci and disease stage, it is necessary to adopt blood concentration techniques, such as the Haematocrit Centrifugation technique (HCT), the Miniature Anion Centrifugation Technique (MAECT), or the Quantitative Buffy Coat (QBC) technique. A study on the treatment of serologically positive patients who are parasitologically negative with one injection of pentamidine is in progress in DRC. The use of the CATT for screening populations in *T. b. gambiense* areas has greatly improved the potential for community diagnosis. Despite the advances, techniques, especially the CATT, have rarely been put into practice in endemic areas except as part of externally funded programmes. This relates in part to the costs (Smith et al. 1998). The Card Indirect Agglutination Test for Trypanosomiasis (CIATT) is another serological test which detects antigens and therefore active infection. However, the very high frequency of positive results in low endemic areas, indicates that it may not be suitable for screening in control programmes. The role of the polymerase chain reaction (PCR) in diagnosis remains to be determined. A test is needed to diagnose late-stage disease, which presently relies on lumbocutaneous that is painful and not well accepted by people. A test is also needed to determine cure after chemotherapy. The current requirement for a 2-year period of follow-up of treated patients is cumbersome, costly, and leads to loss of many patients.

**Chemotherapy**

The chemotherapy of African trypanosomiasis is unsatisfactory, relying on a few drugs which have adverse side effects. Pentamidine, a diamidine developed in 1937, is used for early-stage *T. b. gambiense* sleeping sickness. Suramin, a sulphanated naphthylamine developed in 1922, is used to treat early stage *T. b. rhodesiense*. Melarsoprol, a trivalent arsenical derivative developed in 1948, is used for the treatment of late-stage of both *T. b. gambiense* and *T. b. rhodesiense* sleeping sickness. All these drugs have adverse effects, melarsoprol causing reactive encephalopathy in 5-10% of patients with a fatal outcome in 1-5%. Increasing numbers of patients - between 20-25% in certain foci - do not respond to melarsoprol treatment, probably due to resistance of the parasite to the drug (Legros et al. 1999). The term ‘drug resistance’ covers host and parasite-related factors. Host related factors include poor distribution of drug to infected tissues and intracellular sites, variation in drug metabolism between individuals, and diminished activity of drug. Parasite related factors that contribute to resistance include reduced drug accumulation in the parasite, a change in enzyme target through increase in enzyme levels, increase in metabolite production or retention, or use of alternative pathways to bypass the site of inhibition. A number of these factors could be involved and therefore the mechanism of resistance needs to be elucidated and strategies to combat it, such as the use of drug combinations, developed. The development of a simple test to quickly diagnose resistance in parasite isolates is also needed for successful chemotherapy. Clinical trials with a combination of melarsoprol and eflornithine, whose synergism has been demonstrated in the mouse model, should be given priority (Jennings 1988). Combinations of other drugs are also envisaged. The varying treatment schedules of available drugs were developed empirically and, therefore, optimization of current treatment regimens is needed. Burri et al. (2000) have shown that, with a new regimen of melarsoprol, it is possible to reduce the duration of treatment from 40 days to 10 days. A study sponsored by TDR on treatment with pentamidine, 7 days versus 3 days, in DRC has been interrupted due to rebel forces taking over that part of the country. This occurrence underscores the difficulties of carrying out field research on African trypanosomiasis.

Eflornithine, developed in 1990, is the only available alternative drug to treat *T. b. gambiense* patients who do not respond to melarsoprol. It is not effective in *T. b. rhodesiense* sleeping sickness. The drug has many drawbacks: a complicated mode of administration (intravenously every 6 hours at a dose of 100mg/kg/day for 14 days), which limits its use to a hospital setting; it cannot be used for mass treatment; and the cost of treatment is US$300-500 per patient, which makes it unaffordable by the affected countries. To reduce costs, a shorter course of eflornithine (7-day treatment) was compared to the standard 14-day treatment. However, the results showed that the 7-day treatment is effective only in patients who have relapsed on melarsoprol and that, for new patients, the 14-day course is superior
(Pepin et al 2000). In recent years, there have been doubts about the future availability of eflo

brine. However, through the collaboration between Aventis, the manufacturer, WHO and Médecins sans Frontières (MSF), potential manufacturers have been identified, and it is likely that a suitable producer will be selected to produce the drug and that funds will be provided through donor contributions to guarantee production for the next five years. Nifurtimox, nitrofurazan, was used for acute Chagas disease. Although it was not registered for human African trypanosomiasis, it has been used experimentally and on a compassionate basis to treat T. b. gambiense sleeping sickness patients. Bayer, the manufacturer, has agreed to continue production for treatment of African trypanosomiasis; however, registration of the drug for this purpose is an urgent issue that needs to be addressed.

Vector control
A variety of traps and screens impregnated with insecticide have been shown to be effective in reducing tsetse populations by 99% in control programmes and are suitable for rural community participation. In any outbreak of sleeping sickness, tsetse control in combination with diagnosis and treatment should arrest transmission. Besides, these devices can also be used as preventive measures to reduce human-fly contact. In vector-borne diseases, vector control plays an important role in reducing transmission. For example, Chagas disease caused by T. cruzi has been successfully controlled by vector control as a result of national government commitment to a long-term programme. In onchocerciasis endemic areas of West Africa, blindness is no longer a public health problem as a result of vector control and donated drug (Mectizan) distribution. The effect of deforestation and climatic changes on tsetse populations in West Africa may be responsible for the lull in sleeping sickness in countries like Ghana and Nigeria. Operational issues, such as motivation of communities and recurrent costs, which militate against sustainability in the use of impregnated traps and screens on regular basis, need to be studied and solutions found.

DRUG DEVELOPMENT
An oral formulation of eflo

brain barrier in humans. The functional integrity of the blood-brain barrier and its role in pharmacokinetics and pathogenesis is pivotal to understanding the disease. Attention should be given to new approaches to drug delivery, such as across the blood-brain barrier. In 1999, two leading compounds, both of them diamidines that gave satisfactory results against trypanosomes in animal models in acute infection, failed to cure the chronic infection, due to inability to cross the blood-brain barrier. A number of lead compounds have suffered the same fate in the past. The ideal trypanocide must be safe and effective, and have a simple mode of administration to allow its use under rural conditions, where health facilities are usually poor, and above all should be affordable. The occurrence of T. b. rhodesiense sleeping sickness outside the traditional focus in south-eastern Uganda (Enyaru et al 1999), justifies fears of mixing T. b. gambiense and T. b. rhodesiense due to human population movements between foci in Uganda, the DRC and Tanzania (Kigoma), and underlines the need for a new drug that can treat both T. b. gambiense and T. b. rhodesiense. A multicentre clinical trial with eflo

nithine showed that eflo

nithine was relatively less effective in Uganda than in three other countries (Pepin et al 2000). It should be noted that Uganda is the only country where both T. b. gambiense and T. b. rhodesiense sleeping sickness foci exist and, therefore, this observation needs further investigation.

SOCIAL AND ECONOMIC IMPACT OF SLEEPING SICKNESS
The social and economic impact of sleeping sickness is often underestimated. Some affected countries have agriculture-based economies, and workers on cocoa and coffee plantations are at risk of contracting the disease; consequently the labour force is reduced. At community and family levels, mental confusion, personality and behaviour changes, which often characterize central nervous system involvement in late-stage disease, may lead to divorce and break up in homes and present an unfavourable climate for bringing up children. In some cases, such people become mentally disturbed, suicidal and violent, and constitute a danger to themselves and to the community. Aroke et al (1998) reported that, in the past, T. b. gambiense sleeping sickness in children had an influence on their physical growth and attainment of sexual maturity. In Central Africa, there are important problems regarding treatment, particularly the severe social consequences of long-term hospitalization. Important behavioural factors contributing to the risk of death from sleeping sickness, such as negative attitudes towards hospital treatment, have often led to the patient absconding and not com-
pleting treatment. In studies on the impact of trypanosomiasis on land occupancy systems, on population movements and social conditions in Burkina Faso and Côte d’Ivoire, the extreme mobility of the people due to migrant labour was identified as one of the major problems in case detection, case reporting and control of the disease. There is need to continue monitoring and mapping population movements, as well as incidence of trypanosomiasis, particularly as the impacts of the infection are rather latent but extremely serious in the long run. Studies in Uganda demonstrated that African trypanosomiasis had an adverse impact on the functioning of households at Iganga, south-east Uganda. Adverse impacts included increased poverty, decline in agricultural activities often leading to famine or lack of basic food security, disruption of children’s education, and general reversal of role obligations, which more often than not enhanced women’s and children’s burdens. The debilitating nature of the disease also poses more problems for women, who may be stigmatized and/or rejected by their spouses even after recuperation. The extent of disruption of household social and economic activities is greatly influenced by such factors as the household’s economic status, composition and level of organization (Kyomunhendo 1995, 1998).

GLOBAL RESEARCH ON AFRICAN TRYpanosomes

The trypanosome has many unique biological characteristics that make it one of the most studied parasites. It offers many opportunities for basic research: it is easy to cultivate and purify to yield large amounts of protein and nucleic acid, it is a eukaryotic experimental model for research on the control of gene expression, etc. Though it is difficult to get funds for the control of sleeping sickness, large sums of money are invested annually, particularly in the North, on basic research on African trypanosomes. There is probably more information on the biochemistry and molecular biology of trypanosomes than on any other non-mammalian cell type, and a great deal is known about the differences between trypanosomes and mammalian cells. Yet no drugs have come out of basic research. TDR provides an essential link between research institutions in the North and endemic countries, through access to a network of national field projects and control programmes, where spin-offs from basic research can be evaluated as tools for the control and prevention of sleeping sickness. It is necessary for TDR to preserve this unique role (Kuzoe, 1993).

For institutions in the South, TDR was conspicuously a major source of funding for research on African trypanosomiasis and institutional strengthening activities. TDR’s initiatives in bringing together scientists in Scientific Working Groups, meetings and workshops to deliberate on specific issues, paid off on several occasions. A few examples follow.

A Glossina trapping meeting held in Brazzaville, Congo, in March 1985, brought together scientists, from West, Central and Southern Africa, interested in developing Glossina trapping technology. This meeting opened the way for better interaction between the scientists and gave impetus to improvement in trapping technology for tsetse control. Five years later, the pyramidal trap impregnated with insecticide used at 10 traps per km² was effectively employed in reducing tsetse populations by over 95 per cent within 3-4 months during the epidemics of sleeping sickness in Busoga, south-east Uganda, at an estimated cost of US$0.9 per head of population protected (Lancien, 1991).

The rational development of control and treatment requires thorough knowledge of the pathology of the disease. At the inception of TDR in 1975, there were less than 25 autopsies of African trypanosomiasis reported in the literature. The need for systematic autopsy backed by expert histopathology led TDR, in collaboration with the University of Glasgow, to establish a network of clinical centres that were provided with kits and protocols for autopsy. From this effort, evidence was provided from both laboratory and clinical data showing that reactive encephalopathy, which occurs in 3-5% of patients treated with melarsoprol, points to a drug-related immune response rather than to toxicity (Haller et al 1986). A set of slides showing the features of neuropathology in African trypanosomiasis was prepared and made available to universities for teaching purposes.

New strategies for managing patients with central nervous system involvement in African trypanosomiasis are needed. In 1986, TDR organized a workshop in collaboration with the Institut de Neurologie Tropicale, Limoges, France, which brought together clinicians, neurologists, neuropathologists and scientists. Following the recommendations of this group, a number of studies funded by TDR in collaboration with other institutions, resulted in significant progress in understanding the molecular mechanisms underlying pathogenesis, including brain dysfunction and neuropsychiatric symptoms associated with the disease. The trypanosome lymphocyte triggering factor, TLTF, a molecule which binds to CD8+ cells and triggers the production of gamma interferon, which is growth factor for T. b. brucei, was reported by the Karolinska Institute, Sweden, in 1991 (Olsson et al 1991). The gene for
T. b. brucei. TLTF has since been identified, cloned and sequenced, and a recombinant TLTF produced (Bakhiet et al 1993; Vadya et al 1997; Hamadien et al 1999). The exploitation of TLTF for immunotherapy needs to be followed up. Other studies elsewhere have shown that proinflammatory cytokines, including tumor necrosis factor (TNF), interleukins 1 and 6 (IL-1, IL-6), and prostaglandins, play an important role in the pathogenesis of central nervous system disease (Hunter et al., 1992; Alafiatayo et al, 1994; Jennings et al 1997). Furthermore, this pathology has been shown to be prevented and ameliorated by drugs such as eflornithine (Jennings et al, 1997) and megazole (Enanga et al, 1998). Consideration should be given to the use of such drugs in the management of African trypanosomiasis. Further, preclinical research with such drugs is needed.

The availability of clinical centres in endemic countries supported by TDR facilitated the evaluation of the diagnostic tests MAECT, CATT and CIATT, as well as clinical trials of eflornithine which produced data that were presented to the US FDA for registration of the drug in 1990.

Under the auspices of TDR and its collaborators, considerable progress was made in research on African trypanosomiasis in: diagnosis and development of diagnostic tests; epidemiology, host-parasite-vector relationships, animal reservoirs; development of tsetse traps and screens; better understanding of the pathology of the disease, and drug targeted biochemistry of trypanosomes. However, this progress has not been matched in the control of the disease, due to lack of capacity to sustain improved interventions as well as civil disorder in some endemic countries. These factors were largely responsible for the current problems of African trypanosomiasis.

From 1994, when reorganization took place in TDR along disciplines instead of diseases, the resources allocated to African trypanosomiasis decreased, and continued to decrease tremendously in subsequent years, up to 64% in 2000. In view of TDR’s unique role in research on African trypanosomiasis, the consequences of this lack of funds were grave. Several trained researchers left trypanosomiasis research for HIV/AIDS and malaria, where they could get research funds. It is not surprising that the current chairman of the Task Force on African Trypanosomiasis now works on a project on HIV/AIDS. One tsetse trap expert has turned his ingenuity to making and supplying bednets for a malaria control project. Clinical research centres are rundown and have reduced capacity to conduct clinical studies. One should, however, not overlook the support, no matter how small, that has been provided by other institutions in the North to these centres.

The Bill & Melinda Gates Foundation, in December 2000, awarded US$15.1 million to treat African trypanosomiasis and leishmaniasis to an international consortium of researchers led by Dr Richard R. Tidwell, a scientist at University of North Carolina at Chapel Hill, to develop new drugs to fight African sleeping sickness and leishmaniasis. This welcome news is without precedence in the history of African trypanosomiasis. Some of the scientists involved in this consortium already collaborate with TDR in drug discovery research and drug development. TDR should establish links and maintain collaboration with this consortium. The primate facility at the Kenya Trypanosomiasis Research Institute (KETRI), that was established with TDR’s support many years ago, is an asset that will be available for the evaluation of potential lead compounds in primates. There are few clinical research centres in endemic areas where clinical trials can be conducted and, therefore, these will be in demand for drug combination trials, oral eflornithine phase 3 clinical trials, and trials with any potential candidate compounds. Two years ago, TDR started an initiative to train clinical investigators and monitors worldwide to conduct clinical trials according to the good clinical practice (GCP) concept. Training will be required for clinical investigators in the clinical centres that will be involved in clinical trials. TDR has a comparative advantage in institutional strengthening and can make a significant contribution to the work of this consortium towards the evaluation of candidate compounds. TDR has links with the outside world, with special reference to product development, which should be maintained.

**BIOINFORMATICS**

During 1993/94, TDR initiated a number of parasite genome networks - for *T. b. brucei*, *T. cruzi*, *Leishmania major*, *Schistosoma mansoni*, *Brugia malayi*. The networks are now oriented towards post genomics, and bioinformatics networks are being expanded for data mining annotation and in-depth analysis. The *T. b. brucei* network should be assessed and the necessary inputs provided to move it forward in the genome analysis of *T. b. brucei*.

**COORDINATION NETWORK**

A Human African Trypanosomiasis Treatment and Drug Resistance Network was formed in 1999 in WHO (Communicable Disease Surveillance and Response unit). It has as objectives: to assess the effectiveness of current treatment regimens; collect/disseminate information on refractoriness to
treatment; ensure availability and affordability of existing drugs; provide guidelines for treatment; promote research on causes of treatment failures, drugs and treatments (WHO 1999). The Government of France provides funds for running the secretariat of the network. Participants in the network come from WHO (Communicable Diseases cluster, the Regional Office for Africa); MSF; the United States Centers for Disease Control and Prevention (CDC), Atlanta; the Swiss Tropical Institute, Basel; the Institute of Tropical Medicine, Antwerp. TDR should play an active role in the network and participate in its meetings.

Acknowledgements
I wish to thank Professor David H. Molyneux, Professor of Tropical Health Sciences, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, and Dr Jacques Pepin, Associate Professor, Infectious Diseases Division, Centre for International Health, University of Sherbrooke, Quebec, Canada, for their criticisms and comments on this article. I also wish to thank Dr Paul Nunn, Dr Johannes Sommerfeld and Dr Charity Gichuki, WHO, for reviewing the paper.

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Annex 3
THE EMERGENCE AND RE-EMERGENCE
OF HUMAN TRYPANOSOMIASIS
(SLEEPING SICKNESS) IN AFRICA
1 THE SITUATION IN ANGOLA*

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Instituto de combate e controlo de Tripanosomiase, Luanda, Angola

Angola is a country situated at the interface between Central Africa and Southern Africa and has an estimated population of 12 million. Sleeping sickness is a major public health problem in the country today, with more than 32,000 new cases having been detected during the last five years. In contrast, in 1974, when surveillance was more active and better organized, there were only three new cases.

Tsetse flies, or Glossina, the vectors of the disease, are present in 14 of the country’s 18 provinces. Two million out of the 4.5 million people living in the seven provinces affected - Zaire, Uige, Kwanza Norte, Malanje, Bengo, Kwanza Sul and the peripheral areas of Luanda - are exposed to the risk of direct infection. The number of patients has been rising continuously for more than ten years. The number of new cases identified in 1997 was 8275, the highest figure ever reported in the history of the disease in Angola. The figures reported reflect the case detection and treatment activities introduced over the last few years. Paradoxically, the rapid increase in the number of patients identified is witness to the efforts made to manage the disease by the national health services with the help of non-governmental organizations (NGOs). In the period 1996-2000, 32,445 patients were treated (Table 1).

Table 1. The number of patients treated in the years 1996-2000

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<td>6786</td>
<td>8275</td>
<td>7373</td>
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Since African trypanosomiasis is invariably fatal when left untreated, the increase in number of treated patients means a decrease in mortality. So the 32,331 patients treated during the period 1996-2000 escaped from certain death. The treatment centres are in areas of military confrontation (war front) and this might explain the fluctuations in number of patients treated each year. In addition, this has led to low surveillance coverage and inadequate screening of the population at risk, suggesting that the true number of patients is very high.

The main reasons for the persistence of sleeping sickness in Angola are inaccessibility of most of the territory affected by trypanosomiasis, the scant surveillance coverage of endemic areas, for reasons already stated, and the continual movement of the population, which includes carriers of the parasite who act as a reservoir for its dissemination. In addition, socioeconomic factors including poverty are without doubt implicated in the spread and persistence of sleeping sickness.

At the symposium organized by Fundanga, the Angolan Foundation for Solidarity and Development, and held in September 1998 on the premises of the Angolan National Assembly in Luanda, sleeping sickness was clearly identified as a major public health problem. As a result, the Angolan Government committed itself financially to taking action against the disease, with annual financing of about $1 million in local currency (Kwanza) and $1 million in convertible currency (dollars) since 1998 for activities of the National Programme for control of human trypanosomiasis.

The National Programme has been gradually strengthened in its leadership role, and its efforts have borne fruit with the transformation of the National Programme into the Institute for the Control of Human and Animal Trypanosomiasis (Instituto de Combate e Controlo das Trypanosomiases, or ICCT) by Government decree 2/00 of 14 January 2000. The newly created Institute enjoys autonomy in management and its position has changed in the structural chart of the Ministry of Health. This autonomy first found expression in the delegation of management of salaries of personnel working at ICCT headquarters and at the Viana Reference Centre.

Control of trypanosomiasis addresses one of five priority endemic diseases (AIDS, trypanosomiasis, tuberculosis, leprosy, malaria) for the Ministry of Health in 2001. In Angola, the inclusion of ICCT national staff in the activities of NGOs is effective, enabling it to be present at most places where patients are managed.

This year, the Ministry of Health assigned five physicians to the ICCT and is presently trying to obtain greater participation by the State in the financing of the Programme. There is also effective financial participation by the autonomous provinces, especially the province of Zaire, which ensures supplies of specific drugs for the disease.

Since the refurbishment of the screening and treatment centre and the Viana Reference Centre, some staff have been transferred from the headquarters of ICCT to the Viana Reference Centre, including one physician in charge, two clinicians, one head of laboratory, and one clinical assistant. This doubling of

* The original manuscript is in French
staff has enabled us to open a reference treatment centre, supported by the research projects of the Swiss Tropical Institute. We can therefore say that, in addition to the refurbishment and the equipment of the laboratories, functional practice and expertise have been developed.

A large number of NGOs operate in Angola. Most of them were originally working independently of each other, applying different methodologies to the control of African trypanosomiasis, but numerous territorial disputes arose and it became necessary for the Ministry of Health to take over direction of trypanosomiasis control. As a result, coordination of the national and international NGOs through ICCT was initiated. Many meetings were held for consultation and coordination (on average, one every three months), while project visits by ICCT staff also helped to enhance the legitimacy and credibility of the Institute, facilitating the coordination of interventions. Currently, there is no more rivalry and the role of the ICCT is understood by all, such that the number of visits to partner treatment centres has substantially increased. The NGOs have accepted standardization of the methods for case detection, treatment and data collection, while a growing number of technical and medical staff belonging to ICCT now work with NGOs, where they are replacing expatriate staff.

The NGOs that have worked, and are still working, in Angola are shown in Table 2.

### Table 2. NGO presence in endemic areas between 1996 and 2000

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### RECOMMENDATIONS FOR ACTION

- Combat poverty at all levels. This is one of the major objective, poverty being one of the factors that aggravate the socioeconomic status of the population, including those suffering from trypanosomiasis.

- Adopt a strategy of permanent, regular, epidemiological surveillance, consisting of active diagnosis of new cases of the disease, with a view to early detection, treatment and effective follow-up of sufferers.

- Mobilize resources to combat the disease, such as drugs, reagents, equipment and traps.

- Strengthen vector control actions, using the most appropriate techniques to combat tsetse flies.

- Draw up an information, education and communication (IEC) plan for the population involving political leaders, leaders of civil society and health officials in order to take concrete steps to control this disease.

- Draw up a plan of work for animal trypanosomiasis and update *Glossina* mapping of the country.

- Strengthen cooperation between the ICCT and its national and international partners.

- Prepare coherent and feasible projects that are guaranteed to receive national and international funding.

- Look for ways and means to motivate ICCT workers by improving working and social conditions.
II  THE SITUATION IN TANZANIA

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National Institute of Medical Research, Tabora, Tanzania

GENERAL OVERVIEW

In the United Republic of Tanzania, sleeping sickness, also known as human African trypanosomiasis (HAT), is one of the major public health problems. Sleeping sickness was first recorded in 1922 in Maswa district, south of Lake Victoria (Kilama et al. 1981). It then spread throughout mainland Tanzania and is currently endemic in eight regions, namely Arusha, Lindi, Ruvuma, Kagera, Kigoma, Tabora, Mbeya, Rukwa. The annual average number of cases for the last decade was 264. There are isolated foci, most of which have been producing cases for many years. Heavy foci exist in Kigoma Region (Table 1).

Table 1. Number of sleeping sickness incidences diagnosed from district hospitals in six regions of Tanzania for the past five years.

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<td>KIGOMA</td>
<td>Kibondo</td>
<td>212</td>
<td>286</td>
<td>206</td>
<td>410</td>
<td>376</td>
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<tr>
<td>ARUSHA</td>
<td>Kasulu</td>
<td>155</td>
<td>198</td>
<td>172</td>
<td>156</td>
<td>191</td>
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<td></td>
<td>Babati</td>
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<td>19</td>
<td>15</td>
<td>12</td>
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<td>Monduli</td>
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<td>6</td>
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<td>Hanang</td>
<td>5</td>
<td>3</td>
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<td>TABORA</td>
<td>Urambo</td>
<td>1</td>
<td>7</td>
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<td>RUKWA</td>
<td>Nkansi</td>
<td>4</td>
<td>1</td>
<td>7</td>
<td>8</td>
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<td></td>
<td>Mpondoa</td>
<td>5</td>
<td>1</td>
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<td>MBEYA</td>
<td>Chunya</td>
<td>6</td>
<td>4</td>
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<tr>
<td>TOTAL</td>
<td></td>
<td>400</td>
<td>531</td>
<td>421</td>
<td>588</td>
<td>627</td>
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Although sleeping sickness was present in eight regions during the past ten years, the disease was more concentrated in Kasolu and Kibondo districts of Kigoma Region, which accounted for about 90% of the cases. These figures are underestimates due to lack of accurate diagnosis and under-reporting.

KIGOMA SITUATION

An outbreak of sleeping sickness is currently being experienced in Kigoma Region of Western Tanzania along Lake Tanganyika. The disease represents a continuing threat to the health and morale of many communities in the area. All the districts of Kigoma Region are affected by sleeping sickness, with Kibondo and Kasulu districts producing a great proportion of the total cases over the past few years. The whole of Kigoma Region is on the Western Fly Belt, a portion of a large forest which extends, with few breaks, over an area of 10 368 sq. km, and lies between 31°E and 24.7°W, 3.5°N and 5.2°S. Here, game is fairly abundant, and the people regard sleeping sickness as an old disease.

The worst affected area is along the Malagarasi Valley, where the vegetation is characterized by big trees overhanging open grassland. The population in this large area is, for the most part, concentrated in villages on the Kigoma–Kibondo trunk road. Sleeping sickness infections are contracted mainly by those going into the bush to hunt, fish, collect honey, beeswax, etc. There are few cases of peridomestic infection.

Each village has a population of over 2000. Some of the villages are remote from the main road, for example Kagera and Mvinza in the north-east and Kitanga and Heru-Ushingu in the extreme northwest.

Villages Affected by Sleeping Sickness in Kasulu District

Thirty-two villages are exposed to the risk of infection in the endemic district of Kasulu. Of these, the following are regarded as highest risk areas: Heru, Kitagata, Kitanga, Makere, Mugombe, Mvugwe, Mwali, Nyachenda, Nyakitonto, Nyamidaho, Nyarugusu, Shingu. Others include Kagera, Kaguru, Kitema, Mvinza, Runwge, Mpya, Titye. Overall, the estimated population at risk of infection is 230 000. Each village has a health clinic. There is one health centre for every six villages.

Villages in Kibondo District

Sleeping sickness occurs in the following villages in this district: Bitare, Biturana, Busunzu, Kanembwa, Kazira mihunda, Kifura, Kilemba, Kingoro, Kitahana, Kumshindwi, Malagarasi, Mkabuye, Mvugwe, Nduta, Nyankwi, Nyaruyoba, Rusohoko. Each village has a dispensary. Each health centre serves six or seven villages.

Refugee Camps in Kigoma

There are about 280 000 refugees, mainly from the Eastern part of the Democratic Republic of Congo (DRC), settled in camps in Kigoma region. Sleeping sickness cases have already been detected in these camps and there is concern that an overlap of gamibiense and rhodesiense sleeping sickness exists in the region.

CAUSES OF EMERGENCE AND RE-EMERGENCE

The current situation of sleeping sickness in Tanzania, especially the outbreak in Kigoma region, is caused by the following factors:

- Poor surveillance due to inadequate funding and staff.
- Inadequate and erratic supply of specific trypanocidal drugs.
- Poorly equipped field laboratories for diagnosis.
• Opening up of new unauthorized settlements and farms in the endemic areas of sleeping sickness. In the past, the people in Nyakitonto area, for example, had wished to live in the valley (Kitome area), which is wooded and well watered but also heavily infested with tsetse fly. Permissions to do so had been consistently denied. However, a few families had occupied the area by 1983 and, as was perhaps inevitable, many infections occurred thereafter.

• Lack of monitoring and health education campaigns.

• Increased forest activities including cultivation outside the villages or on the buffer zone. Some of the planned villages were, unfortunately, located in a rather dry and infertile area causing the villagers to open new farmlands outside the villages.

• Apparent bush regeneration with resultant tsetse encroachment.

• The highly probable introduction of a virulent strain of *T. b. rhodesiense*.

The serious outbreaks of sleeping sickness in the district are a disappointing setback in the disease control efforts of the country, and indicate a necessity for review of sleeping sickness control measures in the area. A study in Kigoma region suggests the following factors are important in the current outbreak:

• Farming activities carried on outside the protected area.

• Inter-village visits through tsetse infested bushes in search of the basic necessities of life.

• Increased forest activities – honey and beeswax collection, fishing, hunting, etc.

• Peridomestic activities e.g. firewood gathering, fetching water, cutting poles for building.

• Visits into the wildlife areas where no human settlement is allowed.

The problem that now confronts Tanzania is that sleeping sickness is still endemic and there is laxity of control measures. Compared to other endemic diseases, the number of human deaths from sleeping sickness appears insignificant, but even temporary exacerbation of the disease frightens the local people.

While it is impossible to predict the future, the possibility of a larger outbreak must be considered. With the evidence currently available, it would be a reasonable precaution to step up control measures.

**IDENTITY OF THE HUMAN INFECTIVE TRYPANOSOME IN TANZANIA**

The occurrence of the chronic syndrome of sleeping sickness, together with the marked variation in efficacy of chemotherapeutic treatments, in Tanzania, may be cited as indications that the trypanosomes constitute a heterogeneous complex of organisms including perhaps a mixture of *T. b. rhodesiense* and *T. b. gambiense*.

Old records show that the Malagarasi focus (Kigoma region), for example, in north-western Tanzania, is an old gambiense sleeping sickness focus with the last case of gambiense infection having been reported in 1958 (Kihamia et al. 1991). The area is now considered endemic for rhodesiense sleeping sickness. In Tanzania there are considerable differences in the clinical types of human trypanosomiasis, with the severity of disease varying according to geographical location and becoming less virulent the further one goes south. While this may be attributed to the heterogeneity of *T. b. rhodesiense* strains (Komba et al. 1997), there is concern that it may be due to the occurrence of the two species of trypanosome. Furthermore, the presence of large numbers of refugees from the DRC, a country known to be endemic to gambiense sleeping sickness, increases this possibility.

Further studies are required to:

• Investigate why the disease is localized in specific foci despite the tsetse fly and reservoir animals being present in large areas of the country.

• Investigate the distribution of *T. b. rhodesiense* strains in Tanzania.

• Investigate the possibility of an overlap of rhodesiense and gambiense sleeping sickness, especially in Kigoma focus, which has an influx of refugees from the DRC.

**References**


III  THE SITUATION IN UGANDA AND SUDAN

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SUMMARY
Sleeping sickness is a disease of the rural poor which tends to emerge and re-emerge in epidemics in some 36 sub-Saharan African countries, where close to 50 million people are at risk of contracting the disease. Together, these 36 countries report only about 25 000 new cases of the disease to WHO annually. This is an obvious underestimate attributed to poor reporting, difficulty in diagnosing the disease, and poor accessibility of the affected areas. The true figure is currently estimated to exceed 300 000 new cases annually.

In the recent past, Uganda and Sudan have not been spared epidemics of this disease. A devastating epidemic of T. b. rhodesiense sleeping sickness has occurred in south-eastern Uganda and one of T. b. gambiense in the West Nile region of the country (Uganda). In Southern Sudan, along the border with Uganda, there is another epidemic of T. b. gambiense sleeping sickness.

The causes of the emergence and re-emergence of epidemics of this disease are varied, but can be conveniently grouped into political, economic, behavioural factors, and the effects of climate and vegetation on tsetse fly distribution.

To limit the impact on human lives in these two countries, external support will be required to implement strategies for disease and vector control. On the one hand, donor agencies, NGOs and mission organizations could play an important role in supporting these control efforts. On the other hand, national authorities will need to control and coordinate these efforts with assistance from WHO and the international community.

This paper presents a general introduction to the disease (sleeping sickness) and a brief account of factual epidemic outbreaks in Uganda and the Sudan. The paper then proceeds to discuss, in general terms, possible factors for the emergence and re-emergence of epidemics of the disease.

INTRODUCTION

Historical Aspects of the Disease
Trypanosoma brucei, and the role of the tsetse fly (Glossina spp.) as its vector, was identified in game animals in Zululand by David Bruce two centuries ago (WHO, 1995). Later, morphologically identical trypanosomes were identified in the blood of a European from The Gambia, West Africa (Dutton, 1902), and transmission by riverine tsetse (Glossina palpalis) was confirmed. Subsequent studies revealed the extensive, often focal, distribution of the disease, the substantial endemicity and the chronic progressive nature of human infection in West and Central Africa.

The disease was called Gambian trypanosomiasis and the parasite, T. gambiense (later T. brucei T. brucei gambiense). In 1908, a severe rapidly fatal trypanosomal infection was identified in the Luangwa valley, Zambia. Further investigation confirmed its clinical severity, the distinct epidemiology with transmission via savannah tsetse, and the zoonotic nature of infection from game animals harbouring T. brucei. This led to the description of Rhodesian trypanosomiasis due to T. rhodesiense (T. b. rhodesiense). Other members of the T. brucei T. brucei group, that are non-infective to humans and occur in game and domestic animals, were designated T. brucei (subsequently T. b. brucei).

The General Epidemiology of the Disease
Trypanosomes, the parasites which cause the disease, occur in the blood of man and animals as the trypomastigote.

All members of the T. brucei group are morphologically identical. The parasites have evolved mechanisms for evading host immune responses through variation of their surface antigen glycoproteins. The zoonotic nature of T. b. rhodesiense was initially established by inoculation of ‘volunteers’ with parasites from a bushbuck (Heish et al, 1958), and later from domestic cattle.

Subsequently, the blood incubation infectivity test (BIIT) was developed to assess the human infective potential of parasites from a range of wild and domestic animals. More recently, a number of molecular techniques, especially isoenzyme analysis (Stevens and Godfrey, 1992) and restriction fragment length polymorphism (RFLP), have been used as markers for parasite strains to explore the molecular epidemiology of this complex group of parasites. These techniques allow T. b. gambiense to be distinguished from T. b. rhodesiense.

In West and Central Africa, sleeping sickness is transmitted by riverine species of tsetse fly (palpalis group), which require sustained levels of humidity and prefer dense riverine habitats. These tsetse flies feed preferentially on man, especially where man-
fly contact is high, such as at water collection and bathing points, river crossings and sacred groves. For the riverine species of tsetse fly, man provides the reservoir of infection, although both wild and domestic animals may play a minor role in particular foci. Throughout most of the range, T. b. rhodesiense transmission is effected by savannah species of tsetse (morsitans group). This group of tsetse flies survives in drier, more open areas of woodland, savannah and acacia thickets, and prefers to feed on game animals and domestic stock. Human infection occurs sporadically in individuals coming into contact with the zoonotic cycle, for example poachers, hunters, honey gatherers, firewood collectors and tourists. A wide spectrum of animals, notably game animals and domestic cattle, provide a reservoir of infection. However, in East Africa, the epidemiology is different in that T. b. rhodesiense is transmitted by a riverine species of tsetse, namely G. fuscipes fuscipes, and domestic cattle are the main reservoir. This situation creates a lot of potential for epidemic outbreaks of the disease.

Clinical Manifestation of the Disease
In most endemic areas, T. b. gambiense causes a protracted, often initially unrecognized, illness with episodes of fever, headache and malaise, accompanied by progressive lymphadenopathy and followed later by the development of a progressive, fatal, meningoencephalitis. This contrasts with the acute, severe, febrile disease observed with T. b. rhodesiense, with rapid progression to meningoencephalitis. There is relentless deterioration to a stuporous state, with cachexia, wasting and progressive malnutrition, deepening coma and death, within a few months in the case of T. b. rhodesiense and extending for months or even years in the case of T. b. gambiense.

AVAILABLE OPPORTUNITIES FOR THE CONTROL OF SLEEPING SICKNESS
The principle of control and prevention of sleeping sickness relies on an integrated strategy of continuous surveillance, involving diagnosis and treatment of the population at risk, and vector control where applicable (de Raadt, 1986). A number of tools for diagnosis and vector control have been developed through research during the past decade and are, indeed, field applicable by national health services. These include the card agglutination test for trypanosomiasis (CATT) (Magnus et al, 1978) for serodiagnosis, and the miniature anion-exchange centrifugation technique (MAECT) (Lumsden et al, 1979) for parasitological diagnosis. The antigen ELISA, developed by Nantulya (1989) and evaluated for detection of gambiense (Nantulya et al, 1992) and rhodesiense (Komba et al, 1992) sleeping sickness, was subsequently modified into a latex agglutination test. Lancien in Uganda (1991) confirmed that insecticide impregnated traps can be used, with community participation, to control sleeping sickness epidemics.

Until recently, the treatment of sleeping sickness relied essentially on three drugs, namely pentamidine, suramin and melarsoprol. Pentamidine, a diamidine introduced in 1937, is currently available as pentamidine isethionate and is effective against early infections of T. b. gambiense. Suramin, which was introduced in 1922, is effective against early infections of both T. b. gambiense and T. b. rhodesiense. Melarsoprol, a trivalent arsenical introduced in 1949, was the only drug available, until 1990, for the treatment of late infections of both T. b. gambiense and T. b. rhodesiense. All these drugs have adverse side effects, melarsoprol causing reactive encephalopathy in 5—10% of patients treated, with a fatal outcome in 1-5%. Resistance of T. b. gambiense to pentamidine, and of both T. b. gambiense and T. b. rhodesiense to melarsoprol, occurs. Nifurtimox, a 5—nitrofuran, is currently being used, either singly or in combination with other drugs, to treat late-stage gambiense infections on a compassionate basis.

Efllornithine (DFMO), a potent inhibitor of polyamine synthesis, was developed for the treatment of sleeping sickness through collaboration between Marion Merrel Dow Inc., USA, and the UNDP/World Bank/WHO Special programme for Research and Training in Tropical Diseases (TDR). However, while this drug provides the best alternative treatment to melarsoprol for gambiense sleeping sickness, alone it is ineffective against T. b. rhodesiense infection. Therefore, no alternative treatment for late-stage rhodesiense infection is yet available.

The availability of all these drugs is currently highly uncertain, with the various manufacturing firms either threatening to stop, or having already stopped, their production. It is evident that the treatment of sleeping sickness is still unsatisfactory. The ideal trypanocide should be safe and effective. It must have a simple mode of administration to allow its use under rural conditions where health facilities are usually of a poor standard, and, above all, it should be affordable.

THE PAST AND PRESENT SLEEPING SICKNESS SITUATION IN UGANDA AND SUDAN

Uganda
The sleeping sickness epidemic which devastated the shores of Lake Victoria at the beginning of the
last century is a famous event in the annals of tropical medicine. It is famous because an estimated one quarter to one third of a million people lost their lives (Langlands, 1967). The same epidemic brought controversy as to whether Dr Castellani or Colonel Bruce first identified the trypanosome as the cause of the epidemic.

The cause of the epidemic was attributed to *T. gambiense* introduced to this part of the country by a party accompanying the explorer Lugard from the Congo basin on relief of the Emin Pasha expedition in 1894 (Christy, 1903). However, it is more accurate to say that the cause lay in the general increase in social, commercial and military mobility which developed throughout tropical Africa in the late nineteenth and early twentieth centuries.

Another outbreak involving about 2500 persons occurred in the same area from Jinja eastwards to the border with Kenya between 1939 and 1945 (Mackichan, 1944-45). The most striking feature of this epidemic was the virulence and rapidity of the fatal course of the disease observed on animal inoculation. Thus, it is believed, the epidemic was caused by *T. rhodesiense*. The first cases detected were among migrant workers employed on Kakira sugar estates. Since these immigrants came from areas of reasonable proximity to the infected areas of Tanganyika (now Tanzania), this epidemic was thought to have been introduced by them.

Since that outbreak, cases continued to be reported from within the infected area, though not in epidemic numbers. In 1971, infection spilled north of the usual focus and involved up to 169 persons.

Following the control of the small epidemic of 1971, surveillance programmes were not instituted because of the prevailing political and economic atmosphere in the country at that time. There was indiscriminate and haphazard movement of people and livestock across the traditional trypanosomiasis barrier zone. Besides, smuggling of commodities, including cattle, between Kenya and Uganda, across the zone, became a means of livelihood. It was therefore difficult for the Ministry of Health teams to enforce surveillance measures. The Tsetse Control Department could not carry out control programmes due to lack of insecticide, transport and human resources. Thus, there was total breakdown of control measures and hence, by 1976, the stage was set for another epidemic outbreak of the disease in the area.

This new outbreak started in Luuka County of Iganga district in August 1976. Unfortunately, in June 1977, the East African Community collapsed and EATRO, the department probably in the best position to help contain this epidemic during its outset, lost valuable logistics to a partner state and became helpless. The Ministry of Health posted microscopists to the area and later opened up treatment centres in the area. However, the epidemic continued to increase in magnitude from 52 cases in 1976 to over 8000 cases in 1980 (Fig. 1).

In the West Nile region, a new outbreak of *T. b. gambiense* sleeping sickness occurred along the Dacha river. During the first year of the outbreak, a total of 12 cases were recorded. In the following year (1958), this new focus produced 7 cases and it seemed as if the outbreak had ceased. However, the 1959 incidence of 30 cases, the highest annual figure since the big epidemics had been finally checked 12 years before, made it evident that the outbreak was by no means under control.

However, this outbreak was finally brought under control and the situation remained largely stable thereafter until the early 1980s, when the current outbreak in the region started. This current outbreak is associated with the war of liberation against Idi Amin in 1979, when most local residents in the region fled into exile in Southern Sudan where there was a ravaging epidemic of *T. b. gambiense* sleeping sickness, then as now. When these local residents returned to the West Nile region in the early 1980s, some of them had the infection, which they introduced into the area. The epidemiological trend of the disease in the region (West Nile), over the years, is shown in Fig. 2.
Sudan
Sleeping sickness due to *T. b. gambiense* has been known to occur in the Southern Sudan since the early 20th century. Epidemics of the disease have occurred mainly in the Southern and South-western parts of the country bordering Uganda, the Democratic Republic of Congo (DRC), and the Central African Republic. In addition, cases of the disease have been reported along the Ethiopian border, in Raga since 1909, in Yei since 1910, in Kajokaji since 1914, in Nimule since 1915, in Tambura since 1918, and in Yambio since 1924.

Almost all the aforementioned foci are still active. Duku (1979) attributed these epidemic outbreaks to: the presence of active foci in neighbouring countries, particularly those where tribal settlements straddled the borders; widespread distribution of the vectors; and political upheavals, instability and civil disturbance.

The current flare-up of the disease started after the signing of the Addis Ababa peace agreement in 1972. The relative peace which followed the signing of this agreement made it possible for some control measures to be instituted with external assistance from WHO and the Government of the Kingdom of Belgium between 1974 and 1978, hence the availability of the information shown in Fig. 3. Otherwise information on the current epidemic in the Southern Sudan is not easily available.

FACTORS RESPONSIBLE FOR THE EMERGENCE AND RE-EMERGENCE OF SLEEPING SICKNESS

Factors responsible for the emergence and re-emergence of sleeping sickness are varied and diverse. Mbulamberi (1989) gave an outline of these factors. A brief account, a modified version of these factors, is given below.

Civil Disturbance and War
Civil disturbance and war cause extensive and often uncontrolled movement of people into areas that may have been previously abandoned because of epidemics, thereby promoting circulation of the parasite in the population and the risk of contact between people and the tsetse flies. Civil disturbance and war will, in the final analysis, lead to a breakdown in vital social services including medical and vector surveillance programmes. This is obviously the most important factor in the case of the current epidemics in both Uganda and Southern Sudan, as is the case in most other sub-Saharan African countries, e.g. Angola, Mozambique, the DRC.

Declining Economies
Declining economies, as is the case in most sub-Saharan African countries, will dictate reduced financing of disease control programmes. This, in turn, will affect field control activities as well as the implementation of advances so far made in diagnosis, treatment and vector control. This is mainly because these new advances are not available on the local market and therefore require importing into the country, for which foreign currency is required. In addition, reduced financing is likely to lead to the temptation of progressively dismantling vertical disease control programmes in preference for integrated, community-based programmes, thus leading to loss of focus.

Behavioural Factors
One of the factors to be considered here is the low priority rating accorded to sleeping sickness on the part of both donors and national governments. This is despite the negative impact of the disease on development. One possible explanation is that sleeping sickness control programmes do not have much appeal for international aid donors due to various factors including: the regional distribution of the disease and mainly rural nature of the problem; the relatively small number of new cases reported annually compared to other diseases; the requirement for long-term input to control programmes for sustainability in the absence of the prospect of eradication. Paradoxically, when epidemics of the disease occur, financial support is made easily available in amounts which are usually disproportionately higher than those required for the regular preventive measures (Kuzoe, 1993).

Another pertinent behavioural factor, in this respect, is the population density of the tsetse flies and their feeding behaviour. A tsetse fly feeding on a number of animals, and possibly also on man, may become infected with many different strains of trypanosome. Most of these strains will be non-pathogenic for man and, even if a man-infective strain is
acquired by the fly, the tendency will be for it to be so diluted by the non-pathogenic strains that it will not be passed on in sufficient number to cause infection in man.

Another important behavioural factor is increased man-fly contact. This phenomenon occurs most commonly during the hot, dry season with the result that transmission is enhanced. Indeed, a period of drought almost invariably means an increase in the number of infections because the few sources of water are shared by man, tsetse flies and game animals, in close association; there is also more hunting and more searching for wild forest products at times when crops are bad. This phenomenon is particularly applicable to *T. rhodesiense* infections (Willet, 1965, and many other workers).

The presence of domestic and wild animal reservoir hosts is another important factor. The pig in the case of *T. gambiense*, and cattle in the case of *T. rhodesiense*, have been incriminated as domestic animal reservoir hosts, while the kob and hartebeest in the case of *T. gambiense* and the bushbuck in the case of *T. rhodesiense* have been incriminated as wild game reservoir hosts. The bushbuck is particularly important because it tends to live in thickets near human habitation, which puts it in close contact with man.

The appearance of different forms of the parasite is another important factor. The appearance of such parasites may be due either to the parasites being introduced from outside the area or to genetic changes in the parasite. There is at least a suspicion, based on field observations, that zymodemes of trypanosome introduced into fresh localities may exhibit an enhanced ability to spread through the community. Scott (1961) reported two instances in which the introduction of infected persons from an established epidemic area resulted in outbreaks of the disease in endemic localities far removed from the original focus of infection. There are other similar observations suggesting that severe local outbreaks which quickly follow the introduction of infected persons to fresh localities are, in some way, connected with enhanced ability of the zymodeme to spread. Indeed, the possible existence of epidemic trypanosome zymodemes has been advanced by some workers.

Another factor of importance are the changes in population movements and population growth. It is generally supposed that population movements are liable to precipitate epidemics. Refugees displaced as a result of war, famine, earthquakes and other similar occurrences are notoriously prone to disease in epidemic form, as are immigrant labour forces recruited for large-scale construction work (tropical aggregation of labour) and pilgrims attending major religious festivals.

A new population in an area may spark off an epidemic outbreak of sleeping sickness as a result of imported cases among them, which may be sufficiently large to increase the reservoir of infection available to the insect vector and so, in a quantitative manner, promote transmission. An imported strain may also show quantitative differences such as enhanced virulence or ability to spread, or may be one to which the indigenous population has not been previously exposed and to which no resistance has been acquired. This phenomenon can also operate vice versa. Further, as with some other diseases, the periodicity of epidemics of sleeping sickness may be associated with the growing up of a new generation of people with no previous experience of the disease.

The occurrence of sub-acute cases of the disease is yet another important factor. The presence of an undetected and perhaps unsuspected reservoir of infection in the form of human “healthy carriers” of the disease, which has been reported by several workers (Buyst, 1977; Rickman, 1974; Woodruff et al, 1982), has important epidemiological implications.

Under conditions in which man-biting tsetse are common and where people congregate, the ambulant human carrier assumes a powerful potential for the onward transmission and spread of sleeping sickness. The occurrence of asymptomatic carriers of rhodesiense sleeping sickness is certainly low. However, sleeping sickness cases with non-specific symptoms (fever, headache) who remain ambulant for several weeks are common, and they too may be important reservoirs of infection where man-fly contact is intense (Wurapa et al, 1984). This threat is also present among many early cases of the gambiense disease, in which the initial stages are generally relatively mild and the victim may continue to work for many months or even years before he is eventually driven, by increasing illness, to seek treatment or to retire to his home. During this time, he is a constant source of infection to tsetse so that the very nature of the illness provides great opportunities for its spread. In both the Ugandan and Southern Sudan situation, the question of delayed diagnosis and treatment is a big factor.

Another critical factor in this category is human behaviour and activities in the fly’s habitat. Often, man becomes infected during travel, hunting, fishing, collection of honey or when working in the bush...
which the fly inhabits. Fishing and “honey-hunting” are particularly hazardous occupations. While fishing in riverine pools surrounded by thickets, people may be in close contact with tsetse flies for many days at a time, a situation in which the association between humans, bushbuck and tsetse fly is likely to be significant and conducive to transmission and spread of the disease. Wyatt et al. (1985), working in north-east Zambia, found fishing to be more common among cases of sleeping sickness than controls. Fishing represented a hazard both while walking to the stream and while engaged in the activity itself.

**Climate, Vegetation and Tsetse Fly Distribution Factors**

Climate appears to be of more than ordinary importance. At higher temperatures, there is an increased salivary gland infection rate in the tsetse fly, and, in addition to this direct effect, there are many ways in which climate influences a closer association between man and fly.

In the current epidemic of sleeping sickness in south-eastern Uganda, heavy rains coupled with the abundant growth of *Lantana camara* thickets provided *G. f. fuscipes* with suitable conditions well outside its usual riverine habitat, so it was able to live and breed in the vegetation surrounding homesteads.

Climate also has an influence on where people choose to live, and on the population density of both flies and human beings, as discussed by Ford (1971). This is relevant to the proper use and full development of land, which is the ultimate aim of eradicating the tsetse fly and trypanosomiasis.

Infection rates in tsetse flies, and their infectivity, are affected by climate. Wijers (1960) observed that infection rates were highest in flies taking an infective blood-meal on the day on which they emerged, somewhat lower on the second day after emergence, and did not occur thereafter. Thus, the fact that flies emerging during the hot season are likely to feed early in their adult life means that infection rates in the fly are maximal during the hot, dry season. However, the number of trypanosomes inoculated by an infected tsetse fly varies greatly, even among flies infected from the same host and in the same fly at different times.

Climate also affects tsetse longevity. Flies emerging at the end of the hot, dry season are particularly receptive to trypanosome infection since they will feed early in adult life. With the onset of the rainy season, the expectation of life of a tsetse fly is maximal, so that a combination of these factors produces a situation in which infected flies are liable to survive for protracted periods. This enhances the potential for these flies to transmit the disease, of course depending on their infection rates.

**References**


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Annex 4

EPIDEMIOLOGY, DISEASE SURVEILLANCE AND CONTROL, AND VECTOR CONTROL
I  EPIDEMIOLOGY AND CONTROL OF HUMAN AFRICAN TRYPANOSOMIASIS

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INTRODUCTION

Human African trypanosomiasis (HAT) is caused by hemoflagellates of the Trypanosoma genus, Trypanozoon subgenus and brucei species, which classically includes three subspecies: Trypanosoma brucei brucei, T. b. gambiense and T. b. rhodesiense. These subspecies are morphologically identical but differ in their ability to infect various hosts. T. b. brucei is essentially a parasite of domestic and game animals and is not pathogenic to humans because it is lysed by a haptoglobin-like molecule (Smith et al, 1995). Only T. b. rhodesiense and T. b. gambiense are considered to be human pathogens. There are two clinical variants: an acute syndrome attributed to T. b. rhodesiense and a chronic one caused by T. b. gambiense. Both diseases result from complex interactions between the parasite and its tsetse fly (Glossina) vector and vertebrate hosts.

During the last couple of decades, considerable progress has been made toward the improvement of epidemiological knowledge. This has led to the development of new tools suitable for control. A recent paper by Pépin et and Méda (2001) provides details of the advances in the epidemiology and control of HAT. However, these advances have not been sufficiently used in the field for what they were intended. The aim of this paper is to try to summarize what we know of the epidemiology and control and to identify some of the most important gaps that need to be urgently tackled by the scientists and programme managers involved in research and control activities.

CURRENT EPIDEMIOLOGICAL SITUATION AND DISEASE BURDEN

HAT is the only vector-borne parasitic disease whose geographical distribution is limited to the African continent. T. b. gambiense is seen in West and Central Africa, and T. b. rhodesiense in East and Southern Africa. Uganda is the only country where both subspecies are found: T. b. gambiense in the north-west and T. b. rhodesiense in the south-east. This distribution has probably remained constant over time.

According to the World Health Organization (1998), there remain within the “tsetse belt” more than 200 active foci, located between latitudes 15° North and 15° South. Within this area, 60 million individuals living in 36 countries are exposed to the infection. Due to shortages of financial and human resources, less than 4 million benefit from an adequate surveillance and control programme; all endemic countries are characterized by shortages of the financial and human resources necessary to implement or sustain a comprehensive control programme. Reports from national control programmes can only give a rough idea of the epidemiological situation, because of the difficult security situation and the decay of communication systems in many parts of high-incidence countries. Year-to-year incidence for the 1977-1994 period in all endemic countries can be found in a recent WHO report (WHO, 1998), where Figure 1 shows the geographical distribution of the cumulative number of new cases reported between 1977 and 1997 for the endemic countries. T. b. gambiense trypanosomiasis is now a major public health problem in Central Africa, specially in the Democratic Republic of Congo (DRC), Angola and Southern Sudan, where the ongoing civil war hampers control efforts to such an extent that national statistics give only a very incomplete view of the problem. In DRC, where relatively better information is available, the total number of people at risk is estimated, by the national control programme, to be 12 500 000. The number of new cases reported each year has now reached levels comparable to those seen in the early 1930s, despite substantial underdiagnosis due to inadequate coverage of endemic regions; this situation may result in the death of as many adults as AIDS (Ekwanzala et al, 1996). Underdiagnosis is also exacerbated by the poor sensitivity of diagnostic methods. The number of cases reported annually increased dramatically from about 10 000 in 1980 to more than 27 000 in 1998. In the most endemic regions (e.g. Equateur and Bandundu), many communities have been found to have a prevalence of over 10% during recent case-finding surveys. In 1994, an extra-ordinary prevalence of 72% in a small village of the Bandundu region was reported.

Angola is the country with the second highest incidence of HAT, respectively 8275 and 6610 new cases were reported in 1997 and 1998 by the national control programme. Variations in the annual number of reported cases must be interpreted with caution due to the impact of the civil war on case-finding. The disease is endemic in the north-west provinces. The prevalence rates reported vary between 1.3% and

9.7%. Uganda is the only country where both *T. b. gambiense* and *T. b. rhodesiense* are found, the former in the north-west of the country, near the Sudanese border, and the latter in the south-east, without overlap. Between 1000 and 2000 *T. b. gambiense* cases were reported annually in 1990-1994, but this has at least stabilized with 978 cases reported in 1998, more than half of them from Arua district. A major epidemic of Rhodesian HAT devastated SE Uganda from the mid-1970s, with a cumulative total of about 40 000 new cases over 15 years (Smith et al, 1998; Hide, 1999). There has been some improvement recently, after many years of efforts by national authorities with substantial external support. Only 271 cases were reported in 1998 from the SE of the country (Busoga). In Sudan, where reliable statistics are not available, the foci of *T. b. gambiense* HAT are located in the southern part of the Equatoria region, west of the Nile, within 100 kilometres of the borders with Central African Republic (CAR), the DRC and Uganda. Extrapolations suggest that there must be at least a few thousand cases per year. In other countries of Central Africa, HAT is more or less an emerging public health problem. Elsewhere in East and Southern Africa, the incidence of *T. b. rhodesiense* trypanosomiasis remains low. In West Africa, the disease has regressed or disappeared from several countries as ecological changes reduced the intensity of man-fly contact. Only Côte d’Ivoire and Guinea still report a significant number of cases. Only a few dozen cases are seen each year in Burkina Faso and Mali, corresponding both to importation of cases and local transmission. The situation is less well known in other West African countries.

It is difficult to estimate the overall burden of HAT. There are about 100 000 new cases per year, with between one third and one half of cases remaining undetected and untreated. Rhodesian trypanosomiasis represents less than 5% of the overall burden of the disease. Recent estimates (WHO, 2000) are rather similar, with 2.05 million disability adjusted life years (DALYs) lost, and 66 000 deaths, in 1999 due to HAT. As a comparison, the number of millions of DALYs lost is estimated at 45.0 for malaria, 4.9 for lymphatic filariasis, 2.0 for leishmaniasis, 1.9 for schistosomiasis, 1.1 for onchocerciasis, 0.7 for Chagas disease.

**EPIDEMIOLOGY OF T. B. GAMBIENSE HUMAN AFRICAN TRYPANOSOMIASIS**

**The Life Cycle of *T. brucei***

The complex life cycle undergone by *T. brucei* in its tsetse fly vector and human host is described in various standard textbooks. The whole cycle lasts about 30 days on average; it varies, according to species and the ambient temperature, between one and eight weeks. When *Glossina* takes a blood-meal from an infected host, it ingests bloodstream trypanomastigote forms in its salivary glands. Trypanosomes then move to the fly’s midgut where, over a few days, they transform into the procyclic stage. The variant surface glycoprotein (VSG), the dominant constituent of the surface of bloodstream trypanosomes, is replaced by an invariant surface protein. After two to three weeks of maturation and multiplication, trypanosomes migrate to the salivary gland, where other transformations lead to their development into metacyclic trypanosomes, which require VSG to be infective for a susceptible human or animal host during the next blood-meal. These forms are the metacyclic trypanomastigotes, the only stage which is infective to vertebrates. Once infected, a tsetse fly remains so for the rest of its life span of between three and four months. While taking subsequent blood-meals, the fly is capable of inoculating another vertebrate host with the metacyclic trypanosomes. Within the human host, the parasite multiplies at the site of inoculation, where a chancre might develop. Inoculation chancres are rarely recognized on African skin. From this site, the parasite gets into the bloodstream and lymph nodes and multiplies through binary scissiparity with three morphological forms: the short and stumpy, the intermediate, and the long and slender forms.

After infection of a human host, there is a switch from the expression of metacyclic VSG to bloodstream VSG. To evade the host’s immune response, trypanosomes can successively express different VSG, but only one at a time. This antigenic variation is a unique feature of trypanosomes (Barry, 1997); it has direct consequences for the epidemiology of the disease. Up to a thousand different VSGs are genetically encoded, and it is thought that the VSG currently expressed protects invariant constituents from the host’s immune response. The mechanisms through which trypanosomes switch to expressing a different VSG are complex, but it allows the parasite to escape from antibodies directed against the previous VSG. This complex process explains the intermittent parasitaemia and the very long, largely asymptomatic, incubation period. Thus, there is an alternation between periods of higher parasitaemia following expression of a new VSG, during which the human host might be more infectious, and periods of lower or undetectable parasitaemia, during which infectivity must be lower. Variations in the virulence of *T. b. gambiense* strains were noted early by Van Hoof (1947). More recently, biochemical, molecular and immunological methods have been developed for the identification of trypanosomes by
examining their genetic material (Gibson, 1994). These methods include two-dimensional gel electrophoresis and isoenzyme, chromosome and DNA analysis using polymerase chain reaction (PCR) techniques, which have substantially contributed to a better understanding of the parasite’s taxonomy and the epidemiology of the disease, especially in studies on animal reservoirs of human trypanosomes. Cellular and molecular characteristics of human trypanosomes have been reviewed elsewhere (El-Sayed and Donelson, 1997; Barry 1997).

The Vectors

Vector species and sub-species
The tsetse fly is a vector of trypanosomes that infect man as well as wild (antelopes, giraffes, etc.) and domestic (pigs, cattle, sheep, goats, dogs, horses, etc.) animals. It belongs to the genus Glossina, which includes about 30 known species divided into three groups: the Glossina sub-genus which includes species of the morsitans group; the Nemorhina sub-genus (palpalis group) and the Austenina sub-genus (fusca group). A software has been developed for the identification of glossinids by species and subspecies and the determination of their epidemiological importance (Brunhes, 1994). Several factors related to the vectors determine the transmission of trypanosomes to humans: vector biology and ecology, vectorial capacity, man-fly contacts, longevity, dispersal, feeding behaviours, etc.

Distribution and ecology of Glossina
A large variety of traps have been tested for tsetse sampling and control, a dozen of which were reviewed by Leak (1999). The favourite technique currently used to study Glossina habitats, densities and ecodistribution is the biconical trap developed by Challier and Laveissière (1973). Turner (1980) proposed the “marking-release-recapture” technique using a radioactive compound (e.g. Fe²⁹) and scintillometer to study its biocological characteristics such as Glossina habitats, behaviour and dynamics, population size, densities, dispersal, survival rates, resting sites.

The palpalis group contains two excellent vector species of T. b. gambiense and of animal trypanosomiases in West and Central Africa: G. palpalis palpalis in forest areas and G. p. gambiensis in savannah areas. The former inhabit the forest areas and the moist savannah, whereas the latter is found in the semi-arid savannah. G. tachinoides and G. fusipes fusipes are related to the palpalis group and are vectors of sleeping sickness in the savannahs of West and Central Africa while G. pallidipes is found in the savannah of Central Africa. The savannah is the exclusive habitat of G. morsitans in various regions of Africa. Its geographical distribution is limited to gallery forests bordering streams, where optimal survival conditions are found. In a given geographical area, the distribution of tsetse flies varies depending on the species and is mainly determined by the climate, presence of water, vegetation and availability of sources of blood-meals (humans and animals). Studies conducted in the forest zone of West Africa have shown that G. p. palpalis is a very mobile vector found in different biotopes and that its distribution is closely linked to human occupation patterns (Baldry, 1980; Challier and Gouteux, 1980; Gouteux and Laveissière, 1982). Highest apparent densities per trap (ADT) per day were observed on the edges of villages where the swine population provides an abundant, easily accessible food source. High densities have also been found near sources of potable water, in coffee and cocoa plantations, especially those located on the edge of forests or gallery forests, and along paths separating plantations from the remaining forest used by humans and some wild animals, especially the bushbuck, which are food sources for Glossina.

The tsetse fly is only active for a short time when looking for blood-meal (35 minutes on average per day). It spends most of the time resting to digest or gestate. The amount of activity of each species determines its chances of encountering a host on which a blood-meal may be obtained and varies depending on climatic factors (temperature, humidity, amount of light, wind, rain), olfactory and visual stimuli (smelling and seeing a potential feeding host), and on intrinsic factors (physiological age, nutritional status, gravidity).

Vectorial capacity, competence and susceptibility of Glossina
The vectorial capacity of Glossina is determined by its ability to infect itself while feeding on a vertebrate host, and to subsequently develop an infection and transmit the trypanosome to another vertebrate host (Challier, 1982). According to these criteria, only the palpalis and morsitans groups contain species and sub-species that are vectors of T. b. gambiense. It has been demonstrated that not all flies in a given area have the same capacity to transmit the parasite. It is therefore important to determine whether or not there are local conditions that may reduce or increase this capacity. The tsetse fly’s ability to infect itself while feeding on a parasitized host depends on several poorly understood factors. The number of trypanosomes ingested by the fly during its blood-meal could be one such factor. However, it has been demonstrated that a single trypanosome
can infect *G. m. morsitans* (Maudlin and Welburn, 1989; Baker, 1991). The concept that a blood-meal taken from an individual with low-level parasitaemia is less infectious for the vector than one taken from a host with high-level parasitaemia needs to be investigated. Under natural conditions, the infection rate among tsetse flies is quite low. On average, less than 1% of tsetse flies are infected with *T. brucei* ssp (Molyneux, 1980b). Teneral flies are more susceptible to infection by trypanosomes.

As reviewed by Leak (1999), it has been suggested that lectins present in the haemolymph and midgut of the fly are responsible for increasing the resistance of the tsetse fly, with age, to infection with *T. brucei*. Lectins are absent or blocked in unfed teneral flies, thus permitting infection, while in older flies lectin production seems to be stimulated by blood-meals, preventing subsequent infections. The trypanosome also has effects on *Glossina*. Jenni et al (1980) have shown that the infected insect has a tendency to bite more often and feed more voraciously, which can have a substantial impact on its transmission potential. According to Maudlin et al (1998), the risk of death increases with age, but much more quickly in infected than non-infected tsetse flies. Infected flies are much more susceptible to insecticides than non-infected flies (Nitcheman, 1990).

**Feeding behaviour and trophic preferences**

The first concern of the teneral *Glossina* is to find a host on which a blood-meal may be taken. The young adult needs a blood-meal to complete its maturation. This first meal takes place between 24 and 72 hours or even later, and depends on intrinsic (diurnal rythm, sex, gravidity, species, etc.) and environmental (climatic conditions, visual, mechanical and olfactory stimuli) factors (Colvin and Gibson, 1992). The tsetse’s feeding ground may be restricted by the relatively limited dispersion of potential sources of blood-meals. In forest areas, *G. p. palpalis* confines itself to the outskirts of villages where swine hide, along paths through plantations, near sources of potable water, and around farm camps and other places used by man (Laveissière and Hervouët, 1981).

Most studies on the trophic preferences of *Glossina* were conducted in West Africa and based on analyses of blood-meals collected by dissecting the insect. Methods with different degrees of sensitivity have been reviewed by Leak (1999), including the precipitin test, the agglutination inhibition test, the complement fixation test, direct and indirect ELISA assays, tests based on the latex agglutination technique, and, more recently, one based on electrophoresis of superoxide dismutase (Diallo et al, 1997) which can only distinguish between meals of human and animal origin. Glossinids of the *palpalis* group are notable for their eclecticism and opportunism: they feed indiscriminately on many species and are therefore very dangerous to man. In the forest zone of Côte d’Ivoire, Gouteux et al (1982) found that at least 75% of the blood-meals were from suidae around the villages. In plantations in the same zone, however, Laveissière et al (1985) found that 46% of the blood-meals were from man. This observation was confirmed by recent results of Sané et al (2000).

A comparison of the results of analyses of blood-meals collected in the five main foci in Côte d’Ivoire showed that the proportion of human blood-meals varies significantly from one focus to the other, although the socio-geographic conditions in these foci appear to be identical: a human origin corresponded to 42% of blood-meals of *G. p. palpalis* at Vavoua, 73% at Daniafla, 55% at Zoukougbeu, 91% at Gagnoa and 27% at Sinfra. Humans, therefore, seem to be the favourite host, in differing degrees, of *G. p. palpalis* in plantation zones of Côte d’Ivoire. This preference for man was greater in the foci with low transmission rates (Daniafla, Gagnoa) than in the more active foci (Sinfra, Vavoua and Zoukougbeu). Domestic animals appear to play an important role in feeding tsetse flies at Vavoua and Zoukougbeu, the most active foci, whereas, in the low-incidence foci, the percentage of animal blood-meals is insignificant. At Zoukougbeu, apart from on humans, *G. p. palpalis* feeds freely on domestic swine (30% of meals), whereas at Vavoua it prefers the bushbuck which provides an equal percentage of its food as man (42%). The diversity of the feeding regimen of *G. p. palpalis* might explain to some extent the variations in levels of incidence of human disease. In foci where transmission is more intense, *G. p. palpalis* feeds on both man and animals and proper case-finding and treatment of infected humans is not sufficient to control the disease. With a diversified regimen alternating between man and animals as sources of blood-meals, tsetse flies are able not only to transmit trypanosomes to humans, but also to maintain the putative animal reservoir. In contrast, in low transmission foci, the tsetse fly depends on man, transmission to and from animals is rare, and case-finding prevents the accumulation of human cases. This West African paradigm should probably not be systematically extrapolated to high-incidence foci of Central Africa, where the role of animals is less clear. If one of its usual mammal hosts is not available, *G. tachinoides* replaces it with reptiles as observed in the gallery forest of the savannah: snakes or lizards account for between 54% and 67% of the blood-meals of *G. tachinoides*, while only 8% of its meals are from man (Laveissière and Boreham, 1976). The feeding
preferences of this vector also vary seasonally because of changes in the availability of hosts. In the hot season, 30%-55% of its meals come from mammal hosts (mainly humans and bushbucks) whereas in the cold season, the only animals available are reptiles, and these provide over 50% of its meals (Laveissière and Boreham, 1976).

Transmission cycles of T. b. gambiense
In the forest zone of Côte d’Ivoire, man-fly contacts occur in almost all biotopes but especially in plantations and periurban areas where it is easier for the fly to find hosts (Challier and Gouteux, 1980; Laveissière et al, 1985). Some authors attribute the persistence of residual foci of HAT to the existence of other cycles adjacent or parallel to the common man-fly-man cycle (Molyneux, 1980a; WHO, 1998), i.e. cycles where the parasite travels between domestic and/or wild animals (swine, sheep, goats, bushbuck, etc.) and man. They identify two such transmission cycles: a) a domestic cycle where the Glossina transmits the parasite between human hosts and domestic animals; b) a sylvatic cycle where the vector transmits the parasite between wild animals, sometimes with humans or domestic animals entering this cycle, resulting in sporadic cases or even epidemic outbreaks. The domestic cycle hypothesis is supported by similarities observed between parasites isolated in humans, animals and the vectors (Gibson et al, 1978; Mehlitz et al, 1982), whereas there is little evidence that wild animals are infected with T. b. gambiense.

Tsetse flies and the epidemiology of African trypanosomiasis
The transmission of infectious trypanosomes to humans depends on many factors: the density of Glossina populations, Glossina longevity, the vector’s susceptibility to infection, Glossina infestation rates and the factors that influence these, and human behaviours and activities in the biotopes of the flies that determine the frequency of man-fly contacts. As described in the previous sections, the vector’s biology, ecology and feeding behaviour have direct consequences on transmission of the parasite. Thus, through its biological cycle, the Glossina allows the maturation, development, multiplication, transmission and dissemination of the parasite. The feeding behaviour of various species of Glossina determines their epidemiological contribution to the transmission of T. b. gambiense to the humans, and to some extent the animals, that constitute the parasite’s reservoir. The female G. p. palpalis, because of its more aggressive feeding behaviour and longer life span, plays an essential role in the transmission of the parasite.

The number of trypanosomes inoculated into the mammal host during a blood-meal is probably one of the factors that determine the probability of transmission. It has been estimated that, to infect man, the inoculum must contain between 300 and 500 trypanosomes (Challier, 1982). Transmission is also influenced by factors intrinsic to the vector (physiological age, nutritional status, gravidity, etc.) and by climatic and other environmental conditions. The low infection rate naturally observed among tsetse flies limits the devastating epidemic potential of African trypanosomiasis, and explains the apparent paradox between the abundance of the G. p. palpalis and G. morsitans vectors, and the relative rarity of human disease. There is no direct correlation between the densities of G. p. palpalis, the major vector living in close contact with man, and the incidence of sleeping sickness.

In endemic foci, the nature, frequency and intensity of man-fly contacts are the major determinants of the risk of African trypanosomiasis. In the forest zone of Côte d’Ivoire, there is virtually no ecological zone where humans are safe from being bitten by G. p. palpalis. Man-fly contacts can occur in all botanical zones and are affected not only by the vegetal environment but also, and especially, by human hosts. Multidisciplinary studies have shown that human activities and behaviours have an important impact on the epidemiology of sleeping sickness, through an increase in the frequency and intensity of man-fly-contact (Laveissière et al, 1985, 1986a, 1986b; Hervouët and Laveissière, 1987; Méda et al, 1993). For example, coffee-growing, which requires the planter to spend more time in the plantation than if growing cocoa or food crops, is associated with a high risk of infection due to the increased contact time with the vector. This risk is heightened by residing in farm camps and by procuring water from natural water sources located near edges of plantations and gallery forests. Many other activities, such as collecting firewood, washing and fishing, bring human hosts into contact with tsetse flies. The availability of other sources of blood-meals (e.g. pigs) reduces the chances of transmission.

The Human Reservoir
Humans are the main reservoir of T. b. gambiense. Four factors potentially influence man’s potential in transmitting T. b. gambiense: the duration of infection, the degree of parasitaemia, the number and distribution of individuals who are infected, and the intensity of contact with the vectors.

The duration of infection in humans
Given that the tsetse fly is a relatively ineffective vector, the very long duration of infection (from a
few months to several years) during which the human host can maintain normal activities, and provide blood-meals for the vectors, must be the key determinant behind the endemic features and epidemic potential of Gambian HAT (Baker, 1974). Fortunately, this long duration of infection offers a golden opportunity for intervention. Control programmes focusing on the identification and treatment of asymptomatically infected humans, and thus on a shortening of the duration of infection, are extremely effective if an overwhelming majority of the population shows up during case-finding surveys and if sensitive diagnostic methods are used.

The distribution of infection in human populations
Prevalence of trypanosome-infected individuals in human populations varies tremendously according to socio-demographic factors. Trypanosomiasis is not found equally in males and females. The apparent preponderance of cases among females would not correspond to substantial differences in incidence or prevalence if reliable denominators were available (which is rarely so). Females often participate more regularly in case-finding sessions, which can also lead to a higher number of cases (Asonganyi and Ade, 1994). Variations in incidence and prevalence between age and ethnic group have also been noted. This probably relates to differences in occupational exposure and other determinants of man-fly contact rather than to genetically determined susceptibility (Laveissière et al, 1986a; Hervouët and Laveissière, 1987; Médé et al, 1993). Other risk factors have been described, which are probably all markers of exposure to infective tsetse flies: lack of formal education, absence of pigs (an alternative source of blood-meals for tsetse) in the habitat, etc. (Médé et al, 1993; Médé et al, 1995).

Occupation is also related to exposure and to incidence. For instance, in Côte d’Ivoire, HAT is more frequent in coffee and cocoa plantation workers or people who fetch water than in other inhabitants (Laveissière et al, 1986a and b; Médé et al, 1993). More than 80% of cases occur in people who not only work but also live in small plantation settlements; a case-control study showed that such people were five times more likely to develop trypanosomiasis than their counterparts who resided in villages (Médé et al, 1993). In some foci of the DRC, a higher prevalence was found in fishermen, while elsewhere, farmers were more likely to get infected (Henry et al, 1982; Mentens et al, 1988).

Human population density influences the risk of epidemics in *gambiense* sleeping sickness (Scott, 1970). If population density is very light, tsetse flies take most blood-meals on non-human sources and transmission to humans is unlikely. On the other hand, high human population density results in modifications of the habitat that reduce the tsetse population. Thus, an intermediate population density is optimal for Gambian trypanosomiasis to prosper. A recent study in Côte d’Ivoire showed a strong correlation between the epidemiological risk and settlement density (Laveissière and Médé, 1999). Whether or not there are secular or seasonal variations in the frequency with which humans become infected with *T. b. gambiense* is unknown but plausible through changes in tsetse densities, man-fly contact, presence of alternative sources of blood-meals, etc.

Familial aggregation
Familial clustering of trypanosomiasis has been recognized since the beginning of the century. Compared to children of mothers without a past history of HAT, the risk of a child having had trypanosomiasis was four times higher if the mother had had the disease, while it was two times higher in brothers and sisters of a case than in their half-brothers and half-sisters. Such clustering could be due to either genetic susceptibility or to shared exposure to the vectors. Several arguments reviewed elsewhere (Khonde et al, 1997) suggest that the latter is the most plausible explanation. Shared exposure could result from simultaneous contact with an infective tsetse whose blood-meal on a first individual is interrupted and resumed on a nearby relative, or from members of a same family sharing an ecological microcosm and being similarly but not simultaneously exposed to the vector bites (Gouteux et al, 1989).

The influence of human behaviour
Human behaviour plays an important role in the epidemiology of Gambian trypanosomiasis. Health seeking behaviour might delay the recognition of an epidemic and enhance transmission of the parasite if symptomatic individuals wait for months before reaching a health facility where trypanosomes can be detected, maybe because they first attributed the disease to other, supra-natural, causes and sought traditional treatment. Participation in case-finding surveys varies from place to place and over time, and is a key determinant in the success or failure of such programmes.

Migrations have contributed to trypanosomiasis epidemics, as they favour the circulation of trypanosomes from high-incidence to low-incidence areas where the population is more susceptible (Prothero, 1963). Although this remains controversial, the explosive epidemics seen in Uganda and the Congo a century ago have been attributed by
many authors to the large-scale circulation of workers organized by the colonizers to suit their needs (Leak, 1999). A recent example of the impact of migrations is the epidemic in NW Uganda, which resulted from the exodus of Ugandan refugees to Sudan and Zaire where they got infected, followed a few years later by the migration to Uganda of infected Sudanese refugees and the return of Ugandans back home (Paquet et al, 1995).

Immunity
It was generally thought that no immunity followed a first diagnosis of HAT, because of the parasite’s antigenic variation and its repertoire of VSG which includes hundreds of different antigens. However, observational and experimental studies in animal models have shown these animals to be more resistant to homologous trypanosomes when re-challenged after adequate treatment of a previous infection. This protection resulted from exposure to metacyclic trypanosomes rather than to bloodstream forms (Nantulya et al, 1984; Akol and Murray, 1985; Vos et al, 1988). The existence of protective immunity in humans was investigated in a very-high incidence community of the DRC where 38% of adults had a past history of trypanosomiasis (Khonde et al, 1995). The results suggest that a first episode of trypanosomiasis confers to adults about 85% protection against subsequent reinfection.

The impact of HIV
Little is known about the interactions between the human immunodeficiency virus (HIV) and Gambian trypanosomiasis. Three studies performed in Central Africa (Louis et al, 1991; Pépin et al, 1992) and Côte d’Ivoire (Méda et al, 1995) suggest that HIV infection has so far had little impact on the epidemiology of Gambian trypanosomiasis, to some extent because the prevalence of HIV remains relatively low in rural communities where HAT is endemic. No data are available for T. b. rhodesiense areas. Given that HIV infection is getting more prevalent in rural areas, it is worthwhile undertaking a well designed nested case control study to further investigate these interactions. It has been observed that HIV co-infected HAT patients respond less well to eflornithine treatment than seronegatives (Milord et al, 1992), but this is unlikely to have any impact on transmission.

The Animal Reservoir
The existence of an animal reservoir of T. b. gambiense has been investigated for a long time (Makumyaviri et al, 1989; Leak, 1999). Earlier studies showed that many species of domestic animal could be experimentally infected with T. b. gambiense: pigs, dogs, goats, sheep and even chickens (Van Hoof, 1947; Schutt and Mehlitz, 1981). These infections generally resulted in low parasitaemia that lasted less than a year. The study of naturally occurring infections in animals was facilitated by the development of appropriate laboratory methods: the blood incubation infectivity test (BIIT), isoenzyme electrophoresis, and DNA analysis. Domestic animals were found to be infected with parasites enzymatically identical to T. b. gambiense. Pigs have generated more interest because they are a frequent source of blood-meal for tsetse flies, and have been found infected with T. b. gambiense in Liberia (a country with little human disease), Côte d’Ivoire, Congo and the DRC (Gibson et al, 1978; Schutt and Mehlitz, 1981; Mehlitz et al, 1982; Noireau et al, 1989; Truc et al, 1991). In Côte d’Ivoire, 52 sympatric T. brucei strains were characterised by isoenzyme electrophoresis: among 12 zymodemes revealed, the most frequent was found both in humans and pigs (Penchenier et al, 1997). In Congo, sheep were also found to be infected with T. b. gambiense, and the prevalence of Trypanozoon infection in domestic animals was estimated to be 0.5% (Noireau et al, 1989; Truc et al, 1991). A dog was found infected with T. b. gambiense in Liberia (Zillmann et al, 1984). A more recent study using PCR showed the simultaneous presence of T. b. gambiense in humans and animals (a dog and a pig) from the Lower Congo province of the DRC (Schares and Mehlitz, 1996). However, the epidemiological significance of the animal reservoir is unknown. Whether or not animals may become a threat for reintroduction or persistence of the parasite in foci where near elimination of HAT has been achieved remains an important research question (Molyneux, 1980a).

EPIDEMIOLOGY OF T. B. RHODESIENSE TRYPANOSOMIASIS
HAT caused by T. b. gambiense and T. b. rhodesiense differs in its epidemiological features. We will only stress these differences. T. b. rhodesiense is a zoonosis sporadically transmitted to humans when they venture into bush infested by tsetse fly vectors whose blood-meals are normally taken on game animals. Humans enter this sylvatic cycle as an incidental host. Except during epidemics, human-fly-human or domestic animal-fly-human transmission is thought to be rare. The epidemics usually involve G. f. fuscipes, a peridomestic fly, and transmission occurs around the villages. The disease, characterized by an acute or subacute malaria-like syndrome with high parasitaemias, progresses over weeks or months rather than months or years. The vectors differ from those of T. b. gambiense. The parasite is transmitted mostly by three species of the morsitans group: Glossina morsitans morsitans and G. m. cen-
trypanosomiasis in Central and East Africa. The existence of animal reservoirs was established in the 1950s. Cattle are the major reservoir (Hide et al, 1996), and played a major role in an epidemic in south-east Uganda, with 23% of cattle carrying human infective strains. Other domestic (dog, sheep, maybe pig) and various game (warthog, bushbuck, hartebeest, impala, lion, zebra, hyena) animals can harbour the parasite in their blood; some of these animals are well adapted to the parasite and can remain infected for more than two years without overt disease. Conversely, some other species (e.g. dog) rapidly succumb from the infection. In contrast to T. b. gambiense trypanosomiasis which results from peridomestic infections in Central Africa, rhodesiense trypanosomiasis is, in endemic situations, acquired far from the village, where the animal reservoir lives. It is mainly an occupational disease of adult men: hunters, fishermen, firewood collectors and honey gatherers have been found to be at higher risk. Tourists visiting game parks are also exposed to the infection. However, in epidemic situations, when transmission becomes peridomestic, all groups, including males and females are at similar risk. Indeed, it has been observed that an increase in the number of cases in children and women is an indication that an outbreak is developing (Apted, 1970). As it is in gambiense sleeping sickness, familial aggregation has been noted (Okia et al, 1994). In contrast to Gambian trypanosomiasis, there is a marked seasonality of disease occurrence, with a higher incidence during the warmer season (Smith et al, 1998). Population movements and political upheavals played an important role in development of recent epidemic in south-east Uganda (Mbulamberi, 1989a; Smith et al, 1998). Changes in agricultural practices also led to more favourable conditions for the development of G. fucipes, and resulted in the recent peridomestic epidemic, which was brought into control by the surveillance and early diagnosis through sleeping sickness orderlies and vector control (Smith et al, 1998; Okoth, 1999)

CONTROL OF HUMAN AFRICAN TRYpanosomiasis
Sleeping sickness control relies on two principles: reduction of the parasite reservoir through case detection and treatment, and reduction of man-fly contact through vector control. In the case of T. b. gambiense, reduction of the human reservoir can be achieved through case-finding and treatment. The limiting factor is the relatively low sensitivity of the standard parasitological techniques. A more frequent use of finer serological and parasitological techniques could lead to a rapid and sustainable reduction of the human reservoir. In order to reduce man’s exposure to infectious bites, the tsetse flies must be destroyed. This must be carried out with the active involvement of the community, who can take on trap laying, spraying, bush clearing, etc. There are a variety of vector control techniques, the choice of which depends on financial and human resources, the epidemiological situation, and the programme duration. Within the African context, vector control must be carried out with cheap, cost-effective and easy-to-use methods. Control integrated into primary health care and targeted on groups at risk has been found feasible, cost-effective and cheaper, provided village health workers (VHWs) are motivated and the beneficiary population participates fully. Community involvement, both in case finding and vector control, through the implication of VHWs, has been successfully tested on a large scale in various epidemiological contexts.

Case Detection
Case detection has been the cornerstone of HAT control. For many years, specialized case-finding mobile teams have relied essentially on the presence of swollen cervical lymphs nodes. Lymph node fluid, when present, is examined for evidence of trypanosomes. In some very active foci, the mobile teams carry out wet film and giemsa-stained thick smear examinations. Because of the low sensitivity of these methods, better serological and parasitological tools have been developed over the last two decades.

Serological methods
Various serological assays were developed to help case-finding teams identify a small number of antibody carriers on whom to concentrate efforts for trypanosome detection using parasitological methods. In the 1970s, the indirect fluorescent antibody test (IFAT) was deemed the most reliable technique for epidemiological surveillance of T. b. gambiense trypanosomiasis. However, it required relatively expensive equipment and qualified staff, and its implementation was possible only in laboratories. Delays in obtaining results were such that some seropositive suspects could not be located again to undergo the parasitological confirmation test. From the 1980s onwards, the IFAT was supplanted by the card agglutination test for gambiense trypanosomiasis (CATT) (Magnus et al, 1978), the advent of which considerably enhanced detection of cases of Gambian trypanosomiasis. The CATT is a latex agglutination test relying on the detection of antibodies using the variable antigen type (VAT) LiTat 1.3 anti-
gen, which is expressed by several T. b. gambiense stocks. This is the only serological assay currently used by control programmes. It can be performed in the field, without electricity and without specialized staff, and the results are available within 10 minutes. It is cheap, at approximately 0.40 USD per test, and is generally performed on whole blood. Its sensitivity varies between 92% and 100% when assessed in patients parasitologically confirmed in hospital or other settings, and its specificity is thought to be 94-97% (Noireau et al, 1988; Miézan et al, 1991). The CATT proved highly specific during testing in non-endemic areas (Bafort et al, 1986). Some strains of T. b. gambiense do not express the LiTat 1.3 antigen, but their distribution seems fairly limited (Dukes et al, 1992). The positive predictive value of the CATT depends on the prevalence of disease in the population. This prevalence ranges between 1 and 5% in most endemic foci and the positive predictive value generally varies between 14% and 40% (Miézan et al, 1991). The CATT can be performed on filter paper, using smaller volumes of reagents, thus reducing costs (Miézan et al, 1991). However, the reliability of this micro-CATT is less satisfactory under field conditions than in a research setting. The CATT can also be used on diluted serum rather than whole blood, resulting in higher specificity at the expense of lower sensitivity (WHO, 1998).

The card indirect agglutination trypanosomiasis test (CIATT) is an indirect assay that was newly proposed for the detection of circulating trypanosome antigens in patients’ blood (Nantulya, 1997). The parasite antigen detected is an internal, invariant molecule that is common to both T. b. gambiense and T. b. rhodesiense. This test can be used to detect current infection in both forms of the disease. It has been found to be highly sensitive in endemic areas (Asonganyi et al, 1998). The test was shown to be easy to use in field conditions and does not need chain maintenance for the storage of reagents. A recent evaluation in non-endemic areas revealed a specificity ranging from 61% to 98% depending on the proximity of the endemic zone (Meda et al, submitted for publication). Specificity is improved by titration of seropositive specimens. Apart from their diagnostic potential, the CATT and CIATT have also been found to have potential for use in patient follow-up to determine chemotherapeutic cure. Further operational studies aimed at testing the usefulness of the CIATT in clinical settings and for field use by national control programmes should be carried out.

**Parasitological methods**

Confirmation of diagnosis among seropositive individuals depends on demonstrating the trypanosome in biological fluids. This confirmation can be obtained through the examination of wet blood or giemsa-stained blood smears, or by direct examination of the lymph node aspirate when a typical lymphadenopathy is palpable (Cattand and de Raadt, 1991; Miézan et al, 1994). These classical methods have a rather low sensitivity in gambiense trypanosomiasis. Demonstrating the presence of trypanosomes in blood and cerebrospinal fluid (CSF) has been considerably facilitated by the development of concentration methods. The micro-haematocrit centrifugation technique (Woo’s test) is much more effective in the rhodesiense disease (and in veterinary medicine) than in T. b. gambiense HAT. The miniature anion-exchange centrifugation technique (mAECT) is deemed at present to be the most sensitive parasitological method for the detection of blood parasites (Miézan et al, 1994). Because of the cost (about US$2) and its relative complexity, it is used selectively to test CATT-positive suspects among whom the diagnosis could not be confirmed by classical methods. Two new parasitological techniques have recently been added to the diagnostic arsenal: the quantitative buffy coat technique (QBC) (Bailey and Smith, 1992) and the kit for in vitro isolation of trypanosomes (KIVI) (Aerts et al, 1992). Neither the QBC, which requires relatively expensive equipment, nor the KIVI, which is a parasite isolation method rather than a screening test, has proved to be superior to the mAECT (Truc et al, 1994).

In the CSF, the most sensitive technique is that of double centrifugation (Cattand et al, 1988; Miézan et al, 1994). A variation of the latter, the single centrifugation of cerebrospinal fluid in a sealed Pasteur pipette, has been recently developed (Miézan et al, 2000). It makes detection of trypanosomes in the CSF simpler, quicker and more sensitive, and is especially suitable for passive diagnosis in suspects who consult in health facilities with symptoms suggestive of sleeping sickness. The combination of all these seroparasitological methods ensures increased sensitivity, but falls short of perfectly reliable parasitological diagnosis in all seropositive persons, hence the need to pursue efforts to develop more sensitive parasitological assays and more specific serodiagnostic techniques.

**Treatment and Drug Resistance**

Pentamidine remains the standard treatment for early-stage patients and melarsoprol for late-stage cases. The treatment for HAT is selected by first establishing the stage of infection. The diagnosis of late-stage trypanosomiasis is based on at least one of the following criteria (WHO, 1998): CSF white cell count (WCC) > 5/mm³ or CSF proteins >37 mg/100
ml (as measured by dye-binding protein assay), or on both criteria, with or without the presence of trypanosomes in CSF. Miézan et al (1998) reported that the CSF WCC is, by itself, as sensitive for diagnosis of central nervous system involvement as is the combination of the above criteria. Therefore, the WCC was recommended in patients with confirmed infection, especially in poorly equipped facilities. Advances have been made in the treatment of HAT. Between 5% and 10% of late-stage patients treated with melarsoprol, an arsenical derivative, succumb to its undesirable effects. Until recently, melarsoprol was the only available treatment for late-stage patients. A new drug, efloornithine, has been developed (Pépin and Milord, 1994). Results obtained with efloornithine are excellent, but its future availability remains doubtful.

Drug resistance has been a relatively uncommon phenomenon in Gambian trypanosomiasis, despite suramin, pentamide and melarsoprol having been used for five decades (Pépin and Milord, 1994), and, as a consequence, has not had any impact on the epidemiology of the disease. Suramin is little used in the treatment of Gambian trypanosomiasis. Suramin and Pentamide are given throughout Africa to patients with early-stage of T. b. rhodesiense and T. b. gambiensensis disease respectively. So far, the treatment failure rate remains fairly low. The situation is quite different for melarsoprol. There are at least two foci where melarsoprol resistance is more frequent than elsewhere; clearly this is an issue that will need better investigation and monitoring over the next few years. One is the Mbanza Kongo focus of northern Angola, close to the border with Lower-Congo: a 40% failure rate was reported 25 years ago (Ruppol and Burke, 1977) and similar failure rates have been observed in recent years. Limited epidemiological data suggest that there has been little spread of resistant strains over time. In the Arua district of northern Uganda, a 27% failure rate has recently been reported among new cases treated with melarsoprol (Legros et al, 1999); a 10-fold lower failure rate has been documented in the adjacent focus of Adjumani. In the DRC, where melarsoprol failures are not specially frequent, statistically significant differences in failure rates were found. It has been also observed that HIV co-infected HAT patients respond less well to efloornithine treatment than seronegatives (Milord et al, 1992), but this is unlikely to have any impact on transmission as efloornithine is little used and relapsing cases of HAT are often not parasitaemic.

Vector Control
The tsetse fly is one of the rare insects for which several control methods have been developed, based on bioecological and epidemiological studies. Before the advent of insecticides, vector control depended primarily on elimination of the wooded vegetation which constitutes the habitat of Glossina. Nowadays, insecticides are applied to various types of traps and screens to destroy the vector. A number of improved biconical, monoconical and pyramidal traps, inspired by the Chalieri-Laveissière biconical trap (Challier and Laveissière, 1973), have been tested for the control of G. p. palpalis, G. morsitans and G. f. quanzensis in West and Central Africa. Lancien designed the monoconical trap (Lancien, 1981), which was followed by the pyramidal trap (Gouteux and Lancien, 1986), and later by the Vavoua trap (Laveissière and Grébaut, 1990). To enhance trap efficacy, especially against G. morsitans, olfactory attractive baits are attached to them (carbonic gas, acetone, urine phenols and host skin secretions) (Leak, 1999). These act at a greater distance than purely visual baits.

Pilot studies conducted in various epidemiological settings have shown that trapping is effective. Efficacy is measured in terms of the apparent density of flies captured per trap per day (ADT), and varies according to the type of trap, the species or sub-species of Glossina, the environmental and climatic conditions, etc. (Laveissière, 1988). In the forest areas of Côte d’Ivoire, it was demonstrated that the black/blue/black screen is about twice as efficient as the simple blue one (Laveissière et al, 1987). In the West African savannah, the ADT of G. tachinoides and G. p. gambiensis populations was reduced by 88-92% using the blue screens (Mérot et al, 1984). The same screens reduced the ADT of G. tachinoides by 98% in only 15 days (Laveissière and Couré, 1981). In contrast, in the forest areas of Congo, the screens did not yield satisfactory results. The ADT was more drastically reduced (99%) after five months in the forest areas of Côte d’Ivoire using biconical traps (Laveissière and Hervouët, 1981). Later on, in the same areas, about 16 000 blue screens sited in coffee and cocoa plantations reduced the ADT of G. p. palpalis by 90% in one week, and by 98% at the end of five months (Laveissière et al, 1986c).

Traps and screens have thus replaced insecticide spraying. These vector control methods have generated much interest; they are effective, simple, environmentally friendly and suitable for use by the communities themselves. Pilot projects in Burkina Faso (Mérot et al, 1984), Côte d’Ivoire (Laveissière et al, 1994b), Uganda (Lancien, 1991) and Congo (Gouteux and Sinda, 1990) have demonstrated the feasibility and efficiency of traps and screens used with the participation of rural communities under
the supervision of specialized teams. The number of traps and/or screens required for a particular location depends on the type of vegetation in the tsetse habitats, and on the presence or number of edges, water sources, paths and encampments which determine the frequency and intensity of man-fly contacts. The communities are in a position to provide information on locations where the villagers are most often bitten. The life span of a trap depends on the quality of materials and of maintenance, and on the environmental conditions. In experiments conducted in West Africa, it was estimated that about 10% and 20% of the traps or screens needed to be renewed each year in the forest and savannah zones respectively (Laveissière, 1988). It is not possible to give the precise number of traps or screens to be installed per hectare, since each microcosm will have its own features. The number of traps or screens to be installed does not depend on the density of the human population to be covered. In the Vavoua pilot study, traps and screens were installed as follows: one screen every 100 metres, one or two screens per water-point or encampment, one screen at each working place in the plantation, one trap every 300 metres in forest areas and one every 100 metres around villages (Laveissière et al, 1994).

Traps are preferable to screens if re-impregnation and surveillance cannot be carried out by the population. Traps need to be re-impregnated once a year, at the end of the rainy season. Screens have to be re-impregnated once during the first year of the campaign, and twice a year later on. Traps should be installed as far beyond the endemic area as possible, so as to provide an effective barrier. In the gallery forest of savannah areas, open and sunny places frequently visited by people, such as washing and bathing places, water collection points, bridges and banks of rivers, are the preferable sites. In forest areas, the tsetse flies must be intercepted at the interfaces between different ecological patterns, i.e. the edges that are considered as epidemiologically dangerous areas (Laveissière et al, 1986b), such as areas around villages, paths separating wooded areas and other types of ecological patterns, especially cocoa or coffee plantations, etc.

Vector control with community involvement, as part of a comprehensive sleeping sickness control programme, was organized in the forest areas of Vavoua (Côte d’Ivoire) where Laveissière et al (1985) recorded a 90% reduction of ADT in the villages and farms a week after setting up the traps and screens, and over 99% after three months. These results remained stable for the first six months. Subsequently, ADTs have increased. This phenomenon was linked to the gradual abandonment of equipment maintenance, especially during the farming season, and to lower efficacy due to the insecticide being washed away by rains and the screens being concealed by weeds which grow quickly during the rainy season. Two years later, the impact of the control programme on the incidence of human disease was nevertheless obvious: no new patient had been detected during the case-finding survey carried out in the study area where the overall prevalence was initially higher than 1%. Globally, this experiment had shown that about a year’s vector control was needed to substantially reduce transmission. Similar results were recorded in Congo (Gouteux and Sinda, 1990) and Uganda (Lancien, 1991). Clearly, efficient case-finding must be conducted simultaneously with vector control, otherwise the persistence of the human reservoir will lead to a rapid resurgence of disease when vector control is pursued less vigorously. The cost of a screen and a trap (Vavoua type) was estimated to be US$3 and US$6 respectively (Laveissière and Médéa, 1992). The cost of one hectare protected was estimated at US$1 for the first year, and much less for the second year. Costs vary depending on the type of trap or screen used and on whether or not the equipment is insecticide-impregnated.

Genetic control by the release of sterile males is unsuitable for control in epidemic situations; it poses a series of technical, financial and logistic problems which do not facilitate its implementation in endemic countries.

GAPS IN KNOWLEDGE AND RESEARCH NEEDS

Over the last two decades, new and much more effective tools for HAT control have been developed compared to those that existed some forty years ago when the disease was almost eliminated. This progress contrasts with the present epidemiological situation. The disease remains a serious emerging public health problem in Central, East and West Africa. WHO, industrial firms, bilateral and multilateral organizations have decided to mobilize adequate resources to combat it. Many gaps in knowledge relevant to control of the disease need to be urgently filled. Some of these gaps have been identified as priority areas by the Working Group on Operational Research on African Trypanosomiasis (Geneva, 1997) and the International Colloquium on Operational research priorities on African trypanosomiasis (Antwerp 1998). Scientists and programme managers are invited to pay much more attention to the multiple questions related to the development of new tools for control or better use of existing ones. Most perspectives in HAT research and control proposed by Kuzoe (1991) still need to
be considered. In the short and intermediate terms, some of the research priorities include the following fields.

**Improvement of Epidemiological Knowledge and Disease Surveillance and Control**

Little is known of the basic epidemiology of HAT disease in many endemic countries, especially Tanzania, Southern Sudan, Angola, Chad, Central African Republic and Guinea. It has been speculated that *T. rhodesiense* sleeping sickness occurs among tourists in East Africa; if this is true, it does mean that maybe the disease is rising to an epidemic level among local populations. Improvement in knowledge of the host-parasite and vector-parasite relationships is of great interest for the development of new tools and products for control, including new drugs for treatment. The zoonotic nature of *T. rhodesiense* was confirmed about forty years ago. The situation is completely different for *T. gambiense*; it has been shown that domestic animals such as pigs, dogs, sheep and cattle can, or do, carry parasites whose isoenzyme features match those of parasites infecting humans. An important question arises as to the epidemiological importance of these animal reservoirs in *T. b. gambiense* trypanosomiasis. At present, the exact role of these reservoirs is unknown; it is difficult to establish the relative importance of transmission between animals and humans. Some studies suggest that such transmission could play a significant role by maintaining a minimum level of transmission when the level of endemicity is low. If this transmission is epidemiologically significant, what are the factors that influence the infective contacts between animals and humans? Is there a sylvatic cycle which contributes to the persistence of Gambian trypanosomiasis? How can this cycle be interrupted? Several methods have been proposed for blood-meal analysis. What is the most reliable, easy to use in field conditions, and inexpensive, technique for the determination of blood-meal origin of the vectors?

**Surveillance and Management of Sleeping Sickness**

The card agglutination test for trypanosomiasis (CATT) is a simple, easy to use, sensitive and relatively effective tool for the diagnosis of *T. b. gambiense* trypanosomiasis, which has been available for two decades. However, there is no equivalent test for *T. b. rhodesiense* sleeping sickness; it has been found that *T. b. gambiense* in Central Africa does not express the antigens used in the CATT. Therefore there is need to improve the reagents presently used. More recently, another simple test, the card indirect agglutination trypanosomiasis test (CIATT), was evaluated. This test has been found to be highly sensitive and sufficiently specific for both types of HAT. Its suitability and predictive value in clinical management need to be assessed in different epidemiological settings. The test is easy to use in field conditions and does not need cold chain maintenance for storage; therefore, it is worthwhile evaluating its suitability for screening in primary health care programmes. The last meeting of the Task Force on Operational Research on African Trypanosomiasis elaborated guidelines for designing the following studies on the CATT and CIATT:

- Assessment of chemotherapeutic cure of sleeping sickness using the CIATT in *T. b. gambiense* and *T. b. rhodesiense* HAT.
- Comparison of the CIATT and CATT in the diagnosis of *Trypanosoma brucei gambiense* sleeping sickness in a clinical setting.
- Applicability of the CIATT in the diagnosis of *Trypanosoma brucei rhodesiense* sleeping sickness in a clinical setting.
- Operationality of the CIATT versus CATT in a field survey of *T. b. gambiense* sleeping sickness.

The usefulness of remote sensing as a tool for supporting vector control and surveillance needs to be explored further (Kuzoe, 1991). Is it a reliable tool for the identification of high risk areas for human trypanosomiasis? Is it an adequate tool for surveillance of the disease? Some questions, specifically related to surveillance and treatment and requiring investigation, are given in the handbook by Leak (1999), including: Is there a level of vector density that ensures a low endemic level of trypanosomiasis? Is trypanosome prevalence, as determined by passive surveillance, a reliable indication that more resources are needed for active surveillance? What should be the interval between visits of mobile teams in the context of active surveillance?

PCR has recently been introduced as a new tool for the identification of trypanosomes; however its usefulness in the diagnosis and management of the disease in humans, and its application in studies on animal reservoirs, remain unsatisfactory. The technology needs to be refined, adapted for field use, and transferred to field workers who need to be trained in its application.

Geographical information system (GIS) and mapping technology offers a cost-effective and rational
tool for monitoring and control of disease, and is now available for spatial analysis of data collected in endemic foci. This tool has been successfully applied in the control of animal trypanosomiasis in savannah areas. In the context of limited resources, GIS is more and more perceived as an efficient tool for the identification and mapping of high risk areas where control efforts should be directed. However, its application in HAT has been very limited so far. Therefore, there is a need to provide opportunities for multidisciplinary research teams, including geographers, to further use GIS in pilot control programmes.

Another gap mentioned by some young researchers is that there is no functional network for scientists working in the field of trypanosomiasis at regional or sub-regional levels. Such a network would link senior and young scientists together, allow efficient use of human resources and strengthening of the scientific capabilities of young researchers.

**Research on Existing and New Drugs, and Drug Resistance**

The ideal drug is one that is safe, effective, affordable, and can be taken orally. Scientists and industrialists are invited to collaborate in the development of new effective compounds for the treatment of HAT. Pharmacokinetic studies have shown that it is worthwhile evaluating pentamidine administered for tree days versus seven days in the first stage of the disease. Studies in laboratory animals, using several combinations of existing drugs such as pentamidine and eflornithine, suramin sodium and melarsoprol, or melarsoprol and nifurtimox, have shown them to be synergistic. However, few data on the effects of drug combinations on either type of African trypanosomiasis are available. In view of the increasing development of resistance to melarsoprol monotherapy in some areas, studies on the available drugs, given separately or in combination (eflornithine/melarsoprol, nifurtimox/melarsoprol, etc.), should be undertaken in humans, based on the latest pharmacokinetic information. Relapses following treatment with melarsoprol, with or without pentamidine, occur on a large scale in the DRC and Angola. Studies on factors associated with treatment failure with melarsoprol should be designed to identify preventive measures. Trials on alternative regimens of melarsoprol (e.g. 14 days versus 10 days) might improve its efficacy and tolerance. Many hypothetical reasons have been given for treatment failure, such as possible reinfection, misclassification of the disease, lack of compliance with treatment, and individual variation in the pharmacokinetics of the drug. Studies on the magnitude of the phenomenon, and on the risk factors and biological and environmental conditions determining the epidemiology of resistance to the drug, are lacking. Nifurtimox is a synthetic nitrofurane which was primarily used for treatment of Chagas disease due to *T. cruzi*. In HAT, it has only been used in the former Zaire and in Sudan, mostly in patients with infection refractory to melarsoprol, in combination with other trypanocidal drugs. Further studies using an improved protocol should be carried out in *T. b. gambiense* areas where melarsoprol resistance is expanding (the DRC, Angola, Sudan) in order to identify effective combinations of drugs. Controlled clinical trials on prednisone versus placebo, to assess reduction of incidence of melarsoprol-associated encephalopathy, might also be interesting. No data are available on the efficacy of new drugs (nifurtimox, steroids) in *T. rhodesiense* sleeping sickness.

**Research on Tsetse Flies and Vector Control**

The biotopes most suitable for tsetse flies are known for almost every biogeographical area, except mangrove swamps. There is a need to collect basic epidemiological information on types of sites in order to determine which areas present the greatest risk to humans in West and Central Africa. A better understanding of the variability of vectorial capacity of the tsetse fly, including age, population structure, and feeding patterns, might lead to improvement of control; populations that are genetically different might have different vectorial capacities in different epidemiological contexts. What are the sociological factors that affect man-fly contact and are responsible for the outbreak of epidemics? A recent meeting held in Harare recommended setting up a quality control system for new insecticides to be used for tsetse control. What is the role of population mobility in the emergence and maintenance of epidemics of sleeping sickness in various epidemiological settings?

Data collected from catching tsetse flies with traps are classically analysed using latin squares. However, this method is questionable; there is a need to identify more adequate statistical techniques for the analysis of these particular types of data.

**Social and Economic Impact of Sleeping Sickness**

Little information is available in the literature on the social and economic impact of HAT. Therefore, there is a need for social and economic studies to evaluate the sustainability and long-term cost-effectiveness of integrated control programmes (Kuzoe, 1991). Some evidence suggests that sleeping sickness can reduce physical attributes and socio-economic potential at individual, family and community levels; but physicians, demographers, social scientists and economists have so far been unable to quantify and quali-
fy the effects of the disease. Operational research is also needed to assess the impact of the current structural health sector reforms on sleeping sickness control programmes. Community involvement in tsetse control and disease surveillance is of crucial importance. However, human factors which motivate communities to participate may vary considerably (according to socio-cultural factors, areas) and need further investigations. What kind of message and what is the best way to communicate information to rural communities? Multidisciplinary research teams including social scientists, economists and basic scientists should be involved in such studies.

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II VECTOR CONTROL IN RELATION TO HUMAN AFRICAN TRYPANOSOMIASIS

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CONTROLLING VECTOR-BORNE DISEASES

Controlling vector-borne diseases represents a special challenge to societies in developing countries. Apart from the sheer size of the public health problems posed by diseases such as malaria and sleeping sickness, vector-borne diseases are especially difficult to control. One of the main reasons for this is that vector-borne diseases are quantitatively different from directly transmitted diseases in that the basic reproductive number ($R_0$ - the number of new infections which arise from a single current infection, introduced into a population of susceptibles, during the period of its infectiousness) for vector-borne diseases is often an order of magnitude greater than for directly transmitted diseases. This means that, generally, vector-borne diseases are more difficult to control and may bounce back from control faster than directly transmitted diseases. However, it follows from our theoretical understanding that targeting control activities at the vector has potentially the greatest impact on disease transmission (see below).

CONTROLLING SLEEPING SICKNESS

It is over a century since it was first recognized that tsetse flies were responsible for transmitting trypanosomes from wild animals to domestic livestock (Bruce, 1895). When it subsequently became clear that tsetse were also responsible for the transmission of human sleeping sickness (Bruce and Nabarro, 1903), control of these insects assumed massive importance for the colonial powers because of the threat the disease posed to the economic development of Africa south of the Sahara. Subsequently the largely anglophone countries of East, Central and Southern Africa aimed a large part of their research and control efforts at elimination of the vector combined with passive case detection. By contrast in West Africa, and particularly among the francophone countries, the more direct approach of controlling the disease in humans became established practice. This followed from the success of Jamot, who pioneered large-scale case detection and treatment with the arsenical drug Atoxyl (de Raadt and Jannin, 1999). By combining large-scale arsenical chemotherapy with institutional interventions such as regulating the movement of people, T. b. gambiense epidemics were brought under control in the francophone region of West Africa. This francophone approach was also adopted during the 1930s to control epidemics in Nigeria and Ghana with the newer arsenical tryparsamide. The different colonial administrations appear to have become locked into ‘their’ strategies of either treating the cases or dealing with the vector (de Raadt and Jannin, 1999). It is, nonetheless, clear that both approaches were effective.

In the case of sleeping sickness, as with other vector-borne diseases, the first line of defence in the face of an epidemic would appear to be tsetse control – why then, was this approach not universally adopted across colonial Africa? And why were the alternative strategies adopted in francophone Africa successful? It is instructive to examine the reasoning behind the decisions governing interventions for sleeping sickness control, not only to explain the events of history but also to inform policy-makers faced with the resurgence of sleeping sickness (World Health Organization 2001).

TWO DISEASES – TWO APPROACHES TO CONTROL

In retrospect, we tend to view this division between francophone and anglophone, in approaching what was apparently the same problem, as merely another example of the failure of communications between two competitive colonial powers. This may, however, be simplistic. Assuming that there was some logic to this dichotomy of approach, it is profitable to first consider how this position arose on the two sides of the continent, as this may influence how we might best proceed to control sleeping sickness in the future.

The reasons for the differing approaches to disease control have their origins in the biology of two quite distinct diseases. These differences may be expressed within a theoretical framework of vector-borne disease transmission, as discussed below.

AN EPIDEMIOLOGICAL FRAMEWORK TO EVALUATE SLEEPING SICKNESS CONTROL STRATEGIES

The basic reproductive number, $R_0$, is central to understanding how the parasite spreads through, and is maintained, in a population. A threshold condition of $R_0>1$ must be achieved if a parasitic species “is capable of invading, and establishing itself within, a host population” (Anderson and May 1992). Ideally, disease control will force $R_0<1$, and so eventually lead to the eradication of the parasite from the population. Determination of this threshold condition for transmission is, therefore,
important for the design and implementation of control strategies. (If the threshold condition cannot be met, then control activities should aim to maintain \( R_0 \) at as low a value as possible.)

The study of vector-borne disease epidemiology and the design of control programmes has benefited from the development of a basic mathematical theory by Macdonald (based on Ronald Ross’ earlier work), which captures the essentials of vector transmission (Macdonald, 1957). From this theory, the reproductive number of a vector borne infection, \( R_0 \), is defined as:

\[
R_0 = \frac{ma^2bc}{r} \exp(-uT).
\]

where \( m \) is the ratio of vectors to humans; \( a \) is the feeding rate of vectors, such that the average time between feeds is \( 1/a \); \( c \) is the probability that a susceptible vector acquires infection after feeding on an infectious human; \( b \) is the probability that a susceptible human acquires infection after being bitten by an infected vector; \( r \) is the rate at which humans recover from infection, with the average duration of infection being \( 1/r \); \( T \) is the time taken for the parasite to mature in the vector; and \( u \) is the vector mortality, such that \( 1/u \) is the average vector life expectancy. A fuller explanation of how this expression is derived, both mathematically and intuitively, is given by Anderson and May (1992).

The epidemiological impact of a particular control intervention may be investigated by assigning its effects to one or more of the parameters in the expression for \( R_0 \). For example, vector control will kill vectors, and so increase \( u \) and decrease \( m \); while active case detection and treatment would increase \( r \). Thus the expression for \( R_0 \) provides insights into vector-borne disease epidemiology and a framework in which to evaluate the relative impact of different interventions. An important qualitative conclusion from the simple expression for \( R_0 \) is that killing adult vectors is more effective than the early treatment of cases. The time a case is infected enters into the expression for \( R_0 \) linearly via the parameter \( r \). In contrast, adult vector mortality enters in a nonlinear fashion via the term \( \exp(-uT) \).

This general model has subsequently been developed to specifically model the African trypanosomiases, in both animals and humans, by Rogers (1988). The model provides a theoretical basis to investigate how differences in the natural history of \( T. b. gambiense \) and \( T. b. rhodesiense \) will effect the epidemiology of sleeping sickness caused by the two pathogens, and how these differences will impact on the relative effectiveness of the same control interventions.

Two well recognized differences between sleeping sickness caused by \( T. b. gambiense \) and \( T. b. rhodesiense \) are the duration of infection and the role of the animal reservoir. \( T. b. rhodesiense \) infections are characteristically acute, with death often occurring within a few months of infection (Odiit et al, 1997; Apter, 1970; WELLDE et al, 1989). The role of a non-human animal reservoir in \( T. b. rhodesiense \) transmission has long been recognized (Heisch et al, 1958; Onyango et al, 1966). Indeed, movement of cattle from the sleeping sickness endemic areas of south-east Uganda has been implicated in the origins of a \( T. rhodesiense \) outbreak in an area previously free from the disease (Fèvre et al, 2001). By contrast, \( T. b. gambiense \) infections tend to be chronic, with the time between onset of symptoms, development of late-stage disease and subsequent death often occurring over several years (Apter, 1970). Also, humans are generally considered to be the main host for \( T. b. gambiense \) infections with the animal reservoir less important than in \( T. b. rhodesiense \).

However, the role of the animal reservoir in \( gambiense \) infections is still unresolved. Rogers (1988) argued that domestic animals may be essential in the maintenance of \( T. b. gambiense \) as \( R_0 \) in humans alone may fall below unity, leading to suggestions that the existence of a non-human animal reservoir in \( T. b. gambiense \) infections may have contributed to the failure of human population surveillance and treatment campaigns to eradicate sleeping sickness in certain settings (Morris, 1946; Rogers, 1988). However, it has also been argued that the existence of an animal population on which tsetse flies preferentially feed may result in humans receiving infectious bites at a lower rate than if flies only feed on humans (Goutex, Laveissière and Brehm, 1982).

These differences in the natural history of the diseases may be incorporated into the expression for \( R_0 \) and their impact on the relative effectiveness (expressed in terms of reducing \( R_0 \)) of strategies to control the two types of sleeping sickness demonstrated (Welburn et al, 2001). It was shown that detection and treatment of cases profoundly reduces the transmission of \( T. b. gambiense \) but has relatively little impact on the transmission of \( T. b. rhodesiense \). This is because the duration of \( T. b. rhodesiense \) is relatively short, and the animal reservoir is a relatively important component of \( R_0 \).

In the light of these theoretical and biological considerations, the different approaches adopted by the colonial authorities of the time are seen to be quite logical.

**CONTROL IN THE 21ST CENTURY**

While, theoretically, vector control will proportionally have the greatest impact on disease transmis-
sion, it may not always be the approach of choice in the field when other variables are taken into account:

- Treatment of infected people has to be carried out for humanitarian reasons, and funding may usually being found for such emergency interventions.
- In the war zones of Africa, sustaining a tsetse control operation is very difficult and logistically more demanding than case finding. Tsetse control operations in these circumstances may be merely token, having little impact when carried out by poorly equipped and under-trained personnel. Given limited resources and societal instability (e.g. as in the current T. b. gambiens outbreak in Southern Sudan, Congo and Angola), it may not be appropriate to divert resources from case finding towards tsetse control.
- Targeted treatment of the animal reservoir, in the case of T. b. rhodesiens, is a potentially effective, inexpensive and sustainable control option (Welburn et al, 2001).

Tsetse control operations, whether top-down or enacted by the community, will always suffer from problems of sustainability, which have been considered in great depth elsewhere (Brightwell et al, 2001). While trapping programmes run by the community are appealing to donors, they may simply be ineffective in the face of an epidemic. Under such circumstances, alternative actions (including aerial spraying) may have to undertaken, which may require very large inputs from the donor community. Moreover, sleeping sickness control has unfortunately become a supply driven endeavour. As more and more smart technology has become available for the control of tsetse, so the need has been felt to apply it to the control of sleeping sickness epidemics – but this may not always be appropriate.

By contrast, treatment of sleeping sickness cases and the domestic animal reservoir form part of the normal activities of the medical and veterinary services. When these services break down, for whatever reason, it is usual for aid agencies, the private sector and even communities themselves to step in to the breach. Therefore, these activities are likely to be more sustainable in the medium and long-term.

CONCLUSION AND FUTURE RESEARCH

From a theoretical perspective, killing tsetse flies is a preferred strategy. However, epidemiological effects are only one side of the story. Financial and logistical resources needed to implement and sustain control interventions also have to be consid-

ered, as in reality these resources are always limited. Indeed, in countries affected by sleeping sickness, the many resources necessary for implementation of certain control strategies are often non-existent or severely constrained. Control policies should be based on the optimal utilization of the available resources. To help determine this optimal utilization, cost-effectiveness analysis is an important tool (Murray et al, 2000; Shaw et al, 2001). Such analysis must be based on a sound understanding of the natural history of the disease, to best predict the likely epidemiological effects of the same resources invested into alternative interventions.

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INTRODUCTION

This discussion paper seeks to identify the main issues involved in designing cost-effective strategies for the control of human African trypanosomiasis (HAT) and to highlight areas where more information needs to be compiled or new research initiated.

Some form of control of the disease has been ongoing in most parts of Africa since the beginning of the last century. Thus many schemes have been funded and budgeted for. However, the economics of the different approaches and their cost-effectiveness have only been studied sporadically. Thus, whilst most people working on the disease have a very clear idea of what the most cost-effective strategies are in their locality, given their resources and price structure, little has been done to bring together the information gained in order to help decision-makers design strategies and prioritize in new areas or under new circumstances.

This paper is the first step in an ongoing exercise to:
- identify the main factors influencing the cost-effectiveness of the different approaches to controlling the disease.
- review the current literature on this subject.
- identify potential research areas and information needs.

For 1999, the burden of HAT was estimated at 66 000 deaths and 2 million DALYs lost (World Health Organization, 2000). Although only 45 000 new cases were reported (World Health Organization, 2001), the likely number of people affected is probably ten times greater, thus approaching half a million. Of the 60 million people thought to be ‘at risk’, only 3–4 million are covered by some form of disease surveillance.
The control of HAT is based on combinations of the four different approaches illustrated in Figure 1. It involves:

- treating those human patients diagnosed with the disease,
- trying to improve diagnosis, by some level of surveillance, to find patients and ensure that they are treated earlier, hopefully while still in the first stage of the disease.
- proceeding to more active forms of case-detection, aimed not only at finding and diagnosing patients but also at reducing the size of the human reservoir of the disease. This applies to the gambiense form of the disease.
- trying to reduce the chance of people picking up the infection from domestic animals or wildlife, as in Uganda, where cattle are routinely treated around new outbreaks to control the rhodesiense form of the disease (personal communication, I Maudlin).
- controlling tsetse fly populations so as to reduce transmission of both forms of the disease.

Finally, as indicated in Fig. 1 by the two boxes marked ‘point of contact’, control of HAT involves:

- avoiding areas likely to lead to infection - a strategy that people have used since time immemorial. This strategy applies, above all, to avoiding contact with the tsetse fly and is an approach that has mainly been employed by livestock keepers to prevent their cattle becoming infected and either dying or becoming less productive. As people become aware of the dangers of working in certain tsetse-infested thickets, they avoid them. In the past it was thought that people could avoid contracting the rhodesiense form of the disease simply by avoiding areas containing wildlife. In the early stages of an epidemic of sleeping sickness, the disease tends to be found among people whose occupations put them at risk by bringing them into contact with infected flies, particularly at certain times of the day (e.g. when collecting water, washing clothes, entering game reserves - as in the case of beekeepers, hunters, park rangers). Thus, avoidance, whilst not specifically discussed here, is a control strategy of sorts, although in practice it has mainly been used by cattle herders to protect their stock.

FINDING AND TREATING PATIENTS

As cited above, WHO estimates are that only about 10% of sleeping sickness patients are correctly diagnosed and receive treatment. This proportion may be larger in endemic foci where active surveillance is undertaken, but it can also be a great deal smaller. Typically, patients who are detected passively have suffered from symptoms for some time, possibly years in the case of gambiense sleeping sickness, and have made several attempts to have their symptoms treated and obtain a correct diagnosis. Usually this will have involved several trips to their rural health centre, possibly also to a nearby hospital or treatment centre, a visit to a local healer, and being treated for malaria and other diseases before being diagnosed as having sleeping sickness. Both during trips to the health centre and while the patient is hospitalized, it will usually be necessary for a relative to accompany and look after the patient. As the disease progresses, the patient in search of a diagnosis will have become more and more of a burden on his family, requiring care and being unable to undertake normal activities.

A very rough estimate of the possible cost to patients trying to obtain a diagnosis was made by Landell Mills (2000) for the rhodesiense situation in Uganda. This came to US$825 per patient, including estimates for the cost of transport to rural health centres and hospitals, treatments for malaria, painkillers, and the time taken by relatives to accompany and care for the person. For those rhodesiense patients who are never correctly diagnosed but who do receive some treatments, this cost would rise to a minimum of US$50, including consultations with a local healer, further trips to treatment centres, possibly a short stay in hospital, and care by relatives.

For those correctly diagnosed, the costs of treatment, as estimated by WHO in 1998, are given in Table 1. These figures are based on the then current drug costs, treatment regimes in use, and estimates of the cost of hospitalization.

<table>
<thead>
<tr>
<th>Item</th>
<th>First-stage disease</th>
<th>Second-stage disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pentamidine</td>
<td>Sulfadoxine</td>
</tr>
<tr>
<td>Estimate of total cost</td>
<td>107</td>
<td>114</td>
</tr>
<tr>
<td>Cost excluding drug cost</td>
<td>87</td>
<td>79</td>
</tr>
<tr>
<td>Drug cost as % of total cost</td>
<td>19</td>
<td>31</td>
</tr>
</tbody>
</table>

However, these costings will need to be re-evaluated in the light of current plans to make drugs available free of cost from WHO for a specified number of years. The costs of transport and administration will remain, but, for the relevant drugs, the cost paid by recipient countries or programmes will consist only of transport and drug administration. As the table indicates, treatment costs could be reduced by significant percentages, but nevertheless, in economic as against purely financial terms, the use of these drugs will still represent a resource cost which should be taken into account. Furthermore, drug
availability on these terms will alter with time. Taken together therefore, this implies that the average cost for patients (a high proportion of whom will already be in the second stage of the disease) passively diagnosed and correctly treated would be in the order of US$50-300 each.

In order to analyse this aspect further, there is a need to:
• analyse case histories of individuals being treated for the disease in order to determine by what process they obtained a diagnosis, how long it took, and how much it cost them and the health services.
• collate information on currently used treatment regimes and the costs of hospitalization at trypanosomiasis treatment centres.

ECONOMICS OF CONTROLLING THE HUMAN RESERVOIR: GAMBIAENSE FORM OF THE DISEASE

Turning next to active surveillance, which has been the mainstay of programmes to combat the gambiense form of the disease, calculated costs are given in the World Health Organization (WHO) Expert Committee report of 1998 (see series of tables in Annex 9 of that report). The discussion below is based on these calculations, as originally presented by Shaw et al (1995) and following the same approach as those prepared for the previous WHO Expert Committee on HAT (Shaw and Cattand, 1985). In order to produce a coherent set of costings, it was necessary to use a real situation as a basis while making some adjustments to produce a scenario in line with generally accepted norms, and the figures and prices used were based mainly on work conducted in the Moyo District of Uganda (John, 1995). The figures used in 1985 were based on WHO’s work in the Daloa area of Côte d’Ivoire.

WHO’s calculations of 1998 were used to create a spreadsheet (Microsoft Excel®) model, so allowing results to be calculated for any starting prevalence. The population covered, number of units involved in surveillance, sampling intensity, sensitivity and specificity of screening and diagnostic tests, as well as all prices and other costs, can also be varied. The results of repeated runs of this model enable the relative cost-effectiveness of different sampling strategies at different prevalences to be analysed. These are presented and discussed in the series of graphs below.

The original analyses were based on five alternative surveillance strategies, including the classic mobile teams, fixed-post surveillance, and the less widely used innovative techniques of filter paper sampling by trained community health workers who either visit the community or are based at rural health centres:
• Fixed-post or passive surveillance. Using this strategy, patients who present with symptoms that are difficult to diagnose, or who don’t respond to treatments e.g. for malaria, are eventually referred to a treatment centre and tested for a variety of diseases, including trypanosomiasis, so that those with the disease are eventually diagnosed. The initial screening test is performed on wet blood.
• Filter paper sampling at rural health centres. Under this strategy, community health workers (CHWs) based at rural health centres receive some training in collecting samples on filter paper and routinely test all new patients presenting themselves at the health centre for whatever reason.
• Filter paper sampling by community health workers. CHWs trained in collecting filter paper samples spend 20% of their time collecting samples and following up seropositive individuals - as based on experience in Uganda (John, 1995) and Côte d’Ivoire (Laveissière et al, 1995).
• Monovalent mobile teams. These are the classic surveillance teams. The card agglutination test for trypanosomiasis (CATT) is performed on whole blood, and all the parasitological tests, except lumbar punctures, are conducted in the field. Monovalent teams work only on trypanosomiasis.
• Polyvalent mobile teams. These operate in the same way as monovalent teams except that only a third of their work consists of screening for trypanosomiasis.

In order to standardize the results, calculations were based on areas with a population of 100 000 people, containing 10 rural health centres, and where 20 community health workers were operating. The numbers screened by each strategy were assumed to be as given in Table 2.

<table>
<thead>
<tr>
<th>Surveillance strategy</th>
<th>Fixed-post surveillance</th>
<th>10 rural health centres</th>
<th>20 community health workers</th>
<th>One monovalent mobile team</th>
<th>One polyvalent mobile team</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number screened per annum</td>
<td>300</td>
<td>3000</td>
<td>24 000</td>
<td>36 000</td>
<td>20 000</td>
</tr>
</tbody>
</table>

The cost calculations covered:
• initial screening using the CATT test.
• parasitological confirmation using gland punctures, the capillary tube centrifugation technique.
(CTC) test, the miniature anion centrifugation technique (m-AECT), and lumbar punctures.

- training of staff in specialized techniques needed for diagnosing trypanosomiasis.
- administrative overheads.
- depreciation (annual cost) of capital items (vehicles, laboratory equipment, etc.).
- travel allowances and a share of salaries of all staff in proportion to the amount of time they spend on trypanosomiasis control.
- running costs for vehicles, specialized equipment and other recurrent costs for each surveillance strategy.

Firstly, the relative performance of the different surveillance strategies was analysed in terms of the cost of finding *gambiense* patients. The results, in terms of US$ per trypanosomiasis patient found, are given in Figure 2. Obviously the cost of finding a patient declines very rapidly as the prevalence increases, since a higher proportion of those screened are infected. This clearly does not reflect any increase in efficiency, simply that more and more of those screened are infected, so the costs of the operation average out over a larger number of individuals. Fig. 2 is given in three sections so that the differentials between the costs of each sampling strategy can be seen more clearly.

At very low prevalences (of 1% or less, see Fig. 2a), such as those encountered in past years in areas where surveillance was reasonably regular and the disease was considered to be under control, the costs (at a 0.05% prevalence) are well over US$2000 per patient identified using rural health centres or mobile teams, and drop to just under US$200 using CHWs. Passive or fixed-post detection costs only US $50 per patient identified since there are virtually no overheads. When the prevalence reaches 1%, the cost of passive detection falls to just over US$20 per person, while surveillance using CHWs costs just under US$100 per person, and using mobile teams or rural health centre costs between US$120 and US$140.

At medium level prevalences (from 1% to 5%, see Fig. 2b), the costs per patient found continue to fall, and the differentials between strategies narrow further, with the cost of passive detection being US$14, of detection by CHWs being US$22, and of the other three options being about US$30.
At high level prevalences (over 10%, see Fig. 2c), the cost of patients found passively falls to around US$10, as it does for all surveillance strategies at a prevalence of 20%. When the prevalence reaches 50%, the cost of detection becomes very low, around US$5 for all surveillance strategies, except passive detection where it remains at about US$10.

Given the way in which the figures were calculated, by independently building up the cost of each strategy using local norms and prices, it is surprising how similar the costs for finding patients using different strategies are. Setting aside passive detection, of the four active detection strategies, CHWs using filter paper is consistently the most cost-effective.

The purpose-built mobile team concerned only with trypanosomiasis surveillance is consistently the most costly.

The almost total convergence of costs at high prevalences is due to the fact that, at higher prevalences, more than half of all costs are diagnostic costs for initial screening and parasitological examinations (ranging between US$3.50 and US$2.50 per person). At these prevalences, most individuals are sero-positive and have to be re-examined, so the running costs and overheads associated with each strategy are spread over a large number of patients.

In order to further examine the relative effectiveness of the different surveillance strategies, Fig. 3 shows what proportion of the population could be sampled in a year using the different approaches and reflecting the assumptions made about the possible workload that each form of active surveillance can tackle. The figure shows the situation in the hypothetical area with a human population of 100 000, a fixed number (10) of rural health centres, and a fixed number (20) of CHWs who can assign a significant proportion of their time to active case detection for sleeping sickness. In such a situation, only the inputs by the mobile teams can be varied, for example increased as illustrated, from spending half their time in the area to having two teams working there full time. Based on experience, it was also assumed that mobile teams were able to undertake further examinations on a higher proportion of CATT-positi-
tive patients than was possible under the surveillance strategies based on CHWs. The filter paper based sampling strategies involve a delay before the results are known, after which individuals testing positive are recalled and taken to a treatment centre for further testing. In contrast, mobile teams can undertake many of the parasitological tests themselves, and transport the suspected patients to a treatment centre for final confirmation of disease status.

Under these conditions, as illustrated in Fig. 3, finding and treating a majority of the patients in the population can only be done using one or more monovalent mobile teams. The other active surveillance strategies could be effective in detecting the presence of the disease, or in gradually eroding the size of the human reservoir, provided that the incidence is not high.

Turning from the costs per individual found with the disease to the total investment required for finding trypanosomiasis patients, Fig. 4 illustrates these for the five different surveillance strategies for prevalences of 1% and 10%. As would be expected, the costs largely reflect the proportion of population screened by each strategy (Fig. 3 and Table 2). Although the costs would vary from country to country, and have probably increased somewhat since these figures were published in 1998, they give an idea of the orders of magnitude involved, ranging from US$50 000 to US $60 000 for a monovalent mobile team, to a minimal investment for fixed post/passive detection.
In order to better understand how the situation evolves at high prevalences, details of the calculation for intervention by a mobile team are shown in Fig. 5. Here, the cost of treating the identified patients has been added to produce the total costs, which have been broken down into four categories. The costs of surveillance (logistics, share of salaries, etc.), which are given as 'sampling strategy', and the costs of diagnostic tests, which cover both initial screening and parasitological confirmation, dominate the total costs at very low prevalences. But once a prevalence of 1% is reached, all other costs are dwarfed by the cost of treating patients. Given the uncertainty about how to value drug costs, these have been included at their recent commercial cost but separated from other treatment costs (administration of drugs and hospital care). Thus the graphs can be read so as to exclude the cost of drugs. A notional figure for transport, or for their economic cost, could be included in subsequent analyses.

Fig. 5a
Breakdown of costs of detection and treatment of patients at low to medium prevalences
(one mobile team sampling 36,000 people)

Fig. 5b
Breakdown of costs of detection and treatment of patients at high prevalences
(one mobile team sampling 36,000 people)
The costs of controlling the disease in the area postulated, with one monovalent mobile team screening 36% of the population in a year, thus range from just over US$50,000 where the prevalence is 0.1%, to over US$4 million where the prevalence is 70%.

This analysis, based on conditions in Uganda and extrapolation of the situation encountered there, thus examines the ways in which the costs of controlling the human reservoir vary with the sampling strategy, sampling intensity, and prevalence. The spreadsheet model produced provides a basis for extending this analysis to cover other surveillance protocols, price sets, test sensitivities, etc. It is likely that the costings for the extremely high prevalences need revising upwards, since in the model, labour and time requirements were not fully adjusted to a situation where virtually all individuals test positive to the screening test and need parasitological confirmation.

To update and validate this analysis in different circumstances, what is required is:

• collation and examination of data from budgets and actual expenditures from a range of surveillance activities in different countries.
• to find out what protocols are used in different field situations and what results are obtained, particularly in terms of what sequence of tests are used to obtain parasitological confirmation and how many patients are detected by each test at different prevalences.

ECONOMICS OF CONTROLLING THE ANIMAL RESERVOIR: RHODESIENSE FORM OF THE DISEASE

Turning to the *rhodesiense* form of the disease, its more acute course means that patients usually present with symptoms shortly after infection. Nevertheless, diagnosis is often slow and inaccurate. Control of this form of the disease has relied less on active surveillance and more on vector control.

Recent research results have added a powerful and cost-effective tool to the armoury of control methods for this form of the disease. The proof that cattle have now become the main reservoir of the disease (Hide et al., 1996) in south-eastern Uganda has, as its corollary, shown that treating cattle would control the reservoir, and, if at suitable level, would stop transmission to humans. Although *T. b. rhodesiense* is not pathogenic to cattle, the drugs used to control it also kill *T. vivax* and *T. congolense*, which are pathogenic to cattle and are prevalent among the cattle in the area. Thus, treating cattle generates an economic benefit which is independent of the control of the disease in humans.

A preliminary and very approximate economic analysis of the control of *rhodesiense* disease in south-eastern Uganda was undertaken as part of a DFID-commissioned review of its research work (Landell Mills, 2000). Although based on extremely approximate assumptions, this analysis highlighted the potentially very favourable situation where it is possible to control the disease by treating cattle. Drug treatments cost US$1.75 to US$2 per dose, and it is thought that between 5% and 20% of cattle carry cattle pathogenic trypanosomes (personal communication, Paul Coleman). Based on work done on the impact of trypanosomiasis on livestock production, and current milk and cattle prices in Uganda, it was estimated that treating 1000 cattle around a disease focus could yield a benefit of between US$500 to US$3000, as compared to a cost of US$750 to US$2000. Furthermore, if this expenditure on cattle treatment was successful in reducing the incidence in humans, the financial benefit in terms of sleeping sickness treatment cost avoided would probably more than justify the expenditure.

In this preliminary analysis of the economics of disease control over the past decade in south-eastern Uganda, four categories of costs were considered: research, vector control, medical surveillance and cattle treatment (Landell Mills, 2000). The benefits were calculated by considering three likely alternative scenarios for what the disease incidence might have been if there had been no control activities. The monetary benefits consisted of costs saved for treating patients, benefits to cattle production, and patients’ costs incurred while seeking treatment (see section Finding and treating patients above). The non-monetary benefits were calculated in terms of DALYs averted. The monetary benefits produced benefit-cost ratios ranging from 1.08 to 1.98. Because the project produced a net financial gain, the cost per DALY was actually negative, ranging from -US$1.50 to -US$7.50. These results should be treated with caution, as they depend on very rough assumptions. However, they do point to the likelihood that this control approach, where it is feasible, could be extremely cost-effective.

Research into the involvement of cattle as a reservoir of *T. b. rhodesiense* is ongoing. From the economic point of view, one key issue is, what proportion of the cattle population will need to be treated, and with what frequency, in order to generate financial benefits which cover the costs of controlling the disease in humans? Another important variable is the ratio of reported to unreported cases of the disease in humans. Obtaining this information depends on the results of epidemiological studies. The economic gains to cattle production need to be
more accurately estimated, using data on the prevalence of cattle pathogenic trypanosomes and their impact on livestock productivity.

VECTOR CONTROL

At this stage of the work, it has not been possible to review existing information about the costs of vector control in detail. Recent years have seen few initiatives that use vector control to deal with human trypanosomiasis. Costs are available from recent work in Uganda, where pyramidal traps were very effective in reducing tsetse density and disease incidence; these costs (personal communication R. Floto) amounted to ECU 781,000, excluding technical assistance - at today’s prices, about US$1.1 million.

The costs of vector control will tend to be very specific to the situation, varying with terrain (especially for target and spraying operations), type of organization (especially for targets, traps or screens), and type of settlements and rural economy (being affected by the availability of labour for maintaining traps and targets, and the presence of cattle where these are to be treated with insecticides). Estimates of the cost of vector control operations, such as those cited below, almost invariably include only the marginal costs, i.e. the extra costs involved in field work, but neglect the considerable overheads, which can double or triple the costs per sq. km. Vector control operations include:

- aerial spraying, using fixed wing aircraft and the sequential aerosol technique, which typically involves spraying the area in a cycle of five at carefully timed intervals. Apart from the sterile insect technique, aerial spraying tends to be the most expensive control method. It has the great advantage of achieving a very rapid reduction in the fly population. Costs are likely to be well upwards of US$500 per sq. km.
- traps, screens and/or targets. The cost of these operations is far more variable than for aerial spraying, depending on the number deployed per sq. km. or per linear km. and on the way in which they are deployed and serviced. The costs for target operations probably range between US$300 and US$400 per sq. km., and low-cost trap- or screen-based operations could cost as little as US$100 to US$150 per sq. km.
- treating cattle with insecticides (not to be confused with treating cattle with trypanocides, as discussed above). Here, various ‘pour-on’ formulations are used, but again the costs are very difficult to estimate since the pour-on formulations also protect against tick-borne diseases, and the number of cattle to be treated per sq. km. is also a variable as is the number of treatments per year.

The cost of pour-on formulations currently ranges around US$1.50 to US$2 for an adult bovine. Insecticide treatment of cattle reduces fly density, but whether or not this would be of use in preventing HAT depends entirely on the local epidemiology of the disease.

A useful series of comparative costings for tsetse control methods for one country, along with details of how they were calculated, can be found in Barrett (1997) for the case of Zimbabwe. Current research and information needs include updating costings for the various strategies, and collating information on the impact that vector control operations undertaken in the past have had on the incidence of sleeping sickness. The extent to which community involvement and inputs, especially of labour, can be sustained over long periods of time also needs to be revisited.

It is difficult, especially for gambiense disease, to separate the effects of controlling the human reservoir from those of vector control, since the two are usually undertaken at the same time. The cost-effectiveness of vector control versus case-finding and treatment was modelled by Shaw (1989). At this time, it appeared that case-finding and treatment was the more cost-effective at lower incidences and when dealing exclusively with a human reservoir. The conclusion, then as now, was that epidemiological models and economic models need to be integrated in order to be of use in decision-making.

DALYS AND THE COST-EFFECTIVENESS OF SLEEPING SICKNESS CONTROL

Finally, in order to examine the wider economics of controlling sleeping sickness in relation to the control of other diseases, an estimate of the cost per DALY averted is needed. So far there have been very few comprehensive attempts to calculate the actual and potential DALYs lost due to either form of sleeping sickness.

Recent work in Uganda has produced calculations of DALYs for rhodesiense (Odiit et al, 2000a and 2000b). This work will be integrated into more comprehensive economic analyses. The authors point out that the age distribution of trypanosomiasis patients very closely follows that of the active adult population, so that the disease tends to hit the most economically productive group of society hardest, affecting family livelihoods and community prosperity very much. These data confirm observations made throughout Africa for both forms of the disease. Odiit et al estimate that, at the time of diagnosis, patients will have been suffering from symptoms of the disease for an average of 61 days
and will then require hospitalization for an average of 34 days. For patients correctly diagnosed and treated, the case fatality rate is 5.3%, but for unreported cases, the outcome is assumed to be inevitably fatal. Based on the age distribution of patients, Odiit et al estimate that the number of DALYs lost for unreported, and thus untreated, patients is just over 20 years (personal communication Dr Paul Coleman). This figure was used to underpin the economic analysis discussed in the section on Economics of controlling the animal reservoir above. In this case, because controlling the animal reservoir reduced the rate of transmission to humans, and it was assumed that for every reported case there was one unreported case, the full twenty years applied to half of the cases averted. This DALY figure thus also means that the cost-effectiveness of controlling rhodesiense sleeping sickness compares very favourably with that of other high priority health control activities, such as malaria (Goodman et al, 2000), the expanded programme on immunization (EPI), and human immunodeficiency virus (HIV).

For gambiense disease, no published DALY figures based on detailed field records are available. However, ongoing work in Southern Sudan indicates that a similar situation probably exists (personal communication Dr Anne Moore). An attempt to look at the economics of alternative treatments for second-stage gambiense patients was based on the age-at-death distribution calculated for rhodesiense patients in Uganda (Politi et al, 1995). These authors also concluded that the standard treatment for second-stage patients represented a very attractive cost per DALY averted, ranking with the most cost-effective interventions such as childhood immunization and blood-screening for HIV.

Figure 6 takes this discussion a bit further by modelling how the situation could be analysed if more data were available. The cost of treatment and detection of patients using a mobile team at different prevalences, as shown in Figure 2, is divided by a conservative estimate of the number of DALYs averted. The ‘zero’ baseline figure, which gives the highest cost, is based on each patient treated, i.e. each premature death prevented, representing 15 DALYs averted. This figure was selected as a conservative estimate, taking into account the long asymptomatic period for gambiense disease, and calibrated to be rather lower than the figure for rhodesiense. On this estimate, once the prevalence has reached 1%, the cost per person found and treated falls to US$330, and thus the cost per DALY averted falls below the ‘good value for money’ threshold of US$25. At higher prevalences, the cost per DALY averted stabilizes at between US$10 and US$12.

Figure 6. US$ per DALY averted at different prevalences (Surveillance for gambiense using a monovalent mobile team)
In order to add another dimension to the analysis, three further lines have been drawn showing what the effect would be if the number of DALYs averted per patient found and treated was increased by 0.5, 1 or 1.5. This analysis needs to be developed when more data have been analysed or collected to show:

- what the actual figure for DALYs averted per patient treated is likely to be.
- how the prevalence evolves from year to year in *gambiense* foci.

The latter brings the discussion back to epidemiology, since, at low prevalences, this multiplier can be seen to be a measure of $R_0$, the basic reproductive rate of the disease.

There is thus a clear agenda for analysing existing data on the progress of epidemics and for collecting new data in order to add to the knowledge of the epidemiology of the disease. The low proportion of all cases actually recorded has meant that knowledge of the year-to-year changes in prevalence is often patchy or anecdotal. Nevertheless, for foci which have been the subject of more intensive control work over a number of years, data do exist. In this context, epidemiological models (e.g. Rogers, 1988) have an important role to play.

**CONCLUSIONS**

This paper has tried to cover the main issues involved in the economics of controlling both *gambiense* and *rhodesiense* sleeping sickness. Some aspects, notably vector control, have only been superficially treated. Nevertheless, it is hoped that the paper provides a sufficient basis for discussion on what research and information gathering is needed to help plan funding and resource allocation and gauge what returns to expect from these investments.

Returning to the information requirements that were noted at the end of each section, these fall into one of two categories: economic and epidemiological. Some of the issues and needs identified have been included in the table below, which provides a simple framework for categorizing the information required.
Table 3
Framework for identifying information requirements and sources

<table>
<thead>
<tr>
<th>Epidemiological</th>
<th>Economic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analyse existing data</strong></td>
<td></td>
</tr>
<tr>
<td>• Analyse changes in prevalence over time, relating to control strategies (including vector control) being used.</td>
<td>• Look at finances of past control programmes, budgets, expenditure, overheads.</td>
</tr>
<tr>
<td>• Collate diagnostic protocols used in different countries/situations.</td>
<td>• Compare prices and costs of surveillance between countries and in different epidemiological situations.</td>
</tr>
<tr>
<td>• Collate information on sensitivity and specificity of different tests in the field.</td>
<td>• Update costings on vector control.</td>
</tr>
<tr>
<td></td>
<td>• Cost out different surveillance strategies, and refine existing spreadsheet model or develop new ones.</td>
</tr>
<tr>
<td></td>
<td>• Cost out different treatment protocols, determine appropriate cost for drugs, estimate hospitalization costs.</td>
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| **Initiate new data collection** | | |
| • Determine the ratio of reported to unreported cases. | • Determine the costs of the disease to the local economy. |
| • Determine changes in prevalence over time and with respect to control work undertaken. | |

From the point of view of those allocating funds within the health sector, the recent emergence of DALY calculations for sleeping sickness has made it very clear that control of this disease represents an extremely cost-effective investment. This is linked to two factors. The first is the inevitably fatal outcome of the disease, which has been discussed above. The second is the focal nature of the disease, which means that although the population at risk is large, the disease is nevertheless location specific, so that control operations can target circumscribed geographical areas where the disease is known to be present.

Acknowledgements
This paper reflects: the inputs and ideas provided over many years by Pierre Cattand, the joint work on the cost of surveillance undertaken with Michèle John, and Paul Coleman’s many suggestions and generous inputs into the analysis of the economics of controlling rhodesiense. Any errors remain the responsibility of the first author, who would also like to thank Ian Maudlin, Anne Moore, Jean Jannin and Martin Odiit for their helpful comments and encouragement.

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Annex 5

DRUG DEVELOPMENT, PRECLINICAL AND CLINICAL STUDIES AND DRUG RESISTANCE
I DRUG DEVELOPMENT FOR AFRICAN TRYPANOSOMIASIS

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INTRODUCTION
There is an urgent and obvious need for novel, effective, non-toxic drugs for treatment of human African trypanosomiasis (HAT). This is evident from:

• Dramatic recent increases in incidence of the disease due to civil unrest, warfare, and general breakdown of health monitoring and delivery infrastructure in large areas of Africa.
• An increase in resistance to standard trypanocidal agents.
• Toxicity of standard agents.

Of the standard agents available, pentamidine is used for early-stage Trypanosoma brucei gambiense infections but is not generally recommended for early-stage T. b. rhodesiense. Pentamidine has been in use since 1940, is available from Aventis, and is also used for treatment of Pneumocystis carinii in AIDS. Resistance is due in part to inability to transport the agent. Suramin, a polysulphonated naphthylurea, has been in use since 1920 and is presently used for early-stage T. b. rhodesiense. It does not penetrate the blood-brain barrier and is not used for the central nervous system (CNS) disease. It has the longest half-life of any routinely used trypanocide due to binding to serum proteins and there is no consistent appearance of resistance. Melarsoprol (Mel B, Arsobal) is the first choice for treatment of late-stage CNS infection. Developed in 1949, it has saved millions of lives but suffers from toxicity problems (encephalopathic syndrome) in about 10% of patients, which are life-threatening in about 5% of patients. Recently, there has been an increase in the number of patients who are refractory to treatment, which can largely be attributed to lack of uptake by the parasite (Burri et al, in press).

New agent in Phase I clinical trials: DB 075/289
A number of 2,5-bis(4 amidinophenyl)furan cabamates are reported to have activity against Pneumocystis carinii (Rahmathhulla, et al 1999). One of these compounds (DB 075/289) has nearly completed Phase I testing (toxicity, pharmacokinetics), aimed at development against Pneumocystis carinii pneumonia (PCP) and African trypanosomiasis. In case of a favourable outcome, first field trials (Phase IIA: proof of principle in patients) are to be conducted starting in May 2001. Studies in trypanosome infected monkeys are ongoing and are thus far promising (C. Burri, Pesonal communication). DB075/289 does not pass the blood-brain barrier and is only active against the first phase of the disease. However, as an orally dosed agent, it would be ideal for treatment of actue disease as an alternative to pentamidine and potential additional means for disease control in high transmission areas. The development of DB 075/289 is conducted by an international consortium lead by Dr Richard Tidwell of the University of North Carolina, and funded by a 15.1 million grant from the Bill and Melissa Gates Foundation.

In a recent development, WHO and Aventis have entered into an agreement in which Aventis will supply 60 000 vials of eflornithine each year for five years. WHO will continue to explore avenues to maintain production after the five-year period. WHO is conducting clinical studies to determine the efficacy and safety of oral dose efloornithine, which would allow its use on a wide scale (FAS Kuzoe, personal communication).

In a recent development, WHO and Aventis have entered into an agreement in which Aventis will supply pentamidine and melarsoprol free of charge for five years. WHO will continue to explore avenues to maintain production after the five-year period. WHO is conducting clinical studies to determine the efficacy and safety of oral dose efloornithine, which would allow its use on a wide scale (FAS Kuzoe, personal communication).
LEADS TO OTHER AGENTS
Antagonists of Polyamine Metabolism. In addition to DFMO, a number of other agents targeting polyamine metabolism have shown promise. These include MDL 73811 (5’-[(Z)-4-amino-2-butenyl]methylamino)-5’-deoxyadenosine), an enzyme-activated inhibitor of S-adenosylmethionine (AdoMet) decarboxylase which supplies decarboxylated AdoMet, the source of aminopropyl groups for spermidine and spermine synthesis. This agent was developed by Marion Merrell Dow in the late 1980s. It cures acute laboratory infections of T. b. brucei and T. b. rhodesiense clinical isolates (Bitonti et al, 1990). It is active against late-stage model infections when used in combination with low dose DFMO (Bacchi et al, 1994). MDL-73811 is not toxic to mice at >10 times the curative dose levels used. It is rapidly transported by the parasite through the P2 adenosine site (Bitonti et al, 1990; Goldberg et al, 1998). It is given intraperitoneally once a day at 10-25 mg/kg. Supplies are now limited. However, the absence of toxicity and the strong activity against T. b. rhodesiense isolates indicate steps should be taken to obtain further supplies and initiate preclinical trials.

CGP 40215 is a bicyclic analogue of methylglyoxal bis(guananyldihydrazone) (MGBG), an inhibitor of AdoMet dc. This agent also resembles the diamidines berenil and pentamidine. It was the most active, both in vitro and in vivo, of a series of derivatives produced by Ciba-Geigy in the 1990s, curing laboratory infections of T. b. brucei, T. b. rhodesiense, T. congolense, and T. b. gambiense - a total of 19 isolates (Brun et al, 1996; Bacchi et al, 1996). Used singly, CGP 40215 was not curative to a CNS model, but was curative when used in combination with DFMO. Unfortunately, when tested in vervet monkeys with a CNS infection, the compound was not curative and pharmacokinetic studies indicated that it did not cross the blood-brain barrier (BBB) (Keiser et al, 2001).

Methionine Recycling. Methylthioadenosine phosphorylase (MTA-Pase) is the lead enzyme of a salvage pathway which regenerates adenine and methionine from MTA, the by-product of aminopropyl group transfer from decarboxylated AdoMet. African trypanosomes have an MTA-Pase with a broad substrate specificity. The MTA substrate analogue hydroxyethylthioadenosine (HETA) is cleaved by MTA-Pase and the ribose moiety is metabolized, possibly to a keto acid, which appears to be the active agent (Bacchi et al, 1999). HETA is able to cure acute T. b. brucei infections and infections caused by 6 of 11 T. b. rhodesiense isolates in mice. HETA was 500 times less toxic to mammalian cells in culture than to trypanosomes (Sufrin et al, 1996; Bacchi et al, 1997). In mice, it has given no evidence of toxicity at doses of 150 mg/kg for seven days (infusion pumps). HETA costs about US$2/g to synthesize under laboratory conditions. It deserves further study and should be examined in preclinical trials.

Trypanothione Reductase Inhibitors. Trypanosomes produce a unique glutathione analogue, N¹,N⁸-bis(glutathionyl)spermidine, for use as a redox defence mechanism in combination with a specific trypanothione reductase, which restores oxidized trypanothione to the reduced state. The latter is thus a logical drug target. Many inhibitors of this enzyme have been developed, and some have proven highly effective in vitro against bloodstream form trypanosomes, yet none have been shown to have significant activity in model infections. Classes of compounds include phenothiazines, tricyclic compounds, diphenylsulphides, phenylpropyl and naphthylmethyl β-substituted polyamines. Difficulties with bioavailability, pharmacokinetics, and metabolism of these compounds need to be addressed (Werbovetz, 2000; Keiser et al, 2001).

Cysteine Protease Inhibitors. Cysteine proteases have been detected in most parasitic protozoans and are considered important potential targets for drug development. Initial studies examined cruzain, the major cysteine protease activity in T. cruzi, while more recently trypanopain-Tb (the major lysosomal cysteine protease of T. b. brucei) has been the subject of extensive study. In this context, treatment with the cysteine protease inhibitor Cbz-Phe-Ala-CHN₂ at 250 mg/kg/day for three days reduced parasitemia to undetectable levels in T. b. brucei infected mice (Scory et al, 1999). However, the parasitemia returned and animals relapsed after treatment had ceased. This study, coupled with similar results obtained with T. cruzi, indicate these inhibitors have potential, but will need extensive refinement to adjust the availability and pharmacokinetics (Werbovetz, 2000).

Nitro-imidazoles. Megazole is a 5-nitroimidazole (2-amino-5-[1-methyl-5-nitro-2-imidazolyl]-1,3,4-thiadiazole) which was first synthesized in 1968, but not studied because of risk of mutagenicity (Keiser et al, 2001). Recent studies, however, have demonstrated significant activity of megazole against acute murine infections of T. b. brucei and T. b. gambiense. Megazole was also effective in eliminating a murine model CNS infection when used in combination with suramin. Suramin prolonged megazole’s elimination half-life and altered other pharmacokinetic parameters including the retention time (doubled) and serum profile (extended peak). In most studies, single-dose oral administration of megazole was used (Enanga et al, 1998). In
a primate model, cerebrospinal fluid (CSF) levels in an animal dosed with a combination of megazole and suramin were found to be 5-10% of plasma levels after a single 100 mg/kg dose - a level about 10 times that of the MIC$_{100}$ for *T. b. gambiense*. This animal was cured of a CNS infection (Enanga et al, 2000). Excretion of megazole is primarily (80%) in the urine in the primate model, and four metabolites were consistently found in significant amounts (Enanga et al, 1999). Although promising, further preclinical studies will be needed to determine the structure of the metabolites and their overall mutagenic potential before clinical studies can begin.

**Inhibition of Glycolysis.** The predominant form of African trypanosomes in the blood is the long slender trypomastigote, which depends on glycolysis for energy production. Recent molecular modelling studies on glyceraldehyde-3-phosphate dehydrogenase (GAPDH) have detected differences in the nicotinamide adenine dinucleotide (NAD$^+$) binding site between the trypanosome and human enzyme, and an overall sequence identity of only 28.5% with the trypanosome enzyme (Suresh et al, 2000). From these observations, adenosine analogues modified in the C2’ ribose and N6 adenine positions have been synthesized. The most active of these analogues was an N$^6$ derivative, N$^6$-(1-napthalenemethyl)-2’-(3-methoxyanazamido) adenosine, with an IC$_{50}$ value of 12 µM against *T. b. brucei* and inhibition of pyruvate excretion in *in vitro* incubations. This compound did not inhibit human GAPDH at 50 µM, but had an IC$_{50}$ of 0.28 µM against *L. mexicana* GAPDH (Aronov et al, 1999). Although promising, no *in vivo* studies have been reported with these compounds.

**SIPI 1029.** This agent is a triazine derivative (Trybizine.HCl) which is used against *T. evansi* in buffaloes in China. SIPI 1029 was effective both *in vitro* and in acute mouse model infections against *T. b. brucei*, *T. b. gambiense* and *T. b. rhodesiense* (Bacchi et al, 1998; Kaminsky and Brun, 1998). In combination with low-dose DFMO, it cured a model CNS infection (Bacchi et al, 1998), although used singly it was not active in this model, or in a vervet monkey CNS model (Keiser et al, 2001). Pharmacokinetic studies in the latter model revealed only low concentrations of the agent in CSF.

Over 200 analogues of SIPI 1029 were tested at the Swiss Tropical Institute against African trypanosomes *in vitro*. Over 40 were tested in an acute mouse model and the most active compounds (5-10) were examined for activity in a CNS model. None showed activity in this model and no additional studies are planned with this series (JRL Pink and FAS Kuzoe, personal communication).

**VSG Synthesis.** African trypanosomes incorporate myristic acid into the glycosyl phosphatidyliositol (GPI), which serves as an anchor for the variant surface glycoproteins (VSGs) which cover the trypanosome. Myristate is obtained by trypanosomes either by scavenging from the host by myristate exchange, or by fatty acid remodelling. The critical nature of VSG in the trypanosome’s existence in the host makes the myristoylation step an attractive chemotherapeutic target ( Werbovetz, 2000). Fatty acid analogues of myristate gave IC$_{50}$ values <100 nM *in vitro* against *T. b. brucei* and *T. b. rhodesiense* bloodform trypanosomes. These analogues were inactive *in vivo* however, because they were rapidly metabolized by the host (Werbovetz et al, 1996). The second pathway is one in which other fatty acids are remodelled to myristate and then preferentially incorporated into GPI and not into other lipids (Morita et al, 2000). This pathway can be inhibited by the antibiotic thiolutacin, which kills trypanosomes *in vitro* with an IC$_{50}$ concentration of 150 µM. However, in pharmacokinetic studies in mice, thiolutacin was found to be rapidly excreted. Peak serum levels were obtained 15 minutes after intramuscular injection and then rapidly declined until, at 60 minutes, they were 1/10th that of peak levels. This compound could be given orally with similar rapid elevation and decline of serum levels and tissue distribution patterns (Miyakawa et al, 1982). It would be important to pursue these lines of study and develop effective myristate analogues which are not metabolizable by the host and/or thiolutacin analogues which have an extended serum half-life.

**Amidine Prodrugs and Cis-platinum Derivatives.** A number of 2,5-bis (4 amidino-phenyl) furan carbamates are reported to have activity against *Pneumocystis carinii*, and one is undergoing Phase I clinical trials for this target. As an orally dosed diamidine, it would be of significant interest to examine these agents for treatment of acute *T. b. gambiense* infections as an alternative to pentamidine (Rahmathulla et al, 1999).

A group of organometallic complexes derived from pentamidine have been evaluated for activity *in vivo* against acute *T. b. brucei* in mouse and sheep model infections. Cis-platinum pentamidine-bromide, -thiocyanate and -selenocyanate were curative in a single dose of 1-3 mg/kg. The bromide derivative was curative at 1 mg/kg and had a chemotherapeutically active index of 200 compared to pentamidine, which was curative at 3.5 mg/kg and had a chemothera-
pneumatic index of 13. Further studies are planned with
a CNS model infection and with pentamidine-resistant
isolates (Loiseau et al, 2001).

Combination Chemotherapy. A number of
compounds have been identified in the past seven-
eight years which are excellent trypanocides both
_in vitro_ and _in vivo_, with the exception that they do
not penetrate the BBB and hence do not cure model
CNS infections. In light of the lack of lead com-
ounds, and the fact that even current promising
compounds have not undergone extensive preclini-
cal toxicity studies, which may very well elimi-
nate them from consideration, it would be prudent
to examine drug combinations for CNS activity.
Which compounds however, should be the starting
points? In light of the many studies and clinical
activity, eflornithine (DFMO) at low dose levels has
proven active in curing model CNS infections in
combination with: suramin, melarsoprol, SIPI 1029,
MDL 73811, HETA, CGP 40215, 9-deazainosine.
Clearly, the ability of DFMO to act synergistically
with so many chemically distinct agents does not
imply a common biochemical basis of action. Rather,
the literature on artificially induced BBB injury
indicates that polyamine metabolism is significantly out
of balance after injury and that DFMO has a role in
correcting this (Croft, 1999). A recent review indi-
cates that a number of different types of injuries to
the brain result, after a complex cascade of events, in
the enhanced synthesis, degradation and release of
polyamines in brain tissue. Ultimately, damage may
be attributable to formation of toxic reaction prod-
ucts as a result of the oxidative degradation of
polyamines (Seiler, 2000). DFMO may ameliorate
this injury, and in so doing a situation may develop
which allows passage of molecules impervious to
the BBB. Since, in most combination studies with
DFMO, only one or two doses of the other agent
need be given, usually within in three-four days of
the onset of the DFMO, there appears to be a short
window during which the other agent will pass the
BBB. There is a real need to explore these combina-
tions and the basis for their activity in primate mod-
els using low-dose DFMO in combination with
known agents, e.g. suramin and melarsoprol. It may
be that other polyamine antagonists, e.g. MDL 73811
which blocks AdoMet dc and hence spermidine pro-
duction, may have similar synergistic activity.

Transport of Nucleosides. Recently, a signif-
cant effort has been made by a number of investiga-
tors concerned with the mechanism of drug uptake
by African trypanosomes. While the normal func-
tions of P₁ and P₂ transporters in bloodstream try-
pinosomes were determined to be nucleoside and
nucleobase transport, the ability of P₂ to take up
diamidines and melarsoprol has been the focus of
much study (Hasne and Barrett, 2000; Barrett and
Fairlamb, 1999). Thus P₂ carries adenosine and ade
nine, while melarsoprol and pentamidine interfere
with their uptake. Trypanosomes resistant to mel-
arsoprol and to berenil have lost P₂. Megazole, a
5-nitromidazole active against African trypano-
somes (see above) is also transported through P₂
(Hasne and Barrett, 2000). It now appears that there
are other transporters, in addition to P₂, for dia-
midines and melarsoprol (DeKoning, 2001).

Another aspect of trypanosome nucleoside uptake
is the demonstration of an AdoMet transporter separ-
able from P₁ and P₂ (Goldberg et al, 1997).
AdoMet transporter is not a usual component of
mammalian cell nucleoside uptake. Its appearance
in trypanosomes should be exploited in terms of
transport of currently active agents and in develop-
ment of novel agents with the ability to transverse a
wide range of transporters. For example, the MTA-
Pase analogue HETA is taken up by both P₂ and the
AdoMet transporter (Goldberg et al, 1998). Overall,
the function and substrate specificity of try-
pansome nucleoside/drug transporters should be
made a priority for study and drug development.

Acknowledgements
I thank Michael Barrett, Reto Brun, Christian Burri,
Felix Kuzoe, and Karl Werbovetz for supplying
recent references, helpful discussions, and/or other
information essential for this document.

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II DRUG RESISTANCE IN SLEEPING SICKNESS

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INTRODUCTION
No vaccines exist against sleeping sickness, and the prospects of prophylactic immunization are poor since the parasites change their surface coat periodically in a process known as antigenic variation. Drugs remain the principal means of intervention. Five drugs are currently used against Human African Trypanosomiasis (HAT) (Pepin and Milord, 1994), the drug of choice depending on which subspecies of T. brucei is involved and on whether the disease is diagnosed before or after the parasites have become established within the cerebrospinal fluid (CSF). Problems associated with the current therapies for sleeping sickness include toxicity, resistance, and a lack of a guaranteed supply (Barrett, 2000) (although it seems likely that licensed drugs will be available for at least the next five years). It is unlikely that new formulations will be available for at least ten years. This report deals with the problem of drug resistance.

Antimicrobial drug resistance is widespread. Many antibiotics are obsolete and the antimalarial drug chloroquine has been rendered useless in many parts of the world due to the emergence of resistance. Drug resistance has also become a serious problem in the treatment of animal trypanosomiasis (Geerts and Holmes, 1998). The epidemiology of sleeping sickness and recommended regimens for the administration of drugs against this disease appear to be unfavourable for the development of resistance. Drug resistance has also become a serious problem in the treatment of animal trypanosomiasis. Currently the epidemiology of the disease is not sufficiently well understood to make solid conclusions. However, recent data have started to clarify the situation in the field, allowing room for some speculation on this topic (MacLeod et al, 2001).

T. brucei parasites are able to undergo genetic exchange inside the tsetse fly, although this process is not obligatory. It has recently been shown that many infected tsetse flies in the field carry mixed populations of trypanosome (MacLeod et al, 1999), thus genetic exchange is possible and studies on the population genetics of trypanosomes have revealed that genetic exchange does occur sufficiently frequently to be an important determinant of genetic diversity.

However, it is not yet clear whether the human infectious Trypanosoma brucei gambiense does engage in genetic exchange. Moreover, in East Africa it appears that T. b. rhodesiense may have a predominately clonal structure (MacLeod et al, 2000), suggesting that it does not frequently engage in genetic exchange, although this does not mean that it cannot do so. While T. b. gambiense (type I) appears to be genetically distinct from other T. brucei sub-species, T. b. brucei and T. b. rhodesiense may simply be considered as host-range variants of local T. b. brucei populations that become genetically isolated. Type II T. b. gambiense found in West and Central Africa also appear to closely resemble T. b. brucei (MacLeod et al, 2001).

Studies on trypanosome population genetics are still in their infancy and it cannot be ruled out that human infectious parasites can engage in genetic exchange with non-human infectious organisms.
Moreover, human infectious parasites are not limited in host-range to humans, and many trypanosomes (around 20%) isolated from cattle in Uganda and Western Kenya are human infectious (Hide, 1999). In West Africa it appears that T. b. gambiense may also have a host range beyond humans, with pigs representing a particularly important reservoir (Penchenier et al, 1999). A critical corollary of the essential zoonotic nature of trypanosomes is that resistant strains selected by drug use in animals could be transferred to humans (this will be discussed later). In addition, human infectious parasites could also acquire resistance from non-human infectious parasites as a result of genetic exchange during mixed transmission in tsetse flies. It is important to stress, however, that this has yet to be demonstrated in experimental or population genetic studies.

However, it is clear that even in instances where trypanosomes in humans are not exposed to the classical risk factors for the development of drug resistance, the possibility exists that human infectious parasites are exposed to these classic factors in animals. Domestic animals do receive large doses of various trypanocides, often administered at the discretion of farmers, and frequently given over prolonged periods as prophylaxis. While trypanocides used to treat humans and animals do differ, the potential to develop cross-resistance between human and animal trypanocides does exist. For example, diminazene is used extensively for the treatment of cattle. Cross-resistance between diminazene and the related diamidine pentamidine can be induced in trypanosomes (Barrett and Fairlamb, 1999). Cross-resistance between diminazene and melarsoprol can also be selected with relative ease (reviewed in Barret and Fairlamb, 1999). Cross-resistance between diminazene and melarsoprol can also be selected with relative ease (reviewed in Barret and Fairlamb, 1999). This is because all of the drugs can enter trypanosomes via the P2 amino-purine transporter. Loss of this transporter can contribute to resistance. One study has shown that removal of the TbAT1 gene, is at least partially responsible for uptake of melamine-based arsenicals. Loss of the transporter can contribute to resistance. One study has shown that removal of the TbAT1 gene renders parasites only fourfold less sensitive to melarsen oxide than wild-type trypanosomes. However, this degree of resistance might be enough to allow survival in the CSF (and relapses appear to depend on CSF involvement).

Many of the isolates from treatment failures have an altered TbAT1 gene.

It is possible that a combination of reduced drug sensitivity (in part due to reduced uptake because of changes to the P2 transporter) and variability in accumulation of drug in the CSF can explain current treatment failures in Africa.

The drug and its use

Melarsoprol (Mel B) is a melaminophenyl-based organic arsenical which was introduced as an anti-trypanosomiasis reagent in 1949 (Friedheim, 1949). It was Paul Ehrlich who promoted the idea that arsenicals could be useful drugs for use against sleeping sickness at the turn of the century. His compound, salvarsan or ‘606’, developed for use against syphilis, is often considered as the prototypic chemotherapeutic reagent. Ernst Friedheim developed the melamine-based arsenicals in the late 1940s after the dangers of serious side effects associated with tryparasmide and high rates of treatment failure decreased confidence in this product. Interestingly, melarsoprol has recently been used in clinical trials against leukaemia (Soignet et al, 1999). The trials were abandoned when it became clear that patients were suffering from seizures at more or less the same rate as in sleeping sickness treatment. However, different regimes might be tried.

Melarsoprol itself is amphipathic and will diffuse across cellular membranes. However, the drug is very rapidly converted to the highly hydrophilic melarsen oxide in plasma (96% clearance of melarsoprol within 1 hr) (Burri et al, 1993). Melarsen oxide levels peak within 15 minutes and have a half-life of 3.9 hours. Relatively little melarsoprol, or its metabolites, accumulate across the blood-brain bar-
rrier, with maximum levels being equal only to around 1-2% of maximum plasma levels. The new pharmacokinetic information (Burri et al., 1993; Keiser et al., 2000) has been very useful in establishing a new regimen for administration of the drug which is likely to engender better patient compliance (Burri et al., 2000). Detailed information on pharmacokinetics may also be crucial in permitting an understanding of the factors that underlie clinical drug failure. It seems that levels of drug that reach the CSF might be insufficient to kill parasites that are only a few fold less sensitive to drug than wild-type trypanosomes.

Of all the trypanocides, toxic effects are worst with melarsoprol; up to 10% of patients suffer a frequently fatal reactive encephalopathy (Pépin and Milord, 1994). A high proportion of leukaemia patients suffered neurological seizures when treated with melarsoprol (Soignet et al., 1999), demonstrating that melarsoprol is itself responsible for the reactive encephalopathy associated with drug treatment. Exfoliative dermatitis has also been observed. Hypersensitivity, renal and hepatic dysfunction are also known. Myocardial damage, albuminuria and hypertension can also occur. Headache, fever, general malaise, urticaria, abdominal pains, vomiting and acute diarrhoea are all less severe but common side effects (Pépin and Milord, 1994).

**Mode of uptake and action**

It has been proposed that both the melaminophenyl arsenicals and diamidines classes of drug enter *T. brucei* by the P2 amino-purine transporter (Carter and Fairlamb, 1993). Trypanosomes selected for resistance to sodium melarsen had lost the P2 transporter (Carter and Fairlamb, 1993), and a *T. equiperdum* line selected for resistance to diminazene, which displayed some cross-resistance to arsenicals, had a P2 transporter with markedly reduced affinity for substrate (Barrett et al., 1995). These data suggested that the P2 transporter is involved in uptake of arsenicals (Carter and Fairlamb, 1993) and diamidines including diminazene (Barrett et al., 1995) (the situation for pentamidine is more complicated, as described later). A simplistic model proposing that loss of the P2 transporter was necessary and sufficient to induce resistance to melaminophenyl arsenicals and diamidines was developed (Barrett and Fairlamb, 1999; Carter and Fairlamb, 1993; Barrett et al., 1995). *In vitro*, unmetabolized melarsoprol is likely to cross the membrane by passive diffusion (Scott et al., 1997) although melarsen oxide does not. The possibility of additional modes of uptake should not be excluded.

Trypanosomes exposed to arsenicals lyse very rapidly. A mode of action has yet to be established. Loss of ATP due to inhibition of glycolysis could underlie lysis caused by the drug, as bloodstream form trypanosomes depend solely upon glycolysis for ATP production. However, it seems that the cells lyse before ATP supplies are seriously depleted, leading several workers to question whether glycolysis is a target for arsenical action (Van Schaftingen et al., 1987).

Another suggested target of melarsoprol was trypanothione, a key low molecular weight thiol found in trypanosomatids but not mammalian cells. Since arsenic is known to form stable interactions with thiols, trypanothione was also proposed as the definitive target for these compounds (Fairlamb et al., 1989). Arsenicals, however, interact more tightly with other thiols including lipoic acid and, at the point of arsenical-induced lysis, only a small fraction of trypanothione is conjugated with the drug (Fairlamb et al., 1992). Thus it seems unlikely that trypanothione is the *in situ* target of these drugs.

**Resistance**

Treatment failure with melarsoprol has been reported in the field. There has always been a cohort of 5-10% of treated patients who relapsed after treatment, although it was never clear what factors were responsible for this. However, in northern Uganda relapse rates after melarsoprol treatment of around 30% have been reported (Legros et al., 1999). Anecdotal reports of similar failure rates in northern Angola are also in circulation. The incidence of relapse after treatment does not, at present, seem to be as serious in southern Sudan, although around 16% failure rate has also been reported here. The recent build up of data on the pharmacokinetics of the drug (Burri et al., 1993; Keiser et al., 2000), coupled with advances in understanding of the molecular and biochemical basis of resistance and an analysis of field isolates from relapse cases in Uganda (Matovu et al., in press), has allowed the development of a model for the likely causes of treatment failure in the field.

Precise quantification of drug levels in a patient’s plasma and CSF is difficult, although best results have been obtained using a bioassay for trypanocidal activity in fluids containing melarsoprol or its active metabolites (Burri and Brun, 1992). The logistics of drug delivery in primary health care centres is difficult, so administration of the drug can differ from patient to patient in terms of both total delivered dose and timing between doses. Moreover, the metabolism of the drug and also its distribution within the body will vary from patient to patient.
(i.e. accumulation of the drug in the CSF and other extravascular compartments can be highly variable).

These variables can be crucial to the success of the drug. Absolute quantities vary between patients; however, maximum serum levels following four injections of the drug according to the “classic” protocol were 5 – 6 µg ml\(^{-1}\) (Burri et al, 1993). This falls to 0.22 µg ml\(^{-1}\) 120 hours after the final injection in the series. Drug detected in the CSF 24 hours after the final injection varied between individuals. The maximum concentration was 260 ng ml\(^{-1}\) but this descended to undetectable quantities in some patients and average levels in the CSF are estimated to be in the order of 50-fold lower than those in plasma (Burri et al, 1993). Melarsoprol is rapidly converted to melarsen oxide (and possibly other metabolites) in plasma (Keiser et al, 2000).

There is also some variability in intrinsic sensitivity to melarsoprol (or melarsen oxide) among different isolates of trypanosome. Sensitive parasites appear to have minimal inhibitory concentration (MIC) values of around 1-30 ng ml\(^{-1}\) (De Koning, 2001).

The quantity of active melamine-based arsenical reaching extravascular parasites, combined with the MIC of the trypanosomes, will determine whether they are killed by the drug. The MIC values of most parasites are only marginally lower than estimated concentrations of drug that are achieved in the CSF of most patients. Data regarding melarsoprol in other extravascular compartments that also harbour trypanosomes are absent. The 5-10% of melarsoprol refractory cases could conceivably represent a cohort of individuals in whom extravascular accumulation of the drug is at the lower end of a normally distributed curve and does not reach sterilizing levels, thus allowing relapse.

Since the achievable CSF dose is close to the MIC of normal sensitive trypanosomes, a mere halving in sensitivity of a line of parasite could mean that a larger number of individuals fail to accumulate sterilizing levels of arsenical in the CSF. As the degree of sensitivity declines, the number of refractory cases will rise in proportion.

According to this model, a combination of parasite drug-sensitivity and host factors, including the permeability of the blood-brain barrier to the drug, will determine whether a case is sensitive or refractory to arsenicals. This model is supported by recent data from northern Uganda which indicate that alterations to the TbAT1 gene that encodes the P2 transporter correlate with resistance to melarsoprol (Matovu et al, 2001).

Experiments that led to the identification of a role for the P2 transporter in resistance (Carter and Fairlamb, 1993) involved laboratory derived isolates selected for high level resistance to sodium melarsen (which, like other melaminophenyl arsenicals, is rapidly metabolized to melarsen oxide in vivo). However, recent data involving genetic manipulation of trypanosomes, removing the TbAT1 gene, revealed that loss of the P2 transporter yields only around fourfold reduced sensitivity to melarsen oxide (Matovu et al, 2001). Additional mechanisms must therefore be at play in determining high level resistance in laboratory derived lines. The modest change in sensitivity relating to impairment of P2 function, however, could render parasites resistant to levels of melarsen oxide accumulating in the CSF and other extravascular compartments. It appears that the majority of relapses reported in Uganda do involve small numbers of CSF parasites re-invading the bloodstream post-treatment (Matovu et al, 2001). It is also noteworthy that genetic removal of TbAT1 had no impact on parasite viability.

Many of the drug resistant T. b gambiense isolates from northern Uganda have mutations in the TbAT1 gene that encodes the P2 transporter (Matovu et al, 2001). Many of these mutations are common with mutations found in laboratory derived drug resistant isolates (Maser et al, 1999). Although some of the resistant isolates apparently do not have changes to the sequence of TbAT1, it cannot be ruled out that other genetic alterations, beyond the open reading frame, would downregulate expression of that gene. One example of a T. b gambiense line lacking the TbAT1 gene has been reported (Matovu et al, 2001). A multi-drug resistant T. b rhodesiense isolate from south-east Uganda (Matovu et al, 1997) had an identical set of mutations as the T. b gambiense series and an Angolan isolate (Matovu et al, 2001).

Many instances of cross-resistance between melamine-based arsenicals and diamidines have been reported (reviewed in Barrett and Fairlamb, 1999). The fact that the P2 transporter appears to be responsible for the uptake of both of these classes of drug has suggested that transporter alterations could be the basis of cross-resistance. The fact that some lines of pentamidine resistant parasite have no reduction in P2 transporter activity was explained by the discovery of additional pentamidine transporters (De Koning, 2001) and by the fact that other, non-transport, related events could underlie pentamidine resistance (Berger et al, 1995). The recent discovery that loss of the P2 transporter through gene knockout rather than drug selection leads to a rather modest decrease in sensitivity to melamine-
based arsenicals suggests that other biochemical changes must be occurring in resistant cells. These other factors contributing to resistance remain to be identified.

**Pentamidine**

**Summary points on pentamidine resistance**

- Use of the drug in the field against early-stage sleeping sickness has been extensive, although its withdrawal from use as a prophylactic in the mid-twentieth century might have slowed the appearance of resistance.
- Anecdotal reports about resistance in the field are on the increase.
- Laboratory isolates resistant to the drug have been selected.
- There can be cross-resistance to other drugs, including melamine-based arsenicals and other diamidines, which may to be related to loss of drug uptake via the P2 transporter.
- Resistance need not necessarily involve cross-resistance. For example, one laboratory derived line selected for melarsen resistance which had lost the P2 transporter was not cross resistant to pentamidine. Moreover, another line selected for pentamidine resistance was not cross resistant to arsenicals or other diamidines.
- Pentamidine can enter T. brucei via several transporters (P2, HATPI, LAP1) and it accumulates to high intracellular levels. Resistance in some cases correlates to reduced uptake, but in others reduced uptake does not appear to be involved.
- The mode of action is not known.

**The drug and its use**

Pentamidine is an aromatic diamidine that has been in use for treatment of trypanosomiasis for over fifty years (Sands et al, 1985). It is supplied as a white powder in 200 mg ampoules. It was developed after the observation that a related compound, synthalin, which induces hypoglycaemia in mammals, had been selected.

Maximum plasma concentrations are reached within an hour of intramuscular injection. Extensive variation in plasma concentration is found between individuals (0.2-4.4 mg l⁻¹ following a 4 mg kg⁻¹ injection have been reported). Each daily dose leads to an increase in residual drug concentration. Elimination is slow, with estimates of 70-80% of the drug reported binding to plasma proteins (Sands et al, 1985). The plasma half life is 12 days (but varies), and the drug is probably metabolized by humans since only around 11% is eliminated in the urine. The drug is thought not to cross the blood-brain barrier, although there are reports suggesting that small amounts may enter the CSF.

Intramuscular injection can cause reactions at the site of administration. Other reactions include hypotension, abdominal pain, hypertension, vertigo, nausea and chest pain. Nephrotoxicity is common; hypoglycaemia is also seen in significant numbers of patients. Diabetes mellitus may ensue several months after therapy. Rapid intravenous injection should be avoided as it induces a number of effects including hypotension, tachycardia, nausea and vomiting (Anon, 1998).

**Mode of uptake and action**

Pentamidine is concentrated to high levels by the parasites. It seems that pentamidine can enter T. brucei via the same P2 amino-purine transporter which accumulates melaminophenyl arsenicals (Carter et al, 1995). Loss of this transporter can render parasites cross-resistant to both diamidines and arsenicals. However, some parasites without P2 remain sensitive to pentamidine (Carter and Fairlamb, 1993). In T. brucei, three transporters that can carry pentamidine into the cell have been identified (De Koning, 2001). In addition to P2, a high affinity transporter HAP1 (K_m for pentamidine = 36nM) and a low affinity transporter LAP1 (K_m for pentamidine = 56 µM) are responsible for the uptake of pentamidine. This could explain why the P2 deficient line, RU15, which is resistant to melamine-based arsenicals and some diamidines, was not resistant to pentamidine (Carter and Fairlamb, 1993). Moreover, another line selected for pentamidine resistance was not resistant to other diamidines (Berger et al, 1995); a better understanding of the different routes of uptake for different diamidines is desirable.

The mode of action of the drug has not been established. As a polycation, the molecule interacts electrostatically with cellular polyanions, including the unique intercatenated network of circular DNA molecules which make up the mitochondrial genome of all kinetoplastid flagellates termed the kinoplast. In the case of African trypanosomes, an interaction with the kinoplast may appear to be of limited interest as these organisms do not have a classical mitochondrial metabolism. However, it is wrong to consider the mitochondrion as inert since some kinetoplast genes are known to be expressed and the membrane is maintained in an energized
form indicating that several mitochondrial enzyme systems must be active.

*T. brucei* can retain viability when the kinetoplast has been removed (dyskinetoplastid). On the other hand, dyskinetoplastid parasites have been shown to be somewhat less sensitive than wild-type cells to diminazene (Agbe and Yielding, 1995). Fluorescent analogues of the diamidines, e.g. DB75 and stilbamidine, accumulate rapidly in the kinetoplast and have also been shown to accumulate in small vesicular structures in the cytosol.

Numerous other potential targets have been proposed but none have been verified. Given that the drug reaches millimolar concentrations within trypanosomes, it could be that its toxic effect arises from inhibition of multiple cellular targets, although the fact that one resistant line (Berger et al, 1995) does accumulate drug to millimolar concentrations without adverse effects might indicate that there is a specific target that has yet to be identified.

**Resistance**

Pentamidine resistance did not emerge during large-scale chemoprophylaxis campaigns in the middle part of the twentieth century in west Africa, possibly because the drug was withdrawn from widescale use in the 1950s thus removing selection pressure. In this regard, it is perhaps of note that one laboratory derived line resistant to pentamidine had substantially diminished viability in mammals (Berger et al, 1995).

It is not clear, nor easy to ascertain from available literature, whether melarsoprol resistant field isolates are pentamidine cross-resistant. However, it seems that both pentamidine and melarsoprol enter trypanosomes via the P2 transporter and anecdotal evidence has indicated that treatment failures with pentamidine are growing more common as they are with melarsoprol. Possibly the presence of transporters, in addition to P2, which can accumulate pentamidine means that changes to P2 will not underlie pentamidine resistance in the field. Mechanisms for resistance to pentamidine are currently not known.

**Suramin**

**Summary points on suramin resistance**

- Use of the drug in the field against sleeping sickness is not extensive.
- Few reports about resistance in the field have been published.
- Veterinary use was more widespread than human use in the mid to late twentieth century.

- Suramin resistance in *T. evansi* lines appears to be stable in the field.
- Numerous laboratory isolates (*T. brucei* and *T. evansi*) have been selected for suramin resistance.
- Most laboratory resistant isolates are not cross-resistant to other drugs.
- Neither a mode of action nor mechanisms of resistance are known.

**The drug and its use**

Suramin, a colourless polysulphonated symmetrical naphthalene derivative, was first used against sleeping sickness in 1922 (Voogd et al, 1993). It is useful for the treatment of early-stage infection due to either *T. b. gambiense* or *T. b. rhodesiense*, when there is no central nervous system involvement. Other naphthalene dyes, including trypan red and trypan blue, were initially developed for their marked trypanocidal activity. The drug has recently been used in clinical trials against hormone-refractory prostate cancer and has also been used in the chemotherapy of some helminth infections. During the 1950s, there were reports that treatment failure in patients infected with *T. b. gambiense* was relatively high (around 30% [NeuJeann and Evens, 1958]). Consequently the drug lost favour as a treatment for *gambiense* sleeping sickness although the factors underlying these treatment failures were never identified with any certainty.

Poor intestinal absorption, and a local irritation if given intramuscularly, mean that the drug should be administered by slow intravenous injection (Anon, 1998). Dosing at 1 g per week over six weeks maintains levels at 150-200 mg l\(^{-1}\). Most of the drug binds to serum proteins. It does not cross the blood-brain barrier to levels capable of killing trypanosomes in the CSF at doses given during treatment of early-stage disease. Plasma concentrations decline exponentially with a half-life of up to 60 days. About 80% of the dose is eliminated in the urine.

Nausea, vomiting, urticaria and loss of consciousness can be immediate side-effects (Anon, 1998). Fever (up to 40°C within a few hours) is common as are photophobia and lacrimation. Renal damage can occur several days after treatment. Other adverse reactions include exfoliative dermatitis, stomal ulceration, agranulocytosis and, rarely, haemolytic anaemia.

**Mode of uptake and action**

The drug is a highly charged molecule containing six negative charges at physiological pH and it binds with high avidity to many serum proteins including low density lipoprotein (LDL), for which trypanosomes have a receptor (Vansterkenburg et al, 1993). Suramin accumulates in trypanosomes relatively slowly and may be taken up bound to
LDL by receptor-mediated endocytosis, although definitive evidence proving this is absent. The same experiments that showed that uptake might depend on LDL also showed that suramin inhibits LDL uptake and that this inhibitory effect could be the cause of suramin’s toxic effect on trypanosomes (these organisms get most of their lipids from the breakdown of exogenous LDL). The highly charged nature of the drug enables it to bind to many proteins through electrostatic interaction. Consequently, when the drug is tested for inhibition of a variety of purified enzymes, it shows activity. This has led to many hypotheses regarding its mode of action.

Treatment of trypanosomes with the drug does lead to a reduction in the glycolytic rate. This inspired a study into its inhibitory effect against the enzyme glycerol phosphate oxidase, which is present in trypanosomes but absent from the mammalian host (Fairlamb and Bowman, 1977). Suramin inhibited the enzyme and some textbooks mistakenly report that this enzyme is the target. The discovery of clusters of positively charged amino-acids within several of the T. brucei glycolytic enzymes led to the hypothesis that the drug’s action was dependent upon this interaction. However, none of these speculative ideas have been proven and, at this stage, it should be emphasized that the drug’s mode of action is not known.

The drug is highly active against bloodstream forms of the parasite in vitro, but around a hundredfold less active against procyclic forms of the organisms (Scott et al, 1996). This indicates that either its uptake, or its direct or indirect targets, are differentially regulated in the different forms of the parasite. Early suggestions that receptor mediated endocytosis did not occur at all in procyclic forms have proven to be incorrect as this form of the parasite does endocytose a number of macromolecules (Liu et al, 1999). However, specific receptors may be different. The fact that procyclic form organisms, unlike bloodstream forms, are not totally dependent upon glycolysis has also been used to fuel ideas on modes of action.

Fang et al (1994) recently re-introduced the old notion that the immune system may play a role in the action of suramin, since immunosuppressed mice needed higher doses (around threefold) to clear T. evansi infections.

**Resistance**

Field reports on sleeping sickness resistant to suramin are rare. In animal diseases, however, parasites of the brucei group resistant to this drug have been reported. Information from these species (particularly T. evansi) might be useful in helping to ascertain resistance mechanisms in man.

The relative scarcity of reports of suramin resistance in sleeping sickness has made it difficult to compile field data on this subject. The large incidence of reported treatment failures of T. b. gambiense in west Africa in the 1950s could have been due to multiple factors of which parasite resistance is just one (Neujean and Evens, 1958). Many laboratory derived lines have been selected for resistance to suramin over the years (since the 1930s). Several studies have focused on T. evansi (Mutugi et al, 1995). One laboratory study pointed to the fact that resistant lines were more difficult to clone and grow than wild-type T. evansi. This prompted suggestions that resistance might not be a stable phenotype (Mutugi et al, 1995). However, T. evansi isolated from the Sudan some twenty years after suramin had been withdrawn from use due to the advent of resistance (El Rayah, 1999) was still highly resistant to this drug, indicating that resistance can be very stable.

No evidence for a reduction in drug uptake associated with resistance has been reported and mechanisms of resistance are not known. Most laboratory studies, stretching back 70 years or more, have failed to identify cross-resistance between suramin and other trypanocides. One study did show that a line selected for resistance to melarsen oxide (33-fold resistance) (Fairlamb et al, 1992) had a nearly sixfold decrease in susceptibility to suramin. However, most other studies have concluded that there is no cross-resistance between suramin and the melamine based arsenicals or other drugs.

**Eflornithine**

**Summary points on eflornithine resistance**

- Use of the drug in the field against sleeping sickness has not been extensive.
- No reports about resistance in the field have been published.
- T. b. rhodesiense is innately refractory to eflornithine, possibly due to a shorter half life of the target enzyme ornithine decarboxylase compared to the susceptible T. b. gambiense.
- Laboratory isolates resistant to the drug have been derived.
- Model organisms (e.g. Leishmania, Neurospora) resistant to the drug have also been derived.
- In model organisms, resistance has been shown to relate to different phenomena e.g.:
  - Increase in ornithine decarboxylase levels (gene amplification).
- Decreased drug uptake (loss of basic amino acid transporter in Neurospora; unknown mechanism behind reduced uptake in procyclic T. brucei).
- T. brucei resistant to DFMO have not been reported to be cross-resistant to other drugs.
- The mode of action of the drug relates to its inhibition of ornithine decarboxylase. A functional immune system is required to kill cells in vivo. Whether it is simply loss of polyamines or other indirect effects, e.g. increase in decarboxylated S-adenosyl methionine and inappropriate methylation, that is behind cytotoxicity is not clear. Uptake has been reported to be via passive diffusion in bloodstream forms and via carrier mediated uptake in procyclic forms, although further studies on this question are needed.

The drug and its use

Efollornithine, or D,L-α-difluoromethyl ornithine (DFMO), is an analogue of ornithine which acts as a specific suicide inhibitor of the enzyme ornithine decarboxylase (ODC). It was developed as an anti-cancer reagent, however, it remains at the trial stage against neoplastic disease. The drug also has activity against sleeping sickness caused by T. b gambiense, even in the late CNS-involved stage. It was registered in the USA in 1990 and the UK in 1991 (Pépin and Milord, 1994). By 1995 it was registered in seven African countries. Fourteen daily intravenous injections of 400 mg per kg of body weight are recommended (Anon, 1998). It has also been licensed for use as a topical application to prevent facial hair growth and is now marketed for this purpose (Hickman et al, 2001). Fifty four per cent of the dose becomes bioavailable after oral administration (Anon, 1998). The mean half-life in plasma following intravenous injection is 3 hours, with 80% of the drug excreted unchanged in urine after 24 hours. Little of the drug binds to serum proteins. Immediately after a 14-day course, the CSF to plasma ratio is 0.91 in adults and 0.58 in children. Children retain less of the drug than adults.

Few side effects are apparent although anaemia and other blood cell reductions (leukopenia, thrombocytopenia) are known. Diarrhoea is a common problem to those on oral efollornithine. Convulsions, fever and vomiting have also been reported in low numbers of cases (Anon, 1998).

Mode of uptake and action

Efollornithine is a specific suicide inhibitor of the enzyme ornithine decarboxylase (ODC), which is a key enzyme in the biosynthesis of polyamines (McCann and Pegg, 1992). Some early studies in mammalian cells indicated that DFMO uptake was a passive process involving simple diffusion across the membrane (Erwin and Pegg, 1982). It has been reported that uptake of DFMO in T. brucei occurs via passive diffusion across the plasma membrane (Bitonti et al, 1986; Bellofatto et al, 1987). These observations were based on the fact that uptake appeared to be unsaturable over a wide range of DFMO concentrations and that internal concentration of drug equilibrated with external concentration. Other features reported in these studies, e.g. temperature sensitivity of uptake, might be interpreted as evidence for transport although the authors did not consider this. A separate report did note a saturable process typical of transport-associated uptake in procyclic organisms with a Kₘ of 244 µM (Phillips and Wang, 1987). In the yeast Neurospora crassa, a basic amino-acid transporter has been implicated in uptake of DFMO (since this transporter is lost in strains selected for resistance to the drug) (Davis et al, 1994). None of the studies in trypanosomes have indicated that DFMO shares an uptake mechanism with ornithine, arginine or lysine. The mode of uptake into trypanosomes remains uncertain.

DFMO has similar affinity for both the mammalian and trypanosomal ornithine decarboxylases. Its specificity against the parasite apparently arises because T. b gambiense ODC is degraded within the cell and replenished at a rate much slower than its mammalian counterpart (Phillips et al, 1987). Thus, a pulse of DFMO can deprive trypanosomes of ODC and polyamine synthesis for a prolonged period compared with mammalian cells, leading to a cessation of growth.

Inhibition of ornithine decarboxylase has other results besides a reduction in putrescine and further polyamine biosynthesis. For example, it leads to an increase in cellular levels of S-adenosyl methionine, which might have toxic effects (Byres et al, 1991). Inappropriate methylation of proteins, nucleic acids, lipids and other cell components is thus being implicated.

Trypanothione is a glutathione-spermidine conjugate unique to trypanosomatids and it plays a critical role in maintenance of cellular redox potential (Fairlamb and Car, 1992). Trypanothione levels are diminished after DFMO treatment, which might render parasites more vulnerable to oxidative stress. A functional immune system is required to kill the growth-arrested trypanosomes (De Gree et al, 1983). It has also been reported that T. brucei lacks polyamine transporters, rendering the parasite auxotrophic for polyamines (Fairlamb and Le Quesne, 1997). Conversely, many mammalian cells can scavenge polyamines from plasma using transporters, allowing them to bypass the lack of endogenous
biosynthesis while *T. brucei* cannot tolerate this situation.

In order to be effective against sleeping sickness, the drug needs to be given in large doses. An additional drawback is the drug’s lack of activity against *rhodesiense* sleeping sickness (Iten et al, 1995), which appears to contain an ornithine decarboxylase that is relatively quickly turned over (Iten et al, 1997). A seven-day course appears to be somewhat less efficacious than the 14-day course; however, the cost-benefit ratio appears to favour the shorter course (Pépin et al, 2000).

**Resistance**

No reports of resistance in the field were found in the literature, which is not surprising since the drug has not been widely used in very large-scale treatment regimes. *T. b. rhodesiense* appears to be innately less susceptible to the drug than *T. b. gambiense* (Iten et al, 1995) since it has a higher overall ODC activity and the enzyme has a shorter half life than the gambiense counterpart (Iten et al, 1997). Another explanation involving relative levels of S-adenosyl methionine, which accumulate to lower levels in refractory but not sensitive cells treated with DFMO, has been proposed. Alternative explanations related to drug uptake, or polyamine uptake allowing bypass of the inhibitory effect, have not been subject to extensive analysis.

In procyclic cells selected for DFMO resistance, putrescine uptake was noted to be three-four times higher than in wild-type lines (Phillips and Wang, 1987). Putrescine at >1 mM allowed parasites to survive DFMO treatment *in vitro* while 0.1 mM did not (Phillips and Wang, 1987). Moreover, *T. brucei* parasites from which the ornithine decarboxylase gene had been removed were also viable and capable of growth provided external putrescine was abundant (Li et al, 1988) (far more abundant than in mammalian serum, where it is around 220 nM [Cooper et al, 1978]). A similar result was obtained with *Leishmania* parasites from which ODC had been knocked-out, i.e. putrescine enabled bypass of DFMO inhibition of polyamine synthesis, and also enabled the cells to dispose of accumulated S-adenosyl methionine (Jiang et al, 1999).

Lines selected in the laboratory for resistance to the drug have been studied. Reduced drug accumulation was noted in procyclic parasites resistant to eflornithine (Bellofatto et al, 1987). However, whether this was due to decreased uptake or increased efflux was not determined.

A *Leishmania* line selected for resistance to DFMO was shown to have an increase in ornithine decarboxylase activity associated with an amplification in copy number of the gene (probably associated with amplification of an episome) (Sanchez et al, 1997). Elevated putrescine uptake in *L. infantum* exposed to DFMO has also been reported (Balana-Fouce et al, 1991).

Increased ornithine decarboxylase activity (associated with a rise in transcript levels but not, this time, gene copy number) has also been reported in arsenite resistance, associated with increased trypanothione biosynthesis in *Leishmania tarentolae* (Haimeur et al, 1999). The significance of this observation lies in the fact that, should a similar route to arsenical resistance be possible in *T. brucei*, the possibility of cross-resistance to DFMO would materialize. However, it should be stressed that so far a similar mechanism has not been noted in trypanosomes, and to date, lines selected for resistance to DFMO were not cross-resistant to other trypanocides.

**Nifurtimox**

**The drug and its use**

Nifurtimox was originally licensed for use against South American trypanosomiasis. The drug contains a nitro group which is central to its activity. It has also been used in trials, with only limited success (50-80% cure), against *T. brucei gambiense* in West Africa, although since it is apparently active against melarsoprol refractory parasites it may still be used and as treatment failures with arsenical increase (Pépin et al, 1992).

Serum levels are reportedly low when given orally, peaking one-three hours after administration. The drug can accumulate across the blood-brain barrier (Anon, 1998).

Toxic effects to the central nervous system and peripheral nervous system have been reported.

**Mode of uptake and action**

No reports about the mechanism of action against *T. brucei* could be found in the literature, although reports relating to activity against *T. cruzi* exist. Uptake of nifurtimox into *Trypanosoma cruzi* has been reported to occur via passive diffusion across the plasma membrane (Tsuhako, et al., 1991). Studies have not yet been extended to *T. brucei* but it is also likely to enter these cells via passive diffusion.

Nifurtimox is a nitrofuran compound. One electron reduction of the nitro-group generates a potent free radical which may interact with cellular constituents or generate reduced oxygen metabolites.
believed to cause death of the parasite (Docampo and Moreno, 1984). The reduction potential of the compound (-260 mV) is such that it is relatively easily reduced in many cell types. The specificity towards the parasite (which is not great, nifurtimox being quite toxic to mammals) is thought to be associated with its being more readily reduced by the parasite than the host cells. Moreover, mammalian cells may have better protection against oxidative damage. Specific targets or enzymes capable of reducing the drug cannot be ruled out. Some pathways which might lead to the preferential reduction of these compounds in trypanosomes have been studied. An intriguing hypothesis was that trypanothione reductase might be responsible for the reduction (Henderson et al, 1988). Certainly, a number of nitro-containing compounds can act as “subversive-substrates” for the enzyme. However, nifurtimox was one of the less successful substrates (Henderson et al, 1988), and it is unlikely that trypanothione reductase-mediated nitro-reduction underlies the activity of the clinically used nitroheterocyclics.

Resistance
African trypanosome lines selected for resistance to nifurtimox have not been reported in the literature. It is not clear why treatment failure is high. Further pharmacokinetic studies should be made to determine the degree to which the drug reaches the CSF.

Trypanosoma cruzi isolates show various levels of sensitivity to the drug (Filardi and Brener, 1987). There appears to be a correlation between drug uptake and sensitivity (with lines accumulating least drug being least sensitive to it). No systematic study on susceptibility of different sub-species or strains of T. brucei have yet been conducted. Interestingly, another nitroheterocycle called megazol (discussed below) was equally active against lines of T. cruzi showing different sensitivities to nifurtimox (Filardi and Brener, 1987).

Megazol

The drug and its use
Megazol is a 5-nitroimidazole which has good efficacy against both T. cruzi (Filardi and Brener, 1987) and T. brucei (Enanga et al, 2000). Its synthesis was first reported in 1968 (Berkelhammer and Asato, 1968). The activity of the drug against African trypanosomes is striking. A single dose clears parasites from the blood of rodents and a primate model. Administration of the drug following a single dose of suramin cleared parasites from the CSF of an infected primate (B. Enanga, personal communication).

The drug can be given orally. Peak plasma levels following a 100 mg kg⁻¹ dosing (Enanga et al, 2000) in primates yielded plasma levels of between 0.2 µg ml and 46 µg ml⁻¹ 24 hr after dosing. The drug or its metabolites can be found in the CSF at levels 5.5%-10.6% of those in plasma. The elimination half time was around 2.5 hours. Suramin substantially increases the half-life of the drug and also increases the amounts which can accumulate across the blood-brain barrier.

No published reports of the effect of megazol on humans exist. When the drug was administered to two patients with Chagas’ disease, anecdotal evidence indicated there was little toxicity. However, the compound is positive in Ames’ tests (Ferreira and Ferreira, 1986) and this fact alone appears to have served as a deterrent to further development, in spite of the excellent safety record of another Ames’ test positive anti-protozoal 5-nitroimidazole, metronidazole. Toxicological studies in mammals should be performed under recognized good laboratory practice (GLP) conditions as a matter of urgency. If toxicity proves to be no worse than for nifurtimox, then the case for pursuing the drug as a potential reagent for clinical use would be strong.

Mode of uptake and action
Megazol possesses part of the motif recognized by the P2 amino-purine transporter which is responsible for the uptake of several anti-trypanosomal drugs (Barrett et al, 2000). Should this drug share the P2 transporter as a portal of entry, it would be of limited use against arsenical resistant parasites. However, strains of parasite lacking the P2 transporter and resistant to other drugs which use this portal of entry into trypanosomes, were not cross-resistant to megazol drugs (Barrett et al, 2000). Uptake of radiolabelled megazol revealed that this drug, although capable of interacting with the P2 transporter, enters cells predominantly via passive diffusion (Barrett et al, 2000).

The mode of action of the drug is not clear. The fact that a nitro group is central to its function does not necessarily imply that the mode of action will be the same as for nifurtimox. Indeed, its reduction potential of -438 mV (Viodé et al, 1999) is far lower than that of nifurtimox (-260 mV), and 5-nitroimidazoles are not normally reduced by aerobic cells. However, megazol is susceptible to nitro-reduction in the presence of several enzymatic systems including some found in T. cruzi extracts. How it exerts a lethal action against parasites, however, is not certain although it seems likely that trypanosomes possess a specific enzyme capable of reducing this compound.
**Resistance**

Both procyclic and bloodstream forms of the parasites have been selected for resistance to megazol in the laboratory (Enanga & Barrett, unpublished results) (procyclic forms with >100-fold resistance, bloodstream forms with about 20-fold resistance to the drug). Modest levels of cross-resistance to the other nitroheterocycle, nifurtimox, were apparent (about 7-fold in both cases). Even more modest levels of cross-resistance to diamidines (2 to 4-fold) and melamine based arsenicals (3 to 5-fold) were also observed. Preliminary evidence suggests that there is not a role for an efflux pump or for increased levels of trypanothione in the procyclic lines. The mechanism of resistance is currently unknown but under investigation.

**DETECTION OF DRUG RESISTANCE**

Treatment failure and drug resistance are by no means synonymous. Resistance is best defined as "the heritable, temporary or permanent loss of the initial sensitivity of the population of microorganisms against the active substance" (Schnitzer and Grunberg 1957). It is essential that drugs are administered according to the recommendations put forward based on regimens optimal for activity, although further studies on optimizing the dose are required for most drugs. This is particularly important in late-stage sleeping sickness where the quantity of drug that accumulates in the extravascular compartment may not extend far beyond the MIC required to kill the parasites. Treatment failure can come about for a number of reasons including administration of sub-curative doses of drug or host factors including metabolism and distribution within the body.

It is important to determine whether patients who have not cleared all parasites after treatment actually carry drug resistant trypanosomes. Therefore, tests for resistance should be performed.

**Isolation of Parasites, Propagation and Drug Sensitivity Testing in Rodents**

Blood (or CSF) can be taken from infected patients and injected directly into a suitable rodent model. In the case of *T. b. rhodesiense*, standard laboratory white mice or rats are adequate for this process. High parasitemias are readily achieved in these hosts and highly parasitemic blood isolated from these hosts can be isolated, mixed with a suitable cryopreservant, and frozen in liquid nitrogen. Preserved stocks can be re-injected into rodents and then treated with trypanocidal drugs over a range of concentrations to determine the MIC and effective dose 50 (ED50) of drug useful against these parasites *in vivo*. Protocols must be standardized for this purpose.

*T. b. gambiense* is much more difficult to grow in rodents, although *Mastomys* rats do allow proliferation of some isolates (only 20% of *T. b. gambiense* isolates from a recent study in northern Uganda could be grown in *Mastomys* - Matovu, personal communication). Standardization of rodent protocols is important, i.e. similar parasite numbers should be inoculated, similar drug dosing post-inoculation should be given, and there should be similar follow up with regard to checking mice for parasitemia.

The protocols established at the Swiss Tropical Institute for each of these procedures may be recommended as the standards which should be followed, although consensus agreement is required for this and other procedures may be considered (for example those recommended for testing of veterinary trypanocides in a recent PAAT document) (Geerts and Holmes, 1998).

**Drug Sensitivity Testing in Vitro**

It is also possible to cultivate some lines in vitro using established culture media. Laboratory adapted lines are relatively easy to establish in culture. However, many field isolates, particularly of *T. b. gambiense*, do not adapt readily to *in vitro* culture and few reports of axenically cultured *T. b. gambiense* could be found. Lines which have successfully been passed through *Mastomys* can proliferate in rich medium (containing human serum) over a monolayer of *Mastomys* embryonic fibroblast cells (Brun et al, 1989). Until better conditions for the *in vitro* cultivation of *T. b. gambiense* are established, it is important that all drug tests against all isolates should be performed under the same conditions. If *T. b. gambiense* is to be compared with *T. b. rhodesiense*, it is of limited use to use different cultivation systems to determine drug sensitivity. This is particularly the case when using mammalian cell monolayers as part of the culture system since these may affect the drug and its activity against parasites.

**Molecular Approaches to the Identification of Drug Resistance**

Alterations to the gene *TbAT1* that encodes the P2 transporter have been identified, (Maser et al, 1999) and many parasite lines which are less sensitive than normal to melarsen-based arsenicals possess similar mutations (Matovu, personal communication). Particular mutations appear to have been selected on multiple independent occasions. This opens the possibility of using a polymerase chain reaction (PCR) based approach to detect the presence of particular mutant alleles which correlate to drug resistance. This approach will be limited if different types of mutation affect the status of expression of the *TbAT1* gene. More studies to investigate the frequency of
particular mutations that correlate to resistance are needed if such a test is to be useful. The possibility that different types of mutation could affect the P2 transporter in such a way as to reduce sensitivity to drug, and the possibility that other biochemical changes not related to the P2 transporter could also induce resistance to arsenicals, means that a simple PCR based test might produce an unacceptably high number of false negative results.

GUIDELINES ON THE DELAY OF THE DEVELOPMENT OF DRUG RESISTANCE

Recent evidence indicates that the rise in the number of sleeping sickness cases proving refractory to melarsoprol treatment (Legros et al, 1999) is at least partially caused by the emergence and spread of parasites resistant to the drug in the field (Matovu et al, in press). However, the global quantities of melarsoprol administered are far lower than, for example, those for chloroquine in malaria prophylaxis, or for many of the antibiotics to which resistance is now widespread. It is also recommended that melarsoprol is administered in a clinical setting, thus improving the likelihood that a full curative dose is given. As well, the dynamics of transmission via tsetse flies make it far less likely that human trypanosomes will be transmitted between human hosts at the same frequency as are Plasmodium parasites by anopheline mosquitoes. Therefore, it is perhaps surprising that resistance to the drug has emerged, albeit apparently with a substantially slower time of onset than with chloroquine (chloroquine was introduced in 1945 and melarsoprol in 1949). These factors also need to be set against the fact that the quantities of melarsoprol reaching the parasites within the CSF are only marginally higher than the MIC of the drug, so that parasites which are only slightly less sensitive than wild-type to drug may be selected with relative ease. While the parameters that can lead to selection of resistance are complex, there is no doubt that it is critical to ensure that the recommended dose of the drug is given to every patient.

The potential of cross-resistance between pentamidine and melarsoprol must also be considered. This is because both of these trypanocides can enter T. brucei via the P2 nucleoside transporter (Barrett and Fairlamb, 1999). Selection of resistance to one drug could therefore, in principle, lead to cross-resistance to the other. Interestingly, the relatively widescale use of pentamidine as a prophylactic in west Africa up until the 1950s appears not to have selected for resistance. Some laboratory derived lines have been shown to be pentamidine-melarsoprol cross-resistant but this has not been shown to be so in all cases. This could be due to the fact that pentamidine appears to have alternative routes of entry into the cells (De Koning, 2001). Nevertheless, pentamidine should also be administered prudently, and curative doses ensured, to restrict the opportunities for selection of resistance to this drug which might cause cross-resistance to melarsoprol.

A Possible Role for Veterinary Trypanocide Use in the Selection of Melarsoprol Resistance in Sleeping Sickness

Microbes selected for resistance to drugs during unsupervised treatment of livestock have become a major route of introduction of resistance and resistance genes into human pathogens.

It seems that cross-resistance between diminazene and melamine based arsenicals occurs more consistently than between the latter and pentamidine (Barrett and Fairlamb, 1999; Barrett et al, 1995). This could be because diminazene appears to enter predominantly via the P2 transporter and not via the alternative transporters that carry pentamidine (De Koning, 2001). The emergence of diminazene-melarsoprol cross-resistance can have profound implications for the development of drug resistant sleeping sickness in the field.

To date, no studies have been conducted to assess whether a drug resistant parasite selected in an animal can be transferred to humans – this is a speculative scenario. However, it is clear that diminazene is administered to trypanosome infected cattle (Geerts and Holmes, 1998) and that several generic versions of this product have appeared on the market which are of highly variable quality. Ad-hoc, unsupervised administration of sub-curative doses of diminazene to cattle is reportedly widespread, and resistance to diminazene (in T. congolense and possibly other trypanosome species) is rife in parts of Kenya and elsewhere.

Up to 20% of trypanosomes isolated from cattle in western Kenya and parts of Uganda are infectious to humans (MacLeod et al, 2001). Therefore, human infectious trypanosomes are present in areas where conditions for selection of resistance to diminazene are prevalent. Indeed, one isolate from south-eastern Uganda was clearly resistant to diminazene and isometamidium (Matovu et al, 1997). It has yet to be demonstrated whether resistance is associated with loss of the P2 transporter, or whether cross-resistance to melarsoprol occurs. In spite of this lack of data, there are reasons for concern about the selection of drug resistant parasites in animals which can then be transferred to human infections. It should be stressed at this point that the wider host range of T. b. rhode-
siense makes it more likely that such a scenario might arise for this form of sleeping sickness more readily than for the gambiense form of the disease.

Studies should be conducted to assess the degree to which drug resistance selected in animals can be transferred to humans. In the meantime, efforts aimed at limiting the spread of drug resistance among trypanosomes in cattle should be given additional impetus so as to minimize the risk of this occurring.

Widespread implementation of the rationally derived ten-day administration protocol for melarsoprol in gambiense patients (Burri et al, 2000) might also reduce the probability of transmission of resistant lines.

GUIDELINES ON THE CONTROL OF DRUG RESISTANCE ONCE PRESENT

Melarsoprol resistance appears to be on the increase. However, the only other drug licensed to treat late-stage sleeping sickness, namely eflornithine, is not currently available, although it seems that Aventis will produce it for the coming five years so melarsoprol refractory patients should be treated with this drug if they are infected with gambiense parasites. If eflornithine is not available, then melarsoprol refractory patients can be treated with recently recommended doses of nifurtimox (Bisser et al, 2000). Combination chemotherapy can also be considered and, in this regard, melarsoprol/eflornithine, melarsoprol/nifurtimox and nifurtimox/eflornithine have all been used to treat drug resistant T. b gambiense (Jennings, 1993).

For early-stage disease, resistance to suramin has not yet been identified. It needs to be confirmed whether contemporary lines of T. b gambiense are susceptible to suramin but, if there is no pentamidine-soramin cross-resistance, then one drug should be suitable to treat parasites resistant to the other drug. Melarsoprol can also be used to treat early-stage disease which is resistant to suramin, as can eflornithine, if available. Caution should be exercised in treating melarsoprol refractory parasites with pentamidine, or pentamidine refractory parasites with melarsoprol, since cross-resistance due to the lack of the P2 transporter is a possibility.

Acknowledgments

I am grateful to Dr Enock Matovu for sharing unpublished results, also to Drs Reto Brun, Mike Turner, Harry De Koning, Dominique Legros and Anne Moore for critical reading of this manuscript.

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INTRODUCTION

In the last 20 years there has been a resurgence of interest in human African trypanosomiasis (HAT) following a recrudescence of the disease in most countries of sub-Saharan Africa. Although there are thousands of new cases every year in countries such as the Democratic Republic of the Congo (DRC), Angola and Uganda, no noteworthy progress has been recorded in the development of new drugs to fight the condition.

The drugs currently used in the treatment of sleeping sickness were first marketed in the 1950s. Pentamidine and suramin are the drugs of choice for the early-stage disease, while melarsoprol (arsobal), despite its toxicity, is the drug of choice for the late-stage disease, when the central nervous system is involved. However, a rise in treatment failures with melarsoprol has been noted in the last few years (Ginoux et al, 1984; De Gros et al, 1999), thereby hampering efforts to combat sleeping sickness despite the development of sensitive diagnostic tools and versatile low-cost anti-vectoral control methods such as insecticide impregnated screens.

Difluoromethylornithine (DFMO, ornidyl, eflornithine) has been undergoing clinical trials in the last two decades but is not yet on the market because its cost is considered prohibitive for user countries. There is therefore an urgent need to use better the available trypanocides and to develop new ones that are cheap, well tolerated and effective at every phase of the disease.

TREATMENT AND CLINICAL TRIALS CONDUCTED ON THE T. B. GAMBIENSE FORM OF SLEEPING SICKNESS

Treatment and Clinical Trials using Pentamidine

Pentamidine, an aromatic diamidine, has a success rate of 95% when used for early stages of T. b. gambiense HAT (Doua et al, 1993), and of 94% when used for patients suffering from the early nervous system phase (Doua et al, 1996). These results indicate that pentamidine passes the blood-brain barrier and is capable of halting the multiplication of trypanosomes in cerebrospinal fluid. This suggests that more than 15% of all patients in the nervous system phase of the disease, treated in Côte d’Ivoire, can be excluded from the fatal side-effects of arsobal therapy.

Treatment and Clinical Trials using Melarsoprol

Treatment regimens using melarsoprol in countries where sleeping sickness is endemic date back to colonial times and consist of the administration of three to four series of two to four injections of melarsoprol spaced out at seven to ten day intervals. These regimens, developed on an empirical basis, have helped control HAT epidemics but could be the cause of the growing number of recorded treatment failures. Recent pharmacokinetic data on arsobal, obtained by bioassay and computer simulations (Burri et al, 1993), have shown that an alternative, more rational, therapy regimen, consisting of ten injections at low doses, can be used without increasing the risk of fatal accidents. The new regimen (Burri et al, 1995) is currently under clinical evaluation in seven African countries.

Treatment and Clinical Trials using DFMO

DFMO (ornidyl, eflornithine) is an irreversible specific inhibitor of ornithine-decarboxylase, a key enzyme in the synthesis of polyamines, which are physiological substances implicated in cell multiplication (Sjoerdsma et al, 1984).

Clinical trials carried out using DFMO show that a seven-day regimen is effective in treatment of T. b. gambiense HAT. The side effects, recorded in parenteral DFMO administration, are generally reversible (Doua et al, 1987; Taelman et al, 1987) and the relapse rates after treatment are normally about 1% (Doua et al, 1993). However, the cost of DFMO remains a limiting factor to its wider use. Clinical trials are ongoing in Côte d’Ivoire, to determine the efficacy after oral administration, and the pharmacokinetics. The intended result is to develop an oral formulation of the product at an affordable cost to patients.

Treatment and Clinical Trials using Nifurtimox

This use of nifurtimox for the treatment of T. b. gambiense HAT has not been studied in large-scale clinical trials, although results obtained with four late-stage patients in the DRC show that the product is effective but very toxic (Jansens et al, 1977).

CONCLUSIONS AND RECOMMENDATIONS

In the last 20 years there has been rapid development of both serological and parasitological HAT
diagnostic techniques, but in 2001, treatment of the disease is still based only on pentamidine and suramin for the early stages of the infection, and on melarsoprol for the central nervous system stages. Treatment schedules with these trypanocides were derived empirically, without pharmacokinetic studies. Thus, the treatments applied to patients vary from one country to the next and sometimes from one treatment centre to another in the same country, which only aggravates the high number of failures observed, in particular with melarsoprol, the drug of choice for HAT.

DFMO, which has given rise to great hopes for the treatment of T. b. gambiense HAT, still has not been commercialized because of its cost. To make efforts to control HAT more effective, and to make for rational treatment of patients, we recommend the following:

• Encouragement of research aimed at developing new, effective, well-tolerated, cheap trypanocides.
• Harmonization of treatment schedules for HAT, particularly those for melarsoprol.
• Conduct of multicentre clinical trials using pentamidine for patients in the early nervous system stage of HAT, with a view to evaluating its efficacy in different HAT foci.
• Conduct of multicentre clinical trials using orally administered DFMO, after the current study on pharmacokinetics is completed.
• Conduct of multicentre clinical trials using melarsoprol-DFMO combination in patients suffering relapse after treatment with melarsoprol.
• Introduction of nifurtimox for the treatment of HAT cases that are resistant to melarsoprol.

References


IV  TREATMENT AND CLINICAL STUDIES

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SUMMARY
Diagnosis and treatment of sleeping sickness remains the major control strategy of the disease. The current diagnostic kits are insensitive, the diagnostic and treatment centres inadequately equipped and staffed, and the patients poor and often in inaccessible rural locations. The drugs used for the treatment of human African trypanosomiasis are toxic, necessitating a positive demonstration of parasites, including the invasive, painful lumbar punctures for disease stage determination. The treatment of the disease is lengthy, parenteral and costly, and the drugs used toxic and not readily available. These traditional problems of sleeping sickness are compounded by the emergence of drug resistance to melarsoprol in T. b. gambiense patients, the increasing uncertainty of sustained production of drugs for sleeping sickness due to economic justification, and the increasing prices of some drugs due to their newly found use for HIV treatment. Clinical studies that address these problems which face the major control strategy for sleeping sickness are urgently needed. For melarsoprol treatment of T. b. gambiense late-stage patients, studies on reduction in length of treatment schedules, and therefore of costs, and improved compliance, are in the advanced stages. Research into cost-effective and sustainable strategies of improving diagnosis and treatment are necessary to reduce the level of under-reporting that is associated with certain death of sleeping sickness patients if untreated. This scientific working paper reviews some of the current problems of treatment of sleeping sickness and suggests clinical studies that may address these problems in the near future.

INTRODUCTION
Human African trypanosomiasis (sleeping sickness) is found in the tsetse belt of tropical Africa, where it is estimated that over 50 million people live (WHO, 1998). Approximately 45 000 cases are reported annually, though it is believed that 300 000 persons are infected each year (WHO, 1998). There are two types of sleeping sickness, caused by: Trypanosoma brucei gambiense, found in west and central Africa, and by T. b. rhodesiense, reported in east and southern Africa. The gross national product per capita for the sleeping sickness affected countries (< US$500) ranks them as amongst the least developed countries of the world (World Bank, 2000). However, the costs of treatment for sleeping sickness are estimated to be more than US$100 per adult patient (WHO 1998). In addition, sleeping sickness patients are almost always from poor rural backgrounds within their countries (Okia et al 1994). Drugs for the treatment of sleeping sickness are not readily available in drug stores and are most often procured by the governments and non-governmental organizations through the assistance of the World Health Organization. Stocks of drugs are carefully monitored at national levels. However, it is conceivable that, in the event of an epidemic, it may not be possible to have enough drugs immediately available, a constraint already experienced by some African countries during sleeping sickness epidemics. Most drug companies appear to be reluctant to produce drugs for sleeping sickness because they will not obtain returns that are adequate to finance further drug development (Barrett, 2000; Stephenson and Wiselka, 2000).

SLEEPING SICKNESS CONTROL
Sleeping sickness control has traditionally been carried out through case detection and treatment, and through vector control when epidemics occur (WHO, 1998). Early diagnosis and treatment is the goal of control programmes, but is often hampered by the paucity of resources required to implement these programmes. Most programmes of sleeping sickness control are donor dependent because the costs of sleeping sickness interventions are beyond the budgets of the affected countries. The need for cheaper control strategies is therefore a priority. Decentralization of services, including health services, is being advocated. In some countries, planning and implementation of the sleeping sickness control programmes has been moved to the district level. However, current sleeping sickness control strategies are expensive and often complicated, requiring qualified personnel. Research into the roles of the national and sub-national levels in the decentralization of sleeping sickness control should be reviewed.

DIAGNOSIS
Detection of the parasite in gland or lymph node aspirate or blood is always followed by a lumbar puncture to determine whether there is involvement of the central nervous system (CNS) (also known as the late stage), because drugs used when the CNS is involved are different from those used when the parasite is still restricted to the haematolymphatic system (also known as the early stage). However, most of the currently used confirmatory diagnostic techniques such as the thick blood smear and the haematocrit centrifugation test have poor sensitivity (WHO 1998). The polymerase chain reaction (PCR) has been found to be very sensitive and specific (Kyambadde et al, 2000; Penchenier et al, 2000), but
it is rather complicated and expensive for application as a routine diagnostic test, which restricts its use to research laboratories and referral hospitals.

The criteria used for determination of CNS involvement are as follows: cell counts above 5 cells/mm³ or the demonstration of trypanosomes or protein levels above normal (25mg per cent) in the cerebrospinal fluid (CSF). The first two criteria are generally used because they are simpler and do not require reagents.

CHEMOTHERAPY OF SLEEPING SICKNESS

Most of the affected countries use broad guidelines for the treatment of sleeping sickness as recommended by the World Health Organization (WHO, 1998). However, there is an urgent need for novel approaches in sleeping sickness treatment and control.

Pentamidine is used for treating the early stage of sleeping sickness caused by T. b. gambiense. It is effective for treatment so long as there is no detectable involvement of the CNS. The dosage used is 4mg of pentamidine base per kg of body weight. A total of seven injections are given daily, intramuscularly. Drug resistance appears not to be an important problem in the use of pentamidine for the treatment of T. b. gambiense. The side effects include pain and induration or sterile abscess at the site of injection, vomiting, abdominal pain, hypertension, syncope, hypoglycaemia, and peripheral neuritis. Treatment failure attributable to resistance to pentamidine appears not to be an important public health problem. Studies of the pharmokinetics of pentamidine are required to determine if the length of the prescribed treatment regime can be reduced.

Suramin is used for the treatment of the early stage of T. b. rhodesiense because, though the duration of treatment is longer than that for pentamidine and it is given by intravenous treatment, no primary resistance to suramin has been reported for T. b. rhodesiense. The dosage used is 20mg/kg body weight. Intravenous injections - a maximum of seven - are given every seven days. Side effects observed include pyrexia, pains in the joints and soles of the feet, skin rash and desquamation, hypersensitivity reactions. Pharmacokinetic studies of suramin should be undertaken to determine if the treatment regime can be shortened.

Melarsoprol is the drug used to treat late-stage sleeping sickness due to both T. b. rhodesiense and T. b. gambiense in Uganda. The maximum dosage for each injection is 3.6 mg/kg body weight. Currently, two regimes for the treatment, comprising several series of injections each separated by an interval of one week, are used (WHO, 1998). One regime starts with 2.5ml and the other with 0.5ml, but both total 35 mls of a 36g/litre solution of melarsoprol.

The most serious side effect of melarsoprol is reactive encephalopathy occurring between the third injection and the beginning of the second course. The onset may be sudden or the condition may develop slowly with fever, headache, tremor, slurring of speech, convulsions, and finally coma. It occurs in 5% of patients, and the incidence of fatality due to these reactions is approximately 1% (Odiit et al, 1997). However, the fatality of untreated disease is believed to be 100%, making the decision to treat an ethical necessity. The treatment of these reactions includes the use of corticosteroids, hypertonic solutions to combat cerebral oedema, rapidly acting anticonvulsants, and subcutaneous adrenaline. There are a few cases of diarrhoea, jaundice and dermatitis, but these are generally not life threatening.

PATIENT FOLLOW-UP

It is mandatory to systematically follow up all treated patients to ascertain that they have been cured. It is recommended that patients be seen every six months over a two-year period. In addition to a full clinical and parasitological examination, a lumbar puncture should be carried out on the patients, to allow the CSF to be examined for possible increase in leukocyte counts or presence of trypanosomes that would indicate a relapse. However, due to the painful lumbar puncture procedure, most patients resent returning for follow-up once they begin to feel better, while active follow-up to ensure compliance is not affordable. Therefore, less invasive and painful methods of ascertaining cure need to be developed. Changes in CATT titres or CIATT titres and haematological parameters such Hb, ESR etc. are suggested for investigation in assessing cure. In addition, the duration of post-therapeutic follow-up should be reviewed, especially for T. b. rhodesiense, in the expectation that it may be shorter than that required for T. b. gambiense.

DRUG RESISTANCE

There is no recent evidence for resistance of T. b. rhodesiense to melarsoprol. In the early 1970s, melarsoprol treatment failure of 12 (3.4%) out of 358 treated cases was reported (Ogada, 1974). It is possible that, with low treatment failure rates and lack of systematic follow-up, patients with treatment failure may be missed. There should be periodic active follow-up of cohorts of sleeping sickness cases to monitor drug efficacy.
Treatment failure of melarsoprol in *T. b. gambiense* disease is emerging. Legros et al (1999a and 1999b) reported a melarsoprol treatment failure rate of 26.9% among 428 patients treated in north western Uganda. Melarsoprol treatment failure rates needs to be monitored and documented regularly to establish the magnitude of the problem. The cause of this treatment failure is under investigation to determine whether it is due to variation of melarsoprol pharmacokinetics between individuals or if it is associated with reduced susceptibility of trypanosomes to melarsoprol. Legros et al (1999b) emphasize the need for second-line drugs to treat patients that have already received one or several full course(s) of melarsoprol. In Uganda, melarsoprol treatment failure is a more significant problem to the west of the river Nile than to the east, in the *T. b. gambiense* affected area in the north-west of the country. Possible risk factors explaining this difference in geographical distribution here and elsewhere on the continent should be examined. Subclinical, inadequate treatments in the history of these melarsoprol treatment failure foci could be an explanation.

**HEALTH SYSTEMS AND SLEEPING SICKNESS CASE MANAGEMENT**

Demonstration of the trypanosome is mandatory before treatment can be administered to a sleeping sickness case, due to the toxicity of the drugs used. Due to the costs of staffing and equipping sleeping sickness treatment facilities, few health units are equipped for treating the disease. The endemic area for sleeping sickness in most countries is vast, with poor coverage by fixed post health units for diagnosis and treatment of the disease. Therefore, most sleeping sickness patients travel more than 5km to receive treatment. The problem of poor accessibility to diagnosis and subsequent treatment may be alleviated by the use of mobile teams. However, this is more rewarding where a valid screening test is commercially available. The card agglutination test for trypanosomiasis (CATT) is such a test. It has good sensitivity and specificity (Magnus et al, 1978) but it is only useful for the *gambiense* form of sleeping sickness. A similar test for the *rhodesiense* form of the disease is required before mobile teams can provide a rewarding strategy for routine diagnosis and treatment. A new test, the card indirect agglutination trypanosomiasis test (TrypTectCIATT®), is reported to have good sensitivity for both forms of sleeping sickness (Nantulya, 1997) and is to undergo further field evaluation (TDR, 1999). In Uganda, mobile teams are used in the north-west with support from a non-governmental organization ( médecins sans Frontières, France); during outbreaks in the south-east, the teams are used to increase the awareness of the population and detect cases who may not yet have sought health care. The costs of maintaining mobile teams are not affordable by the countries affected by sleeping sickness, and therefore the teams are often used in epidemic situations and with donor support. Operational research is required to optimize the distribution of fixed post diagnosis and treatment. This strategy may be a more sustainable strategy, especially in between epidemics of sleeping sickness.

**NEWLY DESIGNED TREATMENT REGIMES**

Pharmacokinetic data from studies carried out on *T. b. gambiense* patients indicate that the course of melarsoprol treatment in an adult can be reduced from a period of 25-36 days (WHO, 1998) to a period of just ten days, greatly cutting the costs of hospitalization (Burri et al, 1995). A randomized trial comparing the standard treatment schedule with this new concise regimen showed the same levels of parasitological cure (100%) and adverse effects (16-17%) as well as better compliance with the new regime (Burri et al, 2000). Adaptation of the new concise regime is to be monitored. It would not be advisable to adapt the model to treatment of late-stage *T. b. rhodesiense* infection because the pharmacokinetics may be dissimilar given that this disease is much more severe than the *gambiense* form. The blood-brain barrier may be more affected, allowing for higher levels of melarsoprol in the central nervous system and possible neurological side effects. A study of the pharmacokinetics of melarsoprol in late-stage *T. b. rhodesiense* infections is therefore necessary. However, a seven-day course of eflornithine for the treatment of late-stage *T. b. gambiense* infection was found to be inferior to the standard 14-day regimen (Pépin et al, 2000).

There have been attempts to adjust the criteria for treatment and so reduce the number of patients receiving melarsoprol treatment that is very toxic. To determine CNS involvement, it has been proposed that the number of cells be raised to 20 cells/mm³ (Doua et al, 1996). There is not yet enough evidence to change the cut-off point for the number of cells/mm³ in the CSF (Doua et al, 1996); therefore the WHO recommended criteria for staging are still maintained.

Chemoprophylaxis is not currently used as a sleeping sickness control strategy. A multicentre evaluation of the positive predictive value of increasing dilutions of the CATT (Magnus et al, 1978) is being carried out; results of this study will be used to see if mass chemoprophylaxis is indicated in instances of high sero-prevalence.
Eflornithine (difluoromethylornithine, or α-DFMO, or Ornidyline®) is not effective against *T. b. rhodesiense* (Iten et al, 1995; Matovu et al, 1997) but is useful for the treatment of late-stage *T. b. gambiense* infection (Doua et al, 1987). It is presently available for the treatment of relapse following melarsoprol treatment, but the production of this drug was recently suspended. It has been suggested that nifurtimox be used in desperate situations where DFMO is not available for treating melarsoprol refractory cases. Nifurtimox is very toxic but is reported to be effective for curing some *T. b. gambiense* patients, including those with late-stage disease refractory to melarsoprol treatment (WHO, 1998).

Nifurtimox (Lampit®, Bayer) has been tested for the treatment of sleeping sickness with conflicting results (Pépin et al, 1992; Van Niewenhove, 1992; Doua and Yapo, 1993). It is a cheap drug and easy to administer but is not yet registered for use in the treatment of this disease. With the current emergence of melarsoprol resistance, the efficacy and safety of nifurtimox in the treatment of sleeping sickness caused by *T. b. gambiense* should be re-evaluated.

**REDUCTION OF DRUG PRESSURE**

In areas where drug resistance to melarsoprol exists, the need to use the drug can be reduced by putting emphasis on preventive control strategies such as vector control and mass chemotherapy of the domestic animal reservoir of the disease.

**TSETSE CONTROL**

Although tsetse control is reported to reduce the transmission of sleeping sickness, it is rarely maintained because of the costs of the methods applied. Currently, tsetse traps and targets are considered the cheapest, and an environmentally friendly option, applicable through community participation, but they are still not widely used to control sleeping sickness. Pour-on insecticide for cattle is another vector control option that may involve community participation. However, use of pour-on insecticides is dependent on the distribution and movement of livestock.

**MASS CHEMOTHERAPY OF LIVE- STOCK IN *T. B. RHODESIENSE* AREAS**

Research on the role of the livestock reservoir in the epidemiology of *T. b. rhodesiense* sleeping sickness indicates that chemotherapy of livestock in endemic areas is an important policy for the control of sleeping sickness. There is molecular evidence for the similarity of the parasites of man and livestock (Enyaru et al, 1992; Hide and Tait, 1991; Hide et al, 1994), suggesting that the parasite population circulating in humans and livestock is similar. The cost-effectiveness and acceptability of the strategy to control *T. b. rhodesiense* sleeping sickness by mass chemotherapy of livestock should be investigated, taking into account other potential benefits to the farmer.

In conclusion, the current problems in the treatment of sleeping sickness in Africa include: melarsoprol treatment failure of *T. b. gambiense* sleeping sickness; unavailability of alternative drugs; unaffordable treatments; poor coverage by diagnostic and treatment facilities. Studies to identify practical solutions to these problems are urgently required to reduce the current high fatality due to sleeping sickness, a disease that is regarded as always fatal if not treated.

**SUGGESTED STUDIES**

- Research into new, safe, effective and cheap drug combinations that are active for all stages of sleeping sickness. Suggested drug combinations to compare are melarsoprol and eflornithine, melarsoprol and nifurtimox, nifurtimox and eflornithine.

- Continued screening of new compounds that are less toxic, easy to administer, cheaper and effective for all stage of sleeping sickness.

- Research on cost-effective means to prevent outbreaks of sleeping sickness through methods such as targeted vector control, and to avoid the high costs of treatment, problems of drug manufacture, and complications associated with treatment failure. Identification of markers for determining the probability of occurrence of sleeping sickness outbreaks in space and time for use as a basis for disease prevention.

- Active follow-up of cohorts of sleeping sickness cases to accurately monitor drug efficacy, and document the results. Mapping of foci of melarsoprol treatment failure to study the possible risk factors of its evolution.

- Research into other methods of control to reduce the pressure on human trypanocidal drugs, given the development of drug resistance. Evaluation of the cost-effectiveness of mass chemotherapy of livestock in the control of *T. b. rhodesiense* epemics.

- Operational research to optimize the geographical distribution of sleeping sickness diagnostic and treatment facilities, taking into account factors such as current disease distribution, population distribution, availability of health infrastructure,
and the effects of distance on case detection and early diagnosis.

- Diagnosis of suspect referrals, not positive by traditional methods and requiring immediate attention, by equipping research institutes and referral centres with PCR technology. PCR can serve as a gold standard for evaluating diagnostic tests.

- Pharmacokinetics studies of melarsoprol and suramin in *T. b. rhodesiense* patients and of pentamidine in *T. b. gambiense* patients.

- Continued monitoring of the ten-day melarsoprol protocol for treatment of sleeping sickness due to *T. b. gambiense*, making the results readily available to all health policy-makers in affected countries.

- Market research aimed at creating interest in the private sector for the manufacture of eflornithine.

- Review of the roles of national and sub-national control programmes.

- Review of the parameters for, and duration of, post-treatment follow-up of sleeping sickness patients.

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Annex 6
PATHOGENESIS, GENOMICS AND APPLIED GENOMICS
I DISCUSSION DOCUMENT ON PATHOGENESIS / APPLIED GENOMICS

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For the purposes of group discussion, I have arbitrarily selected several research areas relevant to human African trypanosomiasis (HAT)-associated pathogenesis; these areas are outlined below. Some background information on trypanosome immunology and cell biology is provided in the text that follows (excerpted from recent reviews by Mansfield and Olivier (2001), Mansfield et al. (2001), Paulnock and Coller (2001), and related resources (Imboden et al, submitted for publication, 2001; Mansfield et al, in press; Paulnock et al, 2000). It is anticipated that Melville and colleagues will provide more detailed and appropriate information on the trypanosome genomics project and its practical applications.

DISCUSSION OUTLINE

1) Stage-specific immunobiological changes that occur during infection, with an emphasis on the following subtopics:
   • Early pro-inflammatory events that trigger innate immune system responses:
     - Glycosylphosphatidylinositol (GPI) substituents of the variant surface glycoprotein (VSG) molecule shed into host tissues.
     - Trypanosome lymphocyte triggering factor (TLTF) release from trypanosomes and induction of interferon-gamma (IFN-γ) production.
     - Macrophage activation events associated with exposure to parasite activation molecules and host activation factors.
   • Early-stage acquired immune mechanisms associated with parasite control in the vascular and extravascular tissue sites:
     - Distinct roles of Th1 cells and B cells in providing tissue specific resistance to trypanosomes.
     - Macrophage release of trypanocidal factors/cytokines.
   • Interrelationship of host innate and acquired immune responses to host pathology throughout infection:
     - Elucidation of immunological events that promote tissue specific pathology at all stages of infection.
   • Late-stage anti-inflammatory and immunosuppressive events that modulate the protective effects of host T and B cell responses:
     - Elucidation of mechanisms that promote type 2 cytokine responses, and that suppress host tissue specific resistance.
     - Role of these modulatory events in controlling or exacerbating tissue pathology.

2. Changes in parasite cell biology during infection that impact on host resistance, with an emphasis on:
   • Molecular basis of changes in trypanosome virulence for the host
     - Relatedness to other clonal differentiation events.
     - Association with tissue specific residence/tropism.
     - Identification and targeting of parasite genes and molecules that are differentially expressed in highly virulent trypanosomes.
   • Molecular basis for the resistance and susceptibility of certain trypanosomes to the cytolytic effects of human serum high density lipoproteins (HDL) and of tumour necrosis factor-alpha (TNFα).

3. Identification of stage-specific parasite molecules through genomics and proteomics approaches that might be exploited, with an emphasis on:
   • Potential trypanosome molecules/cell biological systems that could be targeted by highly specific chemotherapeutic agents:
     - BSF trypanmastigote specific.
     - Tsetse fly stage specific.
   • Highly conserved invariant antigens that could be used for either more accurate immunodiagnosis or for targeted immunotherapy.

BACKGROUND INFORMATION

The background information that follows is an opinionated view of trypanosomiasis, excerpted from this author’s writings and other resources, that is meant to serve only as background information for group discussion on immunology and selected aspects of parasite biology. As pointed out in the passages below, there may be some disagreement as to the biological significance of certain observations. Additional background information on the genomics projects will be provided by others.

Stage-Specific Immunobiological Changes that Occur During Infection

Macrophages and innate immunity
Cells of the macrophage lineage provide the first line of host defense against infectious diseases, and also
modulate downstream events that impact on the development of acquired immunity. Macrophages are present in all tissues and possess the ability to recognize and eliminate many microbes. It is well established that recognition of microbes by macrophages results in cellular activation following the uptake or binding of microbial components to specific membrane receptors (Hoffman et al, Mosser, 1992; Mosser and Karp, 1999). Receptor-mediated activation of macrophages represents one of the first events in the innate immune response to many microbial infections, leading to the production of pro-inflammatory cytokines that initiate an inflammatory response and affect the downstream development of activated T cells as well as other parameters of host immunity. Cytokines produced by activated T cells, primarily IFN-γ, provide additional activation signals for macrophages, unleashing effector functions that can destroy a wide range of intracellular and extracellular microorganisms (Bendelac and Fearon, 1997; Fearon and Locksley, 1996; Medzhitov et al, 1997). Thus, the processes of innate resistance and acquired immunity are intimately interdependent, with macrophages playing a dual role as the initiators of acquired responses and as a major effector component of cell-mediated immunity.

**Macrophage activation in trypanosomiasis**

Macrophage activation is one of the hallmarks of infection with the African trypanosomes (Askonas, 1984; Askonas, 1985; Bancroft et al, 1983; Beschin et al, 1998; Borowy et al, 1990; Clayton et al, 1979; Darji et al, 1992; De Gee et al, 1985; Fierer and Askonas, 1982; Fierer et al, 1984; Grosskinsky and Askonas, 1981; Grosskinsky et al, 1983; Mayor-Withey et al, 1978; Murray and Morrison, 1979; Paulnock and Coller, 2001; Paulnock et al, 1989; Sacks et al, 1982; Schleifer and Mansfield, 1993; Sileghem et al, 1989; Wellhausen and Mansfield, 1979). There is extensive evidence that the numbers and activity of macrophages increase dramatically in the tissues of trypanosome infected animals, and are associated with tissue pathology. Within the first two weeks of experimental *Trypanosoma brucei rhodesiense* infection, for example, a large percentage of cells in the enlarged spleen exhibit membrane and functional characteristics associated with activated macrophages. These include: increases in the release of interleukin-12 (IL-12) and IL-18, known to be important in the development of the early polarized Th1 cell responses to trypanosome antigens (Mansfield, 1994; Mansfield et al, submitted for publication; Schleifer et al, 1993; Schopf et al, 1998), an enhanced ability to serve as antigen processing cells coupled to increases in expression of membrane I-Aα, B7-1 and B7-2 (Imboden, submitted for publication, 2001; Paulnock et al, 1989); and, upregulation of mRNA or proteins for other markers that include TNFα, IL-1, IL-6, inducible nitric oxide synthase (iNOS), prostaglandins and IL-10 (Beschin et al, 1998; Darji et al, 1992; Imboden et al, submitted for publication, 2001; Magez et al, 1999; Mathias et al, 1990; Schleifer and Mansfield, 1993; Sileghem et al, 1989). More importantly, the expression or release of several of these activation markers is associated with modulation of host immunity and resistance. For example nitric oxide (NO), prostaglandins and TNFα have been implicated in the suppressor cell activity exhibited by macrophages at different time points of infection (Beschin et al, 1998; Borowy et al, 1990; Hertz and Mansfield, 1999; Schleifer and Mansfield, 1993; Sileghem et al, 1991; Sileghem et al, 1989; Sternberg and McGuigan, 1992; Sternberg and Mabbot, 1996); although NO and TNFα have been shown to kill trypanosomes in vitro and are thought to be important for trypanosome control in extravascular tissue sites (more below on this topic) (Lucas et al, 1994; Magez et al, 1997; Magez et al, 1999; Mnaimneh et al, 1997; Vincendeau and Daulouede, 1991; Vincen-deau et al, 1992), neither factor alone has been linked definitively to protection in vivo (Hertz and Mansfield; 1999; Magez et al, 1999) and the expression of these factors may be linked to pathological changes during infection (Mabbot and Sternberg, 1995; Mabbot et al, 1994; Sternberg and Mabbot, 1996). However, the pro-inflammatory pattern of macrophage activation appears to change over the course of infection to become a counter-inflammatory pattern of activation in which IL-10 predominates and Type 2 cytokine responses appear to emerge (Namangala et al, 2000; Namangala et al, 2000; Namangala et al, 2001); these events have been associated with late stage disease (Imboden et al, 2001; Paulnock and Coller, 2001; Sternberg, 2001).

![Figure 1. Glycosylphosphatidylinositol (GPI) membrane anchor substituents of the trypanosome variant surface glycoprotein (VSG) molecule.](image) GPIs have been termed “...one of the most potent microbial pro-inflammatory agents known” (Almeida et al, 2000). The GPI anchor of the *Trypanosoma brucei rhodesiense* LouTat 1 VSG component of cell-mediated immunity.

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molecule is depicted in this figure, showing glycosylinositolphosphate (GIP) substituents associated with sVSG after cleavage from membrane-anchored GPI-mfVSG by a trypanosome membrane-associated phospholipase C (GPI-PLC), as well as dimyristoylglycerol (DMG) substituents that remain associated with the trypanosome membrane. Figure adapted from Menon (1999 and 1994), Magez (1998) and others (Varela-Nieto et al, 1996).

**Role of the variant surface glycoprotein GPI anchor in macrophage activation**

The source(s) and mode of action of activating factors delivered to macrophages during trypanosome infection are only partially understood. One major activation factor is of parasite origin; this is the glycosylphosphatidylinositol (GPI) membrane anchor of the trypanosome VSG molecule (see Figure 1). The GPI anchor precursor is synthesized in the endoplasmic reticulum and subsequently is covalently attached to newly synthesized VSGs after proteolytic cleavage of a VSG C-terminal GPI attachment signal sequence (Bangs et al, 1988; Cross, 1990; Doering et al, 1989; Doering et al, 1990; Englund, 1993; Ferguson et al, 1988; Masterson et al, 1989; Menon, 1999; Menon, 1994, Menon et al, 1997; Menon et al, 1990; Menon et al, 1990; Patnaik et al, 1993; Raper et al, 1993; Sharma et al, 1999; Vidugiriene and Menon, 1995; Werbovetz and Englund, 1997). After further modifications in both the glycoprotein and GPI anchor residue, the mature VSG is transported to and anchored in the trypanosome plasma membrane as membrane-form VSG (GPI-mfVSG). During the course of infection, a trypanosome membrane-associated phospholipase C (GPI-PLC) becomes activated and cleaves the GPI anchor as shown in Figure 1; this results in the release of substantial soluble VSG (GIP-sVSG) that retains only the glycosylinositolphosphate (GIP) substituent of the original GPI anchor and leaves the dimyristoylglycerol (DMG) lipid component remaining behind in the membrane (Armah and Mensa-Wilmot, 2000; Bulow et al, 1989; Butikofer et al, 1996; Hereld et al, 1988; Hereld et al, 1986; Mensa-Wilmot and Englund, 1992; Mensa-Wilmot, 1995; Paturiaux-Hancoq et al, 2000). Parasite numbers routinely fluctuate during infection, both in the blood and extravascular tissues, primarily as the result of host B and T cell responses to variant determinants of the VSG molecule. Since a) trypanosomes numbers may approach 10^8 organisms per ml blood and may also be quite high in the extravascular tissues during peak parasitemias, b) there are approximately 10^7 VSG molecules per cell, c) GIP-sVSG is clipped and released from both viable and stressed/damaged trypanosomes, and d) trypanosomes are episodically destroyed by antibody-(Ab-) and Th1 cell/macrophage-dependent effector mechanisms, the amounts of GPI substituents (GPI-mfVSG, GIP-sVSG and DMG) saturating host tissues during infection are quite substantial. Conservative calculations estimate that experimentally infected animals may be exposed to 15-20 μM VSG with each wave of parasitemia, which is an inordinate amount of parasite material with intrinsic macrophage activation potential to be released into host tissues.

Limited in vitro studies by several labs have begun to characterize the activating effects of GPI substituents on macrophages. It appears that GIP-sVSG and GPI-mfVSG (containing the DMG lipid substituent) have similar macrophage activating capabilities in terms of TNFα, IL-6 and NO production, but that there may be subtle differences in the ability of GPI-mfVSG to more effectively induce IL-1 and IL-12 production (Magez et al, 1998; Schofield et al, 1996; Schofield and Tachado, 1996; Tachado et al, 1996; Tachado and Schofield, 1994). An extension of these initial studies regarding the ability of GIP-sVSG to interact with macrophages demonstrates that the GIP-sVSG component binds directly to macrophages and induces expression of a specific subset of activation genes in an IFN-γ independent manner (Imboden et al, submitted for publication 2001; Paulnock and Coller, 2001). Studies have also shown that GPI substituents exhibit signaling activities (Tachado et al, 1997). That specific signal(s) are delivered to the cell is apparent from the resultant activation phenotype of macrophages exposed to GPIs, and by recent in vitro studies showing that GIP substituents (specifically the core glycan sequence) activate a specific protein tyrosine kinase, while the DMG substituent may independently activate a protein kinase C isoform in macrophages (Tachado et al, 1997). However, it is not yet known how GPIs interact with the macrophage membrane nor what receptor(s) may be important in delivering GPI-mediated activation signals to the cell nucleus.

During trypanosome infection, however, it is clear that host cells are exposed to biologically active levels of GIP-sVSG, DMG and GPI-mfVSG, although the timing of exposure to these molecules and the nature of their delivery to the macrophage membrane could be very different (see Figure 2). While the effects of such GPI substituents on uninfected macrophages have been partially characterized in vitro, the impact of tissue saturation with these activating agents in vivo during infection is just being fully appreciated (Imboden et al, submitted for publication 2001; Paulnock and Coller 2001). Also, macrophages are not the only cell type that can be targeted by GPIs; recent evidence demonstrates that natural killer (NK) 1.1 T cells may...
express T cell receptors (TCR) specific for GPI determinants and that B cells may be stimulated to undergo cell differentiation by GPis (Bento et al, 1996; Schofield, et al, 1999). Thus, GPI substituents released by pathogens such as the African trypanosomes may have broad effects on the host immune system that surpass central activating effects on macrophages.

**Role of IFN-γ in macrophage activation and early host protection and/or pathology**

The other major macrophage activation factor produced during trypanosomiasis is IFN-γ, which is of host origin. Small amounts of IFN-γ may be produced very early during infection as the result of TLTF activation of CD8 T cells (Bakhiet et al, 1996; Bakhiet et al, 1993; Schleifer and Mansfield, submitted for publication 2001; Vaidya et al, 1997). First described by Bakhiet, Olsson and colleagues (Bakhiet et al, 1993; Bakhiet et al, 1996; Bakhiet et al, 1990; Olsson et al, 1993; Olsson et al, 1992) and subsequently cloned by the Donaldson laboratory (Vaidya et al, 1997), TLTF is a 453 amino acid protein with potentially important biological effects. TLTF was discovered when researchers noted that rodents injected with *T. b. brucei*, or lymphoid cells cultured with trypanosomes in vitro, exhibited an increase in the number of antigen non-specific secreting cells; depletion of CD8⁺ T cells in animals or cultures abrogated the effect and, interestingly, also resulted in less trypanosome growth (Bakhiet et al, 1990). Use of a chamber system separating lymphoid cells and trypanosomes showed that a soluble factor was responsible for induction of IFN-γ synthesis (Olsson et al, 1991).

Several *Trypanosoma* species appear to express TLTF but may possess different IFN-γ stimulating abilities as measured by the relative increase of IFN-γ producing cell numbers in the presence of extracts or culture filtrates of species including *T. evansi*, *T. b. rhodesiense*, and *T. b. gambiense* (Bakhiet et al, 1996). Subsequent characterization of CD8⁺ T cell IFN-γ activation by TLTF showed that tyrosine protein kinases are necessary for activation but protein kinase C and protein kinase A specifically are not (Bakhiet et al, 1993). Interestingly, TLTF may stimulate other cells to release IFN-γ, such as rat dorsal root ganglia, and this secretion apparently also is dependent on tyrosine kinase(s) (Elayeb et al, 2000). These types of studies and their experimental extension over the past decade have led investigators to posit the following hypotheses with respect to the role of TLTF: trypanosomes secrete TLTF which binds to CD8 molecules expressed on CD8⁺ T cells, thereby inducing antigen non-specific activation and production of IFN-γ; TLTF-induced release of IFN-γ subsequently serves as a growth factor that promotes trypanosome growth (Bakhiet et al, 1996; Bakhiet et al, 1990; Hamadien et al, 1999; Olsson et al, 1991; Olsson et al, 1993; Vaidya et al, 1997). Thus a factor secreted from the parasite, TLTF, is visualized as inducing an essential trypanosome growth factor, IFN-γ, from host cells.

Experiments partially in conflict with these proposed hypotheses exist, however. First there has been no independent confirmation that IFN-γ serves as a growth factor for African trypanosomes; unpublished studies from several labs have failed to substantiate the claim that IFN-γ serves as a growth factor and no critical biochemical studies of IFN-γ binding to or utilization by trypanosomes have yet been published. Furthermore, parasitemias are higher in IFN-γ knockout mice, which are highly susceptible rather than more resistant to infection with *T. b. rhodesiense* (see discussion below on the role of IFN-γ in early host resistance [Hertz et al, 1998]). One might reasonably expect that parasitemias would be lower in IFN-γ-deprived animals if this cytokine served in any substantial manner as a growth factor. Additionally, TLTF expression is identical in trypanosomes expressing high and low virulence attributes (Mansfield, 2001), suggesting that there is no modulation of the gene or protein in organisms known to exhibit rapid growth characteristics and virulence attributes for mammalian hosts.

Initial studies asserted that TLTF was a secreted protein (Vaidya et al, 1997), but subsequent characterizations showed that the amino acid sequence does not contain a hydrophobic region typical of membrane-transported trypanosome proteins, though it did appear to be targeted to the flagellar pocket region through an apparent conformational signal within a specific 144 amino acid domain (Hill et al, 1999). To date there is no clear evidence that TLTF is a secreted protein, though the predicted protein does possess unique internal targeting sequences. More recent studies on the cell biology of TLTF have suggested an alternate (or coincident) role for the protein. The gene sequence identified as TLTF is expressed in both insect and bloodstream forms of *T. b. brucei* and the protein appears to be tightly associated with the flagellar cytoskeleton (present in detergent-resistant and Ca²⁺-resistant cytoskeletal fractions of trypanosome extracts) (Hill et al, 2000); modification of TLTF gene expression in the procyclic form resulted in an unusual motility defect, suggesting that TLTF may be an integral part of the trypanosome cytoskeletal architecture. Surprisingly, TLTF-like genes are present in a number of divergent eukaryotes including *Drosophila* and zebra fish. Notably, the human growth arrest specific gene (GAS)11, closely related to TLTF (Vaidya et al, 1997),...
is a possible tumor suppressor molecule with a sub-
molecular region that may localize to cellular micro-
tubules.

Thus, it is difficult to see how TLTF, a tightly bound cytoskeleton-associated molecule, would be secreted or released in biologically active levels during infection or in cell cultures containing viable trypanosomes to affect the release of IFN-γ from host cells. Yet, it is clear that trypanosome infections and trypanosome extracts are capable of inducing IFN-γ release from naive host lymphoid cells in an antigen nonspecific manner; the levels are low and occur independently of antigen specific induction of IFN-γ from host Th1 cells (Mansfield, 2001; Schopf et al, 1998). Thus, IFN-γ secretion induced by parasite material(s) has been a repeatable phenomenon and is clearly of some interest; there is the distinct possibility that release of biologically active TLTF (or a similar molecule with closely related effects) occurs during periods of cataclysmic elimination of trypanosome variant antigen types (VATs) by host Ab and Th1/macrophage cell responses throughout infection (see below), rather than by an active secretory pathway, to induce IFN-γ. Given that trypanosomes / trypanosome extracts are capable of inducing nonspecific IFN-γ release from host cells, and that IFN-γ may be an early and critical factor in host protection (probably important in regulating parasite numbers in the extravascular tissues—see Figures 2 and 3), it may be that TLTF is important in a different context than originally suggested: by inducing low levels of IFN-γ that control an early potentially explosive spread of trypanosomes in infected host tissues, regardless of the genetically-based resistance status of the host. Such an early control over parasite expansion and, in susceptible animals or individuals, a delay in host death, would permit the host to survive for a period of time so that the possibility of trypanosomes being taken up in a blood-meal and transmitted to new hosts could occur. Regardless of one’s view of TLTF, the hard work of providing functional and genetic linkages between TLTF expression and biological effects on the host remain.

Clearly, however, the major source of IFN-γ during infection appears to be parasite antigen stimulated Th1 cells, which appear in significant numbers somewhat later than early TLTF induced IFN-γ responses. Th1 cell responses to VSG (and to invariant antigens of the parasite) have now been characterized and result in strong IFN-γ responses in genetically more resistant animals but not in susceptible animals (Hertz et al, 1998; Hertz and Mansfield, 1999; Schleifer et al, 1993; Schopf et al, 1998). The IFN-γ response has been definitively linked to early host resistance during T. b. rhodesiense infection, and is thought to be associated with macrophage activation characteristics responsible for control of the parasites in extravascular tissue sites (De Gee et al, 1985; Hertz et al, 1998; Imboden et al, submitted for publication, 2001; Paulnock and Coller, 2001).

Figure 2. Macrophage activation in African trypanosomiasis is mediated by exposure to host and parasite factors.

(Figure adapted from Paulnock and Coller, 2001)

The theoretical timing of exposure to host- and parasite-derived macrophage activating factors during an early peak of parasitemia in trypanosome infection is shown here. GIP-sVSG homodimers, released from the trypanosome plasma membrane by the action of GPI-PLC, are detectable within several days of infection throughout the body (Diffley and Jayawardena, 1982; Diffley and Straus, 1986; Diffley et al, 1980; Norton et al, 1986). Although DMG is liberated by the same GPI-PLC cleavage event as GIP-sVSG, host tissues may be exposed earlier to GIP-sVSG since effective membrane transfer of DMG would largely be dependent on high concentrations of parasites in contact with macrophage membranes. GIP-sVSG can activate macrophages independently of residual DMG (Imboden et al, submitted for publication 2001; Magez et al, 1998; Paulnock and Coller, 2001; Schofield and Tachado, 1996; Tachado et al, 1997). During immune clearance of trypanosomes, macrophages take up parasites and presumably are also exposed to intact GPI-mVSG as well as DMG remaining from GPI-PLC cleavage of the anchor. Low levels of IFN-γ may also serve as an early activating agent during infection, presumably derived from TLTF-stimulated CD8 T cells. Cytokine levels subsequently rise substantially over time due to CD4 Th1 cell stimulation by VSG and other parasite antigens. The patterns shown here may differ markedly later in infection.
The IFN-γ/IFN-γ receptor interaction and downstream subcellular signaling pathways have already been well characterized in other model systems, and macrophage activation events triggered by IFN-γ have been extensively studied (Adams and Hamilton, 1984; Adams and Hamilton, 1987; Adams and Hamilton, 1986; Hamilton, 1989; Paulnock, 1992; Paulnock, 1994; Paulnock-King et al, 1985). In African trypanosomiasis, it is clear that some macrophage activation events are dependent on IFN-γ exposure and others on exposure to trypanosome-derived molecules, including GIP-αVSG (Imboden et al, submitted for publication 2001; Paulnock and Coller, 2001). The activation patterns observed in the presence of both factors are different from each one alone, are dependent on the genetic background of the infected host, and may be important in the control of infection. Why trypanosomes induce such broad macrophage activation effects is unknown. It may be important for trypanosomes to induce early temporal protection against the infection regardless of the genetically-based resistance status of the host (e.g. so the host isn’t killed by infection before natural transmission of the disease can effectively occur). Alternatively, it might be linked to the early generation of suppressor macrophage activity so as to depress host T cell responses to parasite antigens. Or it may result in deregulation of IFN-γ-induced activation events in macrophages in order to avoid parasite elimination. Clearly, a goal of unraveling the cellular and molecular basis of macrophage activation by trypanosome-derived antigens or other factors (e.g. DNA released from dead trypanosomes [Shoda et al, 2001]) in the context of overall host resistance to infection and tissue pathology is important.

**Tissue specific immune control mechanisms in early infection**

There are clear differences in the ability of various host species, and strains within species, to display relative resistance to African trypanosomiasis (Levine and Mansfield, 1981; Mulligan, 1970). Studies over the past twenty years have revealed that the host Ab response plays only a partial role in such relative resistance against trypanosomes. While VSG-specific Ab clearly is responsible for the cataclysmic elimination of VATs from the bloodstream of infected hosts, it is now known that this event is not linked, functionally or genetically, to overall host resistance (De Gee et al, 1988; De Gee and Mansfield, 1984; Mansfield, 1995; Mansfield, 1990; Mansfield and Olivier, 2001). The seminal studies were those in which H-2 compatible radiation chimera mice, reconstituted with reciprocal bone marrow cell transplants from relatively resistant or susceptible donors, revealed the following: that susceptible mice, which normally do not make a sufficient Ab response to VSG and do not clear VATs from the blood, were afforded by donor cells from resistant mice a functional B cell response that enabled them to clear parasitemia during infection; however, despite the ability to eliminate trypanosomes from the blood, these animals were just as susceptible as mice receiving susceptible donor bone marrow cells that failed to make protective VSG-specific B cell responses (De Gee and Mansfield, 1984). Subsequent genetic studies with crosses between Ab+ resistant and Ab− susceptible mouse strains showed that the F1 hybrids all were able to make VAT-specific Ab responses and control parasitemias, but all such hybrids were as susceptible as the susceptible parental strain (De Gee et al, 1988). Taken together, these types of results showed that the VSG-specific B cell response, although linked to trypanosome clearance from the blood, was not by itself functionally or genetically linked to overall host resistance.

**Th cell responses to trypanosome antigens**

This information led the way to studies that first elucidated Th cell responses to VSG and other trypanosome antigens during infection (Hertz et al, 1998; Hertz and Mansfield, 1999; Mansfield, 1994; Schleifer et al, 1993; Schopf et al, 1998). T cell responses to trypanosome antigens were not discovered previously because of several interesting characteristics of trypanosome infections. First, a non-specific immunosuppression of T cell responses in trypanosomiasis had been recognized for many years (Mansfield and Wallace, 1974), and, although earlier studies revealed that T cell responses to trypanosome antigens could be induced in immunized animals (Campbell et al, 1982; Finerty et al, 1978), such responses were not readily detectable in infected animals (Mansfield and Kreier, 1972; Paulnock et al, 1989). For example, not only were spleen or lymph node T cells from infected mice unable to proliferate in response to mitogens or antigens, they also failed to produce significant amounts of IL-2 or IL-4, and these events could be shown to impact on T-dependent B cell responses to a variety of antigens (Mansfield and Bagasra, 1978). This generalized immunodeficiency was shown to result in part from the presence of macrophage “suppressor cells” in lymphoid tissues (Sacks et al, 1982; Wellhausen and Mansfield, 1980; Wellhausen and Mansfield, 1979; Wellhausen and Mansfield, 1980); in fact, macrophages from infected mice had the capacity to actively suppress the proliferative responses of normal T cells to mitogens and antigens in vitro and in vivo. A breakthrough in recognizing that Th cell responses to trypanosome antigens occurred during infection came with the finding that functional com-
partamentalization of such responses occurred (Schleifer et al, 1993). It was revealed that Th cells reactive with VSG were predominant in the peritoneal T cell population; when stimulated with VSG, these cells made a substantial IL-2 and IFN-γ cytokine response but failed to proliferate. Subsequently, it was discovered that Th cells in the peripheral lymphoid tissues also made an IFN-γ response (but little IL-2) when stimulated with VSG. Thus, it was apparent that VSG-reactive T cells were present in infected animal tissues but that they exhibited a restricted cytokine response and minimal evidence for clonal expansion (Schleifer et al, 1993). Since these VSG-reactive T cells displayed a CD4+ αβ TCR+ membrane phenotype, expressed Type 1 cytokines, were major histocompatibility class II antigen (MHC II) restricted and antigen presenting cell (APC) dependent (Hertz et al, 1998; Schleifer et al, 1993; Schopf et al, 1998), it was clear that they represented a classical Th1 subset of T cells that recognized VSG during infection. More recent work has begun to elucidate the submolecular targets of VSG-reactive Th cells. In preliminary studies it has been shown that Th cell specificities are directed against a defined hypervariable subregion of VSGs that is not exposed when VSG homodimers are assembled into the surface coat structure (Mansfield, 2001; Mansfield and Olivier, 2001), fulfilling earlier predictions that VSG sequence variability in nonexposed regions of the molecule might be driven by T cell selection (Blum et al, 1993; Field and Boothroyd, 1996; Reinitz et al, 1992).

The extreme polarization of the Th1 cell cytokine responses seen in some experimental systems is due in part to the early production of IL-12 by macrophages exposed to trypanosome GPI substituents (Mansfield et al, submitted for publication). That IL-12 is not the only polarizing factor is seen from preliminary studies with IL-12 knockout (KO) mice and mice exposed to Abs against IL-12; in each case, early temporal depression of the Type 1 cytokine response did not result in a compensatory Type 2 cytokine response and, after a period of 10 days or so, the Th1 cell response emerged in both groups (Mansfield et al, submitted for publication). That IL-12 is not the only polarizing factor is seen from preliminary studies with IL-12 knockout (KO) mice and mice exposed to Abs against IL-12; in each case, early temporal depression of the Type 1 cytokine response did not result in a compensatory Type 2 cytokine response and, after a period of 10 days or so, the Th1 cell response emerged in both groups (Mansfield et al, submitted for publication). Thus, there are complex features of infection that promote the production of Type 1 cytokines and the outgrowth of antigen-reactive Th1 cells. While reasons for the relative tissue compartmentalization of Th cell cytokine responses (e.g., IL-2 and IFN-γ production by peritoneal Th cells, but mostly IFN-γ production by Th cells in the peripheral lymphoid tissues) have not been elucidated, the reason for inhibition of T cell clonal expansion has now been resolved.Suppressor macrophages were shown to elaborate several factors that inhibited the proliferative (but not the cytokine) responses of VSG activated Th1 cells: NO, prostaglandins and TNF-α (Darji et al, 1996; Hertz and Mansfield, 1999; Schleifer and Mansfield, 1993; Sternberg and McGuigan, 1992). Macrophages were activated to produce these suppressive factors primarily as the result of exposure to GIP-sVSG and to IFN-γ released by parasite antigen stimulated Th cells (Hertz and Mansfield, 1999; Mansfield et al, submitted for publication; Schleifer and Mansfield, 1993). The full impact of NO and prostaglandins on host immunity to trypanosomes has not been completely resolved, but studies with iNOS KO mice have shown that, although NO is the main “suppressor” factor that limits clonal expansion of T cells (and maybe also modulates cytokine responses to a degree) the absence of NO did not affect overall host resistance (Hertz and Mansfield, 1999).

Early and strong trypanosome-specific Th1 cell responses may provide an essential component of host resistance; this realization emerged from studies with cytokine gene knockout mice. The central finding in one study was that mice with a resistant genetic background but which lacked a functional IFN-γ gene were as susceptible as scid mice to trypanosome infection, even though those mice produced Abs sufficient to control parasitemia (Hertz et al, 1998). In contrast, the same genetic strain of mouse with the IL-4 instead of the IFN-γ gene knocked out were as resistant as wt mice to infection (Hertz and Mansfield, 1999). These results underscored earlier studies demonstrating that the VAT-specific Ab response and control of parasitemia were not capable of providing resistance alone, and that the production of a single cytokine, IFN-γ, in response to infection was found to be a critical element in host resistance. The mechanism(s) associated with IFN-γ-mediated resistance are not yet clear, but seem to involve macrophage factors induced by IFN-γ activation. Several candidate factors have been proposed, such as NO and TNFα, both of which have been shown to kill trypanosomes in vitro (Lucas et al, 1994; Lucas et al, 1993; Magez et al, 1997; Magez et al, 1999; Mnaimneh et al, 1997; Vincendeau et al, 1992). Recent studies suggest, however, that neither factor alone is capable of mediating resistance in vivo; results with trypanosome infected iNOS KO mice and TNFα KO
mice showed that such mutations on a resistant mouse genetic background do not significantly affect the course of infection (Hertz and Mansfield, 1999; Magez et al, 1999; Millar et al, 1999), although it is possible that the combination of NO and TNFα is required for functional resistance. Clearly, IFN-γ inducible events in macrophages must carefully be evaluated for their impact on trypanosomes during early stages of infection. Since these events occur independently of B cell mediated resistance mechanisms that are known to control trypanosomes in the vasculature, and since IFN-γ activated macrophage control mechanisms are presumed to be important in regulating trypanosome numbers in the extravascular tissue spaces (but this by itself is inadequate to provide protection [Mansfield, 2001]), it appears that multiple arms of the host immune system are required to control trypanosomes and to provide relative resistance during infection.

Figures 3A and 3B. Innate and acquired immune elements in early- versus late-stage trypanosomiasis.

These figures portray the cell and molecular elements important in controlling trypanosome infection of host tissues. In early infection stages, IFN-γ cytokine responses activate macrophages to produce factors cytotoxic for trypanosomes, limiting the spread or survival of parasites outside the vasculature. B cell responses appear to selectively control trypanosomes within the vasculature. In late-stage infections, there appears to be a shift from the phenotype of pro-inflammatory responses and parasitidal macrophage activation to a phenotype of counter-inflammatory responses and Type 2 cytokine responses that do not promote parasite elimination from tissues and perhaps also from the blood. Adapted from Mansfield and Olivier (2001).

Thus, relative resistance to African trypanosomes may be mediated by two major components of host immunity, neither one of which by itself is adequate to provide resistance (Figure 3A). First, VSG specific Ab responses control trypanosomes present in the blood. Second, T cell production of IFN-γ and subsequent macrophage activation events are necessary to control trypanosomes in the extravascular tissues. Animals that make weak B cell and/or T cell responses to trypanosome variant antigens invariably will demonstrate relative susceptibility; in contrast, animals making pronounced B and T cell responses (including appropriate macrophage activation events) will display relative high resistance. However, this picture is evolving considerably due to evidence that the later stages of experimental infection display a different pattern, a pattern that is associated with loss of resistance to trypanosomes (see Figure 3B).

Overview

Events that impact on B, T or macrophage cell responses during infection can be expected to cause modulations in host resistance and tissue pathology. These events need to be clarified in both animal model systems and in clinical HAT.

Changes in Parasite Cell Biology during Infection that Impact on Host Resistance

The African trypanosomes display considerable biological variation during their life cycle. This biological variation is directed by specific patterns of gene and protein expression. For example, bloodstream trypomastigote forms display a number of different surface antigenic phenotypes during infection of their mammalian hosts; this phenotypic variation has as its basis the differential expression of VSG genes and molecules (Borst et al, 1998; Borst and Rudenko, 1994; Cross, 1990; Donelson, 1987; Van der Ploeg et al, 1992; Vickerman and Luckins, 1969). Additionally, the differentiation of long slender (LS) trypomastigote forms to short stumpy (SS) trypomastigotes during infection results in profound morphological and functional changes in these cells. Such changes include mitochondrial biogenesis and the acquisition of new metabolic pathways; it is the differential expression of specific genes and proteins that presage these biological changes (Bienen et al, 1983; Bohringer and Hecker, 1975; Hua et al, 1997; Mulligan, 1970; Mutomba and
Wang, 1998; Tschudi, 1995; Vanhamme and Pays, 1995; Vickerman, 1971; Vickerman et al, 1993). Furthermore, the differentiation of SS forms to procyclic forms in the insect vector also results from the differential expression of specific genes and proteins (Butikofer et al, 1997; Roditi, 1996; Ruepp et al, 1997; Vanhamme and Pays, 1995; Vickerman et al, 1988). Finally, the transformation of insect forms to metacyclic trypanmastigotes results in different morphological and functional changes in the parasites that permit infection of a new mammalian host; these changes also occur as the result of differential gene and protein expression (Turner et al, 1986; Vanhamme and Pays, 1995; Vickerman, 1985; Vickerman et al, 1993). Thus, all trypanosomes exhibit considerable clonal plasticity in terms of their antigenicity, morphology and biological function during the life cycle; this plasticity occurs as the direct result of differential expression of specific subsets of genes and proteins. It follows that the failure of trypanosomes to undergo biological variation at critical points in the life cycle may result in elimination by the mammalian host, failure to differentiate within the intermediate host and vector, or inability to establish new animal infections.

The molecular mechanisms that regulate changes in VSG phenotype and stage of cellular differentiation are shared in common among African trypanosomes; however these are not the only mechanisms that regulate biological differences in these parasites. New evidence is emerging that differential susceptibility of *brucei* group trypanosomes to host factors such as trypanosome lytic factor (TLF) (De Greef and Hamers, 1994; Hager et al, 1994; Hajduk et al, 1992; Rickman and Robson, 1970; Riffkin, 1978; Smith et al, 1995), may occur as the result of clonal variation among trypanosomes (Hager and Hajduk, 1997; Hajduk et al, 1995); the basis for such changes has not yet been defined, but is believed to encompass specific clonal modifications in gene or protein expression. Host specificity may also be defined by the clonal expression of different transferrin receptor genes (Bitter et al, 1998). While these examples reflect on trypanosome infectivity for a host species, related observations and mathematical modeling predict that considerable biological variation occurs among trypanosomes during infection that is not related directly to infectivity or cyclical differentiation in the life cycle (Turner et al, 1995; Vassella et al, 1997). For example, it is well known that different isolates, species and subspecies of trypanosomes exhibit remarkable variation in pathogenicity and virulence for genetically defined host species (Mulligan, 1970). A key question has been whether such differences in virulence are immutable characteristics associated with genetically distinct populations of trypanosomes, or whether there is intraclonal biological variation within trypanosome populations that impacts on the course of disease in a genetically defined host.

This question was addressed in an earlier study in which mice of a relatively resistant phenotype were infected with a single trypanosome of *T. b. rhodesiense* clone LouTat 1 (Inverso and Mansfield, 1983). Several different VATs were isolated from parasitic peaks at intermediate and late time points during infection of a single mouse; these VATs were subcloned and characterized as to VSG phenotype. Three different VATs, which represented antigenically distinct daughter cell populations clonally derived from a single LouTat 1 parental cell, were used to infect the same mouse strain; the courses of infection were monitored in comparison with mice infected with LouTat 1. The interesting result was that each of the daughter cell populations exhibited a different virulence profile compared to the parental clone (Inverso and Mansfield, 1983). For example, LouTat 1 caused death in approximately 62 days post-infection, while LouTat 1.3, LouTat 1.4 and LouTat 1.5 caused death in approximately 44, 30, and 28 days, respectively. These results demonstrated that VATs arising during infection expressed virulence phenotypes different from the infecting VAT. In essence, daughter cells arising within a trypanosome population expressed the capacity to transcend host genetic resistance characteristics and render a relatively resistant animal into a more susceptible one.

These seminal observations have been repeated with consistent results, and related observations on clonal heterogeneity among trypanosomes have been made in other studies (Diffl ey and Mama, 1989; Inverso et al, 1988; Joshua, 1990; Mamman et al, 1995; Ortiz et al, 1994; Reinitz and Mansfield, 1988; Sacks et al, 1980; Seed, 1978; Seed and Sechelski, 1996; Turner, 1990; Turner et al, 1995). Additionally, the general observation has been made that many other daughter cells/VATs arising naturally from LouTat 1 also expressed differences in virulence (see Table 1, below), and that the most virulent VATs seemed to arise at later time points in infection, just prior to host death. Thus, the apparent result of infection with relatively low virulence trypanosomes is a progressive increase in population virulence with time, rather like the turning up of a “virulence rheostat”. Based on these observations, it was speculated that the longer a trypanosome population existed in a mammalian host, the more virulent that population might become. Such a virulence rheostat may have evolved as a programmed mechanism to overcome different levels of host resistance that might be
encountered in nature, where there is a pool of genetically disparate mammalian hosts available for infection. Implicit in this speculation, however, is the idea that a virulence rheostat must somehow be reset, perhaps upon cyclical passage through the intermediate host and vector, the tsetse fly.

Since virulent trypanosomes seem to arise after a prolonged period of replication in an infected host, it may be possible to generate highly virulent trypanosomes by rapid subpassage of low virulence trypanosomes through mice for a substantial time period; in essence, the effect would be one mimicking a single, prolonged course of infection. This was achieved by infecting irradiated mice with LouTat 1 and subpassaging the trypanosomes into different mice every three days for approximately six months. At the end of this time, trypanosome stabilates were made from sublines and subclones, and were assessed for their virulence characteristics. One representative subclone, designated LouTat 1A, was examined in some detail. These trypanosomes displayed a single uncontrolled peak of parasitemia and were able to kill a resistant mouse strain (as well as all other resistant or susceptible strains of mice) in approximately four days post-infection; in contrast the parental clone LouTat 1 gave rise to multiple peaks of parasitemia and a prolonged survival time of over 60 days in the same mouse strain (Inverso et al, 1988). Thus a model system of comparative trypanosome virulence was developed from this approach, in which the relatively low virulence clone LouTat 1 and the relatively high virulence subclone LouTat 1A represent different ends of a virulence spectrum, with the virulence of other naturally arising VATs existing somewhere between these two extremes (Table 1).

A natural question that arose from these types of studies was whether the VSG molecules expressed by virulent VATs acted as virulence factors, with specific VSG isotypes exerting defined biological effects on the host. This idea was not unfounded since several biological traits associated with VSG molecules have been described in the literature (Mathias et al, 1990; Musoke and Barbet; 1977; Schofield et al, 1999; Tachado et al, 1997; Tizard et al, 1978). Alternatively, one could also speculate that expression site-associated genes (ESAGs) co-transcribed with certain VSG genes at specific chromosomal expression sites may be responsible for virulence expression or regulation. This idea was based on observations of others concerning potential growth or differentiation regulatory roles associated with ESAGs (Cross, 1990; Vickerman et al, 1993). However, an analysis of the LouTat 1/LouTat 1A model system revealed that both organisms displayed the same antigenic surface coat structure (Inverso et al, 1988), transcribed identical VSG genes (Reinitz et al, 1992; Uphoff et al, submitted 2001) and expressed their VSG genes by a duplicative transposition event from the same chromosomal telomeric expression site (Uphoff et al, submitted 2001).

Table 1. Virulence phenotypes of LouTat 1-derived VATs.

<table>
<thead>
<tr>
<th>Variant Antigenic Types</th>
<th>Virulence (Mean Survival Time)</th>
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<tbody>
<tr>
<td>(A) VATs isolated from natural infections*</td>
<td></td>
</tr>
<tr>
<td>LouTat 1 (parental clone)</td>
<td>62</td>
</tr>
<tr>
<td>LouTat 1.9</td>
<td>46</td>
</tr>
<tr>
<td>LouTat 1.3</td>
<td>44</td>
</tr>
<tr>
<td>LouTat 1.4</td>
<td>30</td>
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<tr>
<td>LouTat 1.5</td>
<td>28</td>
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<tr>
<td>LouTat 1.7</td>
<td>25</td>
</tr>
<tr>
<td>LouTat 1.2</td>
<td>13</td>
</tr>
<tr>
<td>LouTat 1.6</td>
<td>11</td>
</tr>
<tr>
<td>LouTat 1.10</td>
<td>6</td>
</tr>
<tr>
<td>(B) VATs derived by subpassage**</td>
<td></td>
</tr>
<tr>
<td>LouTat 1A</td>
<td>4</td>
</tr>
<tr>
<td>LouTat 1D</td>
<td>4</td>
</tr>
<tr>
<td>LouTat 1X</td>
<td>4</td>
</tr>
<tr>
<td>LouTat 1n</td>
<td>6-60</td>
</tr>
</tbody>
</table>

Therefore, it is unlikely that either VSG molecules or the active VSG gene expression site are important for virulence regulation in trypanosomes (see Figures 4-6). Confirmation that VSG genes and VSG gene expression sites are not involved in virulence came from additional experimental approaches. New sublines and subclones were derived by rapid subpassage of LouTat 1 through irradiated mice, as above, and examined for VSG phenotype and gene expression. Many of the subclones expressed the same VSG gene as LouTat 1 and all were highly virulent like LouTat 1A (Table 1). In another approach, LouTat 1A was used to infect rabbits and goats; although these animals exhibited pathology sooner than LouTat 1 infected controls, they did not die as early as mice and trypanosomes were able to undergo antigenic variation. The VATs generated from LouTat 1A in these animals were isolated and subsequently used to infect mice; the results showed that the LouTat 1A-derived VATs were as virulent for mice as LouTat 1A (Inverso et al, 1988). Thus high levels of virulence, once expressed in mammalian hosts, appear to be a constitutive trait that is unaffected by further VSG gene switching.
Amino acid sequence of the *T. b. rhodesiense* LouTat 1/ LouTat 1A VSG molecule. The protein sequence was deduced from the identical full-length nucleotide sequences (Accession no. X56643) (Reinitz et al, 1992) as well as from partial amino acid sequencing data (Inverso et al, 1988). The N-terminal signal sequence and the C-terminal hydrophobic extension are underlined; threonine-27 is the start site of the mature protein.

Genomic DNA from LouTat 1, 1.5 and 1A was digested with *Xmn*I and hybridized in a Southern blot with a labeled LouTat 1 VSG cDNA probe (BC, basic copy of VSG gene; ELC, expression linked copy).

The expressed copies are in a telomeric site while the basic copy of the VSG gene is found at an internal chromosomal site that is the same for both trypanosomes. The solid bar denotes the VSG gene.

Subsequently LouTat 1 and 1A were examined for non-VSG related cellular differences (Mansfield, 2001). Comparative analysis of several traits revealed significant differences between the two clones. A few chromosomes were altered in size, as determined by pulsed field gel electrophoresis; however, there was no net loss of cellular DNA nor were any differences apparent in restriction fragment length polymorphism (RFLP) patterns utilizing a number of random and known non-VSG cDNA probes. Thus, the chromosomal size variations observed may largely be subtelomeric or simply do not involve chromosome regions to which the probes hybridized. Two-dimensional gel electrophoresis of 35S-labeled proteins showed not only that different proteins were expressed in LouTat 1A compared to LouTat 1, but also that there were different proteins expressed in LouTat 1 compared to LouTat 1A. Competitive Northern analyses in which labeled total cDNA from LouTat 1A, in the presence of excess unlabeled LouTat 1 competitive total cDNA, was hybridized to mRNA from LouTat 1A showed that that there were numerous mRNA species unique to LouTat 1A. Taken together, these observations suggested that a subset of genes and proteins was being expressed in LouTat 1A that was not being expressed at the same level in LouTat 1.

Overall, preliminary observations on the biological behaviour of LouTat 1 and LouTat 1A, and on the subcellular differences detectable between LouTat 1 and LouTat 1A, as well as biological variation observed with other subspecies of *T. b. brucei* in terms of TLF susceptibility or TNFα sensitivity, have led to the hypothesis that African trypanosomes have the capacity to regulate clonal expression of virulence. Specifically, it has been proposed (a) that trypanosomes have evolved the programmed capacity for clonal upregulation of virulence as a means to successfully subvert host resistance mechanisms, regardless of host genetic background; (b) that this capacity to modify virulence phenotype occurs independently of changes in VSG gene expression; and (c) that the level of virulence expressed is determined by differential gene and/or protein expression during trypanosome growth in an infected host.

**Overview**

It seems therefore an important goal to characterize the molecular mosaic associated with the virulence phenotype and to determine whether or not specific subsets of genes or proteins linked to virulence can be identified; the prediction is that such approaches may open new doors in the understanding of trypanosome-mediated pathogenesis, and candidate virulence factors could be targeted for specific immuno- or chemotherapies.
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II APPLIED GENOMICS: PROSPECTS FOR CONTROL OF AFRICAN TRYPANOSOMIASIS VIA THE TSETSE VECTOR


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INTRODUCTION

Despite many decades of research on vector-borne parasites and their development in mammalian hosts, effective strategies have yet to materialize for control of any of the diseases with which they are associated. At the same time, a heavy reliance on insecticides and therapeutic drugs has resulted in the spread of insecticide resistance in many vectors and the emergence of drug resistance in parasites, threatening the availability of effective tools to combat these diseases. This scenario is also apparent for sleeping sickness in Africa where there are currently at least half a million people estimated to have contracted the fatal disease. Antigenic variation in the mammalian host has hampered efforts for vaccine development. The current strategies for management of trypanosomiasis depend on active surveillance and treatment of infected hosts, and on limited vector control measures. These efforts are, however, restricted by the lack of effective drugs, their high cost, adverse side effects, and the emergence of drug resistance in patients (McNeil, 2000). Thanks to recent international effort, there are now several programmes in place that will allow continued provision of the existing drugs for treatment and for research on the development of new drugs. While this is encouraging news, treatment alone for a zoonosis for which there is no evidence of acquired immunity is likely to be extremely costly in the long run even if effective drugs were to be available. The fact that the parasite relies on a single insect for its transmission opens up many avenues for control via the control of its vector. It is important to note that trypanosomiasis is a disease based on interactions among at least three organisms, the human, the parasite and the tsetse fly. Interference with any of these interactions can prevent disease. In fact, tsetse control strategies have been widely used for management of animal diseases. Most of these efforts however have been on a small scale, involving trapping and the use of insecticide sprays, and have been costly and difficult to sustain. A recent study looking into the cost/benefit analysis of various strategies clearly identifies large-scale, area-wide methods as being far more efficient and affordable in the long run for tsetse control (Budd, 1999).

In Lome, Togo, July 2000, the Organization of African Unity (OAU) endorsed a strategy of tsetse fly and trypanosomosis eradication on the African continent by using an integrated approach including area-wide control strategies. The recent advances in molecular technologies and their application to insects have revolutionized the field of vector biology although progress in the tsetse field has been slow. In this paper, we review the current status of knowledge on the molecular aspects of tsetse interaction with trypanosomes. We highlight areas where research efforts are needed and are likely to contribute either to the development of new vector control strategies or to the improvement of the efficacy and affordability of existing approaches.

TSETSE GENOMIC STUDIES

The trypanosome has been most studied in efforts to develop a mammalian-based vaccine. While these research efforts have turned trypanosomes into a model eukaryote to study novel mechanisms of gene expression and cell biology, there are no effective products forthcoming for disease control in the foreseeable future. There are now genome sequencing programmes for the parasite, and the genome of the human host is being deciphered. In contrast, to date, only a handful of tsetse genes and proteins have been characterized. This is also in sharp contrast to the vast level of knowledge available for mosquitoes such as Anopheles gambiae, for which an international genome sequencing effort is now in place, and Aedes aegypti, where a similar genome project is currently being planned. The many molecular markers developed in A. gambiae have formed the basis of a rapidly increasing number of popula-
tion genetics and ecological studies. The availability of contemporary genomics information will provide us with essential tools for investigating parasite-host interactions as well as vector population structures. This information is central for vector-based control strategies to be effectively developed.

Tsetse Population Genetics

Population Structure

The recent availability of several microsatellite loci and their application to population analysis has revealed extensive genetic structuring in tsetse populations of the *morsitans* and *palpalis* groups at various geographical scales (Krafsur et al., 2000; Krafsur and Griffiths, 1997; Krafsur, et al., 2000; Luna, et al., 2001; Solano, et al., 2000; Wohlford, 1999). In the case of *G. palpalis gambiensis*, these populations were shown to transmit different species of trypanosomes with different efficiencies (Solano, et al., 2000; Solano, et al., 1997). Additional microsatellite loci and the development of efficient oligonucleotide primers are required to achieve the level of genetic resolution necessary to measure accurately the differentiation of taxa and populations and gene flow within and among them. In addition, randomly amplified polymorphic DNAs (RAPDs) have been used successfully to examine paternal lines of gene flow, an important tool in studying historical lines of dispersion (Malacrida, et al, 1999). These studies are important for vector control approaches such as the sterile insect technique (SIT), which is being considered for use in upcoming tsetse eradication campaigns. For the successful application of SIT, information is required on the tsetse species represented in the area, the degree of reproductive isolation among field populations, the existence of natural barriers to dispersion, and the locations of dense, stable populations from which immigration is likely.

**Hybrid Sterility**

Hybrid sterility has been considered as a potential control method for tsetse ever since Vanderplank eradicated an isolated population of *G. swynnertoni* by release of *G. m. centralis* into its habitat (Vanderplank, 1948; Vanderplank, 1944). In the two subgenera of *Glossina* that contain the most important trypanosome vectors, *Glossina sensu stricto* and *Nemorhina*, there are several groups of closely related taxa that hybridize readily both in the field and in the laboratory (Gooding, 1990). Hybridization of tsetse almost always results in production of hybrid females with lower than normal fecundity and males that are completely sterile (Gooding, 1990). The sterility in males is attributable mainly to incompatibility between sex chromosomes from two taxa, but autosomal genes are also involved in some cases. In the subgenus *Glossina*, but not in the subgenus *Nemorhina*, maternally inherited factors appear to be involved in hybrid male sterility (Gooding, 1990). Success in hybridizing members of the subgenus *Glossina* is often highly asymmetric, with one cross being significantly more productive than the reciprocal cross. Gooding (1987) attributed this asymmetry to maternally inherited factors, which are now believed to be *Wolbachia* (O’Neil, et al, 1993). These factors can lead to unusual situations. For example, using *G. m. morsitans* and *G. m. centralis* it has been possible to produce backcross males with maternally inherited factors from *G. m. morsitans* and chromosomes from *G. m. centralis*, and yet such males can fertilize *G. m. morsitans* but not *G. m. centralis* (Gooding, 1987). It was suggested that such flies could be used as genetic control agents against *G. m. centralis*, but the phenotypic expression of these maternally inherited factors is unstable (Gooding, 2000; Gooding, 1990). However, if the stability problem can be overcome, this approach may be useful for control of certain members of the subgenus *Glossina*.

Most hybridization studies on tsetse have used closely related species and subspecies. There have been few “intra-taxon crosses”, using flies from colonies that were established from widely separated geographic areas. Without exception, the latter studies found that F1 males were fertile and this has been interpreted as indicating that, within a nominal taxon, there are no genetic barriers between geographically separated populations. However, hybrid breakdown has recently been found in tsetse (Gooding, unpublished). When *G. p. palpalis* from colonies that originated from widely separated geographic areas were crossed, the first generation offspring had normal fertility, but a high proportion of males in the second and subsequent generations, and in backcrosses to the parental lines, were sterile. The molecular basis for the hybrid breakdown is unknown, but its elucidation will be most helpful if hybrid breakdown is to be exploited for tsetse control. Hybrid breakdown may be a useful genetic control approach for tsetse, especially if its manifestations include reduced fecundity of females and/or reduced longevity of adult flies. In any case, the existence of hybrid breakdown in tsetse suggests that there may be cryptic species of tsetse, a possibility that could have implications for evaluating what now appears to be simply geographic variation in vector competence. Further work with other widely distributed species is clearly needed.

**Polytene Chromosomes**

Polytene chromosomes have proven especially valuable in studies of chromosome structure and function. They provide a means for the accurate map-
ping of chromosome rearrangements and for the precise localization of genes by using both chromosome rearrangement analysis and the technique of in situ hybridization. This has been technically challenging in tsetse, but photographic polytene maps have now been constructed for three species, *G. austeni*, *G. m. submorsitans*, and *G. pallidipes*. Comparison of the homologous chromosomes between the three species indicates that, in addition to similarities in their banding patterns, there are also various major differences, especially between *G. austeni* and the other two species (Dr A. G. Papalexiou, University of Patras, unpublished). Studies can now be undertaken to identify and characterize chromosomal rearrangements in field populations to understand genetic variation. The development of in situ hybridization using FISH analysis with cloned tsetse genes and selected molecular probes such as microsatellites will facilitate the eventual mapping of genes of interest (e.g. genes affecting vector competence, refractoriness, etc.).

**VECTORAL CAPACITY OF NATURAL TSETSE POPULATIONS**

Trypanosome infections with *T. brucei* ssp. complex parasites are typically detected in less than 1% of the field population of tsetse flies (Lehane, et al, 2000; Msangi, et al, 1998; Woolhouse, et al, 1994). Even under ideal conditions in the laboratory, transmission rates are between 1-10% depending on the fly species/strain and parasite strain (Moloo, et al, 1994; Moloo and Kutuza, 1988; Moloo, et al, 1992). The basis for this refractoriness is not known, but depends on tsetse species/strains and their symbiotic bacteria as well as the genotype of the strain of the parasite acquired. In laboratory infections, the age and sex of the fly and the source of the blood-meal also contribute to the outcome of the infection. Field data are limited, however infections with multiple parasites are common, indicating that they were acquired in different blood-meals, hence fly age may not be as significant a factor as once thought.

The life cycle of *T. brucei* in the tsetse fly begins when it feeds from an infected mammalian host. The non-proliferating short stumpy parasites that are pre-adapted for life in tsetse fly rapidly differentiate into procyclic forms in the gut lumen, lose their variant surface glycoprotein, and express a new coat composed of procyclin proteins. The procyclin coat contributes to the establishment of infections in the fly (Ruepp, et al, 1997). It has recently been shown that procyclic cells express different procyclin coats during establishment in the gut and that the N-terminal domain of all procyclins are quantitatively removed by proteolysis in the fly, but not in culture (Acosta-Serrano, et al, 2001). It has also been shown that the binding of a lectin (concanavalin A) to the procyclin coat molecules of the procyclic forms induces multinucleation, a disequilibrium between nuclear and kinetoplast replication and a unique form of cell death (Pearson, et al, 2000). Those surviving procyclic cells eventually proliferate in the gut (establishment phase) and flies can be scored with infections seven-ten days after acquiring an infectious meal. The subsequent maturation phase occurs in the salivary glands for *T. brucei* (Vickerman, et al, 1988). Here, they first differentiate into attached proliferating epimastigote forms which then yield the infective, free-living metacyclic forms which are transmitted to the next host during blood-feeding by the fly (Vickerman, et al, 1988). It is at this stage that parasites are thought to undergo genetic exchange (Gibson and Whittington, 1993). The factors triggering this differentiation step are unknown. There is believed to be a critical period for maturation between days 8 and 11 after infection (Ruepp, et al, 1997; Sinkins, et al, 1995). One suggestion is that lectins have a role, since feeding lectin inhibitory sugars can block maturation (Sinkins, et al, 1995). However, it is clearly crucial to investigate the role of other components of the immune system.

The first physical barrier to ingested parasites in the gut is the peritrophic matrix (PM), which is a prominent feature of the digestive tract of insects. While in many adult insects, PM components are secreted by midgut cells in response to a blood-meal, tsetse adults have a PM constitutively synthesized from cells in the proventriculus (cardia) in the foregut prior to feeding (Lehane, et al, 1996; Lehane and Msangi, 1991). How trypanosomes cross the PM to move from the endo- to the ectoperitrophic space of the gut has been controversial, with penetration of the thick chitinous PM and migration around its open posterior end in the hindgut both having been proposed (Ellis and Evans, 1977; Welburn and Maudlin, 1999). It is generally thought that during normal development in the fly there are no intracellular stages, although reports of intracellular *T. b. rhodesiense* (Ellis and Evans, 1976; Evans and Ellis, 1975) and *T. congolense* (Ladikpo and Seureau, 1988) in anterior midgut cells have been published. It is also thought that during normal infection, trypanosomes do not cross an epithelial barrier in the fly, although once again there are several reports of trypanosomes in the hemolymph of flies (Mshelbwala, 1972; Otieno, 1973). Also unknown is how procyclic parasites move from the gut to the mouth parts and/or the salivary glands of the fly. The classical route involves crossing back into the gut lumen across the proventriculus in the foregut and thence to the mouthparts and salivary glands (Ruepp, et al,
This is indeed an incredible journey, which few trypanosomes apparently embark on or complete (Ruepp, et al, 1997). Presumably the increased length and motility of the trypanosomes observed en route are adaptations enabling them to succeed (Ruepp, et al, 1997).

**TSETSE VECTOR COMPETENCE**

At the center of vector competence in insects are immune reactions, which include a diverse set of mechanisms ranging from phagocytosis, activation of proteolytic cascades, such as coagulation and melanization and production of various antimicrobial peptides (Barillas-Mury, et al, 2000; Dimopoulos, et al, 2001). While much of this immune response is initiated in the fat body, effector molecules expressed in the midgut are increasingly being recognized as playing a role in immune reactions (Dimopoulos, et al, 1997; Dimopoulos, et al, 1998; Lehane, et al, 1997). Discrimination between pathogen groups is well known in *Drosophila* and mosquitoes, where it is explained by the use of different receptor/signalling pathways. Because dipterans including mosquitoes and tsetse are largely refractory to parasite transmission, much effort has gone into understanding the immune mechanisms of mosquitoes. If the genetic basis of this refractoriness could be understood, then flies might be engineered to completely block parasite transmission using various recombinant approaches currently being developed. Immunity genes are also being studied as they are strong candidates to confer such transmission blocking phenotypes for inducing refractoriness. As a result of the application of molecular approaches, a good deal of information is now available about the response of mosquitoes to *Plasmodium* species.

There is little known about the genetic basis for refractoriness in tsetse or the molecular basis for the wide ranging differences observed in parasite transmission rates of different tsetse species. Lectins, which are known immune molecules, might play a role in the attrition of trypanosomes during establishment (Welburn and Maudlin, 1999). There is also some information demonstrating antimicrobial activity (Kaaya and Darji, 1988; Kaaya, et al, 1987; Kaaya, et al, 1986), the presence of the phenoloxidase cascade (Nigam, et al, 1997) in hemolymph. Preliminary results indicate that the immune response to different pathogens in tsetse is specific. These results also suggest that trypanosomes may use unprecedented novel mechanisms to achieve their transmission through the fly by blocking the expression of some of its immune responsive genes early in the infection process (Zhengrong, et al, 2001). When the immune system is upregulated prior to an infectious meal, the transmission of trypanosomes can be significantly reduced (Zhengrong, et al, 2001). Further characterization of these immune mechanisms or products and their interaction with trypanosomes are needed. In addition, a study of other tsetse molecules, such as receptors involved in interactions with parasites, or molecules that are simply required for survival of tsetse or that influence their fecundity, will be important for developing strategies to interfere with transmission of trypanosomes. These studies are of applied interest as they support the development of strategies to block parasite transmission *in vivo* via transgenic approaches.

**TRANSGENESIS IN TSETSE**

Much effort has gone into developing genetic transformation systems for medically and agriculturally important insect vectors. There is no doubt that the availability of this technology will revolutionize insect genetics by allowing basic studies relating to the functional characterization of various genes and their products. It might also allow for the development of alternative control strategies such as the use of transgenic refractory insects. It is thought that such genetically engineered refractory insects can be driven into natural populations to replace their susceptible counterparts and hence reduce disease transmission. While the efficacy and feasibility of this strategy are being widely debated among scientists at large, in the case of tsetse, the development of trypanosome-refractory strains will have an immediate application for at least one effective control strategy, the sterile insect technique (SIT), as described below.

At the core of transgenesis is the process of genetic transformation, which in many insects relies on the microinjection of transposable elements that insert themselves into insect DNA, resulting in germ-line transformation. Marker genes carried by the transposable element help identify transgenic individuals. It has now been possible to introduce foreign genes into several important insect vectors including one important malaria vector in Asia, *Anopheles stephensi* (Cateruccia, et al, 2000), and others are likely to follow using similar technologies. Tsetse, however, have an unusual reproductive biology. There is no free egg stage, females retaining each egg within the uterus. Following hatching and *in utero* development of the larva, one young larva matures and is finally expelled as a fully developed third instar larva. Each female can deposit four-six offspring during its five-eight week average life span in the field. This viviparous reproductive biology would undoubtedly complicate any attempts to transform tsetse through egg microinjection. However, tsetse flies naturally harbor a number of symbiotic microorganisms that have been exploited to express for-
eign gene products (Aksoy, 2000). Through such an approach, the insect cells are not transformed as in germ-line transformation, but instead, foreign genes are expressed in the symbiotic bacteria. Since the symbionts naturally live in close proximity to the developing trypanosomes, anti-pathogenic gene products introduced and expressed in these cells could adversely affect trypanosome transmission.

**TSETSE SYMBIONTS AND EXPRESSION OF FOREIGN GENES IN VIVO**

Many insects with limited diets such as blood, plant sap or wood, rely on symbiotic microorganisms to fulfill their nutritional requirements. It has been shown that tsetse harbour three distinct microorganisms (Aksoy, 2000). Two of these are present in the gut tissue: genus *Wigglesworthia* (Aksoy, 1995; Aksoy, et al, 1997) and genus *Sodalis* (Aksoy, et al, 1995; Cheng and Aksoy, 1999; Dale and Maudlin, 1999) and both are closely related to *Escherichia coli*. The third symbiobiont harboured in certain tsetse species is *Wolbachia*, an obligate intracellular bacterium which is closely related to the genus *Ehrlichia* (O’Neill, et al, 1993). During its intrauterine life, the tsetse larva receives nutrients along with the two gut symbionts from its mother, via milk-gland secretions (Aksoy, et al, 1997; Ma and Denlinger, 1974), while *Wolbachia* is vertically transmitted by transovarial transmission.

It has been possible to culture the *Sodalis* symbiont in vitro (Beard, et al, 1993; Welburn, et al, 1987) and a genomic transformation system has been developed (Beard, et al, 1993). Recently, a homologous recombination approach has also been established so that foreign genes can now be directly inserted into the symbionts chromosome (Dale, et al, 2001). It has also been possible to reconstitute tsetse with the recombinant *Sodalis*, which has been found to be successfully acquired by the intrauterine progeny when microinjected into the haemolymph of the female parent (Cheng and Aksoy, 1999). It is now necessary to identify effective gene products which have anti-trypanosomal effects when expressed in *Sodalis* in tsetse midgut. Using a similar symbiont-based insect transformation approach, it has been possible to block the transmission of *Trypanosoma cruzi* in *Rhodnius prolixus* in vivo by expressing the antimicrobial peptide cecropinA (cecA) in its symbiont, *Rhodococcus rhodnii*, in the hindgut of the bugs (Durvasala, et al, 1997). It has also been possible to express a single-chain antibody gene fragment in the bacterial symbionts of *R. prolixus* (Durvasala, et al, 1999). If the tsetse immune-responsive molecules, which the trypanosomes apparently down-regulate to achieve their transmission, can be identified, they could be constitutively expressed in the symbionts to prevent parasite survival in the midgut. The identification of monoclonal antibodies (mAbs) with parasite transmission blocking characteristics and their expression as single-chain antibody gene fragments in the symbionts provides an alternative approach. Towards this end, several transmission-blocking antibodies targeting the major surface protein of the insect stage procyclic trypanosomes have already been reported (Nantu-lya and Moloo, 1988). Recently, midgut-specific monoclonals with transmission blocking activity have been characterized from *A. gambiae* (Lal, et al, 2001). It seems likely that similar molecules can be identified in tsetse and ultimately expressed in the gut symbionts. The relative ease of transformation and gene expression in bacteria, and the multitude of potential antiparasitic targets which can be explored, make this a desirable system for transgenic approaches. Should resistance develop in parasites against any of the expressed foreign gene products, it could be relatively easy to switch to express a different gene product. Alternatively, several target genes can potentially be expressed simultaneously in the symbionts to prevent the development of resistance against any one individual target.

**FIELD APPLICATIONS OF TRANSGENESIS**

In order to interfere with disease transmission, the eventual goal of any transgenic approach is to replace the naturally susceptible population with their engineered refractory counterparts in the field. At present, there are no proven mechanisms to achieve this spread. One powerful potential driving system involves the use of *Wolbachia* symbionts, which confer a reproductive advantage to their hosts, including the engineered females.

The functions of *Wolbachia* in their various hosts are variable. One common reproductive abnormality they induce has been termed cytoplasmic incompatibility (CI). This, when expressed, results in embryonic death due to disruptions in early fertilization events (Bandi, et al, 2001). In an incompatible cross, the sperm enters the egg but does not successfully contribute its genetic material to the potential zygote. In most species, this results in very few eggs hatching. *Wolbachia* infected females have a reproductive advantage over their uninfected counterparts because they produce progeny after mating with both infected and uninfected males. This reproductive advantage allows *Wolbachia* to spread into populations. In *Drosophila simulans* in the central California valley, a natural *Wolbachia* infection invading naive uninfected populations has spread at a rate of over 100 km per year simply through the expression of CI (Turelli and Hoffmann, 1991). To understand the functional role of *Wolbachia* in
insects, most insects can be cured of their Wolbachia infections by administering antibiotics in the diet. This approach, however, is not feasible in tsetse since antibiotic treatment results in the clearing of all bacterial symbionts, including the obligate symbiont Wigglesworthia, in the absence of which the flies become sterile. In order to study CI expression in tsetse, uninfected flies need to be collected from the field and colonized so that appropriate mating experiments can be performed in the laboratory. As Wolbachia infected insects replace naïve populations by virtue of the CI phenomenon, they can drive other maternally inherited elements of tsetse, such as the maternally inherited gut symbionts Sodalis, into that same population (Beard, et al, 1993). It has been proposed that multiple Wolbachia infections, in which an insect contains two or more different Wolbachia strains that are incompatible with each other, could be used to generate repeated population replacements or to spread Wolbachia into target species that already contain an existing infection (Sinkins, et al, 1995; Sinkins, et al, 1997). The analysis of Wolbachia strain types infecting different species of tsetse has shown that they are different, and as such represent independent acquisitions (Cheng, et al, 2000).

In addition to CI, certain Wolbachia strains, such as wMelPop characterized from Drosophila melanogaster, induce an age-shortening effect in their host (Min and Benzer, 1997). This age-shortening effect was reversed when infected flies were cured of their Wolbachia infections with antibiotics (Min and Benzer, 1997). It remains to be seen whether such Wolbachia infections can be documented in tsetse. It has also been possible to introduce Wolbachia into insect species which do not harbour natural infections, and these approaches can be pursued in tsetse. Since the T. brucei group parasites require 14-30 days to complete their developmental cycle in the fly, tsetse flies need to be at least this age to transmit disease. Reductions in the life span of individual flies might have a large effect on disease transmission in the field (Sinkins and O’Neill, 2000).

STERILE INSECT Technique AND TRANSGENIC INSECTS
SIT is a genetic population suppression approach and involves sustained, systematic releases of irradiated sterile male insects among the wild population. The sterile males fertilize wild females, which are then unable to produce progeny. By continually releasing sterile males in high numbers over a period of three-four generations, after having previously reduced the population density by other techniques (trapping, insecticide spraying, etc.), the target population can be eradicated (Politzar and Cuisance, 1984; Vreysen, et al, 2000). Improvements in two aspects of current tsetse SIT technology have the potential to enhance the efficacy of future programmes (Aksoy, et al, 2001).

The first is the development of parasite refractory strains. Since the large numbers of male flies released can potentially contribute to a temporary increase in disease transmission, the incorporation of refractory traits into the SIT release strains will greatly enhance the efficacy of this approach, especially in human disease endemic foci. In the current field SIT programmes, male tsetse are provided, before release, with a blood-meal containing a trypanocide; no infections have so far been found in released sterile males caught in traps. The second is the use of Wolbachia mediated CI, or hybrid sterility, as a method of inducing sterility - as an alternative to irradiation. With CI, the released strain of tsetse would carry a Wolbachia infection that would induce CI when males mate with wild females. The competitiveness of these males would be expected to be much higher compared with irradiated males, and, as a result, fewer insects would need to be released in order to achieve the same level of sterility in the wild population. This strategy is dependent on the use of a very efficient sexing system. If Wolbachia-infected females were released in sufficient quantities, then Wolbachia would have the opportunity to invade the target population, which would render subsequent releases ineffective. If it were impossible to guarantee extremely low quantities of released females, then it would be possible to incorporate low levels of irradiation with Wolbachia induced sterility to prevent released females from successfully reproducing. This approach has been successfully tested in Culex mosquitoes (Shahid and Curtis, 1987).

CONCLUSIONS
• Understanding the molecular/cellular basis of trypanosome transmission in tsetse is of fundamental significance, and will allow development of new applications for vector control. Since the symbiont-based transformation system can be used to express gene products in tsetse midgut, such will aid in the identification of candidate genes that can be expressed to confer refractoriness in tsetse.

• A research plan should be developed to coordinate information on expressed sequence tag (EST) sequences, genomic sequences and the physical map locations of selected genes. For EST analysis, the cDNAs to be sequenced can be obtained from tsetse exposed to infected blood-meals containing eukaryotic pathogens, including trypanosomes,
and from tissue specific (midgut, fat body and salivary glands) normalized libraries as well as from different developmental stages (larvae). The ESTs deemed to be of interest should be physically mapped to metaphase chromosomes using in situ hybridization (FISH) technology. A bacterial artificial chromosome (BAC) library could be constructed in order to obtain single pass sequences. Some of these resources are already available and these efforts would promote and foster collaboration and intellectual input from the international tsetse community. The availability of the complete and annotated D. melanogaster genome, and the anticipated genome projects for A. gambiae and A. aegypti, will constitute an important resource for gene discovery efforts in Glossina. It follows that a proteomics approach to protein identification will allow further exploitation of the genomic information. This aspect of molecular entomology will be a powerful approach in the post-genomic era.

Molecular approaches for population genetics stand to improve our understanding of vector populations and are of significance for the success of area-wide control strategies. These field-based studies could easily be coupled with efforts to better understand the prevalence and phenotypic effects of natural Wolbachia infections in tsetse.

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III APPLIED GENOMICS AND BIOINFORMATICS

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OVERVIEW OF THE T. BRUCEI GENOME AND THE HISTORY OF TRYPANOSOME GENOMICS

The African trypanosome genome network was formed by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) with a brief to coordinate the analysis and sequencing of the nuclear genome. It consists of research laboratories worldwide, contributing to different aspects of genome analysis. At the time of its formation, some aspects of genome structure and organization were known, especially from studies on antigenic variation and transcription, but only one group had actively initiated global genomic analysis. In the last five years, much has been learnt about the organization of the nuclear genome of T. brucei, which consists of at least eleven diploid megabase chromosomes (1-6 Mb), a variable number of intermediate-sized chromosomes of indeterminate ploidy (200-900 Kb), and 50-100 minichromosomes (25-100 Kb) (Melville, et al, 1998).

With the exception of some variant surface glycoprotein (VSG) genes, most expressed genes are located in the megabase chromosomes. These are inherited in a Mendelian fashion (Turner, et al, 1990) and have been assigned Roman numerals in order of increasing size in TREU927/4 (Turner, et al, 1997). Many, perhaps most, genes are expressed in cistronic transcripts (Vanhamme and Pays, 1995). Some chromosome ends carry VSG expression sites: only one is active at one time, resulting in a uniform surface protein coat. Gene- or promoter-switching results in antigenic variation (Barry and McCulloch, 1996), and was used to determine the structure of a megabase chromosome (Melville, et al, 1998). Despite remarkable genomic plasticity (Melville, et al, 1999), studies reveal a significant level of conservation of genome structure. Initial apprehension that the genome was so polymorphic as to make sequencing of one strain of little use for cross-comparison to other strains has been assuaged – although further studies could investigate the role this plasticity is playing in the survival strategy of the trypanosome.

Most megabase chromosomes differ in size from their homologues by up to 15%, but homologous chromosomes in different stocks vary more - considerably more than reported in other organisms. Nevertheless, mapping studies show that syntenic groups are maintained in all stocks studied (Melville, et al, 1998). Despite remarkable genomic plasticity (Melville, et al, 1999), studies reveal a significant level of conservation of genome structure. Initial apprehension that the genome was so polymorphic as to make sequencing of one strain of little use for cross-comparison to other strains has been assuaged - although further studies could investigate the role this plasticity is playing in the survival strategy of the trypanosome.

There is no perfect isolate that should form the basis of all molecular experiments on T. brucei. It is indeed preferable that some genomic analyses are carried out on multiple stocks, for example karyotyping and random gene sequencing. However, we can only aim to sequence one complete genome in the immediate future. T. brucei stock TREU927/4 (GPAL/KE/70/EATRO1534) was cloned from a population of trypanosomes isolated from a tsetse fly in Kenya and was chosen as the reference genome for a variety of reasons, some optimal and some pragmatic. The original stock exists and the history of its isolation is documented. It was not isolated from an infected human, but laboratory tests indicate that it has intermediate resistance to human serum (CMR Turner, personal communication). It is pleomorphic and may be replicated as the bloodstream or procyclic form in laboratory animals, tsetse flies or culture. It has been used as a parent in laboratory-controlled genetic crosses with other stocks, and cloned hybrids have been isolated (Turner, et al, 1990; Tait, et al, in press), allowing the creation of a genetic map. A high quality, arrayed genomic library of the megabase chromosomal DNA of TREU927/4 already existed (Melville, et al, 1996), and was used to determine the structure of a megabase chromosome (Melville, et al, 1999). Participation in the network is dynamic because, as priorities change, researchers with different skills are required. Many contribute without direct funding, and the network is dependent on the support and active participation of the research community. It has been vital to promote universal commitment to the idea that this huge task can progress most efficiently if the community works together with open sharing of data and resources.
The availability of the DNA sequence will benefit many research programmes. Some benefits are already obvious, some will only become clear as more researchers begin to use the available data. This is our task: to be forward-looking and imaginative in our plans for the use of this tremendous resource.

CURRENT STATUS OF THE TRYPANOSOMA BRUCEI GENOME PROJECT

Expressed Sequence Tags (ESTs)

Rapid gene discovery was achieved in the early phase of the genome project by sequencing of randomly selected cDNA clones (El-Sayed, et al, 1995; Djikeng, et al, 1998). At the time of writing, there are 5133 T. brucei ESTs in the public databases from four cloned stocks of T. brucei (dbEST: http://www.ncbi.nlm.nih.gov/dbEST/index.html). Most EST sequences were generated from cDNA clones of bloodstream-form mRNA. There has been no concerted effort to produce large EST datasets from each of the life-cycle stages as it was decided to divert effort and resources to sequencing of genomic DNA. The determination of life-cycle stage-specific expression will be undertaken by other methods (see section 3.5). In addition to discovery of many novel T. brucei genes, the ESTs have provided a rich source of markers (Melville, et al, 1998) and aid sequence annotation.

Genome Survey Sequences (GSSs) for Gene Discovery

In a pilot project, approximately 500 random genomic clones were sequenced (El-Sayed and Donelson, 1997) to show that this led to equally efficient gene discovery, due to the lack of introns and the close spacing of genes in the T. brucei genome. Therefore, it was decided that a portion of the funds obtained for high-throughput sequencing at The Institute for Genomic Research (TIGR) should be allocated to sequencing of random, short pieces of DNA (GSSs). Sequencing of both ends of almost 25000 clones (almost 50 000 short sequences) provided a total of 29 Mb of the TREU927 genome (http://www.tigr.org/tdb/mdb/tbdatabase/status.html). A proportion of these sequences derive from the ends of large genomic clones (in bacteriophage P1 and bacterial artificial chromosome (BAC) vectors), and these contribute to the mapping and sequencing of whole chromosomes (see section 2.4) by providing paired markers of about 500 base pairs every 2.5 Kb across the chromosomes (excepting the minichromosomes). Many researchers have reported finding T. brucei homologues of known genes in the GSSs, and this generated great enthusiasm for the rapid provision of more such sequences. In response to requests from the community, the Sanger Institute has provided a further 47 000 single-pass sequence reads to aid gene discovery and facilitate the completion of contiguous chromosome sequences (http://ftp.sanger.ac.uk/pub/databases/T.brucei-sequences).

The EST and GSS sequences have been clustered by collaborators at the Sanger Institute to provide contigs. In total, 96 474 sequences (~45.87Mb) were used for the clustering, achieved using the sequence assembly programme Phrap - 12 251 contigs were generated and 8242 sequences could not be placed in a contig (singletons). One of the contigs has 1926 constituent members (contig length 9.342Kb), but this is probably due to repeated DNA and is an example of the care that must be taken in interpreting these automatically generated data. The cluster data are available as a searchable subcomponent of the T. brucei BLAST server (http://www.sanger.ac.uk/Projects/T_brucei_Toolkit/blast_server.shtml) and from the ftp site as a gzip file (http://ftp.sanger.ac.uk/pub/databases/T.brucei_sequences/GSS/clusters).

Physical and Genetic Mapping

One chromosome was mapped thoroughly and to completion (Melville, et al, 1999) prior to submitting sequencing grants. However, rapid progress in methods for completion of genome sequences has reduced the requirement to produce prior contiguous physical maps of each chromosome (El-Sayed, et al, 2000). Nevertheless, mapping information is required for seeding BAC sequencing and to aid final reconstruction of the chromosome. Many hybridization data have been amassed by the Cambridge group and also by the community through using centralized resources. So far, these data have been made freely available but only by email request. A database is being established to make the data web-accessible (see 4.4).

TDR and The Wellcome Trust have also supported the creation of a genetic map using classical genetic analysis of F1 hybrids (Tait and Turner, Glasgow). F1 hybrids have been isolated following simultaneous passage of genotypically distinct stocks through tsetse flies. This group has developed amplified restriction fragment polymorphism (AFLP), mini- and micro-satellite markers for use in genetic analysis and, together with the genomics group (Melville), has been able to combine some of the data with chromosome maps. The aims are to determine crossover frequency, estimate the physical size of the recombination unit (Centimorgan), and investigate variation in crossing-over in different genomic regions. Initial data of this type indicate that it is indeed feasible to obtain sufficient hybrid progeny and genetic markers to aim towards using a genetic
map for positional cloning of genes underlying complex phenotypes (Tait, et al, in press).

Genomic Sequencing
Sequencing of chromosome I of T. brucei strain 927/4 GUTat 10.1 commenced at the Sanger Institute in 1998. Sequence was obtained from shotgun clones of chromosomal DNA eluted from a pulsed field gel (PFG) (> 8 X coverage) and from mapped P1 clones (1 X coverage), a combination of methodologies pioneered by the malaria sequencing consortium. While approximately 75% of the chromosome sequence was contiguous by year two, 25% of the chromosome has proved difficult due to repetitive DNA: genes in VSG expression sites, gene families, retrotransposons, and some tandem repeats (Melville, et al, 1999). Following the random sequencing phase (see 2.3), TIGR commenced the sequencing of chromosome II in 1998. This sequence is derived from BAC clones, mapped to chromosome II using cDNAs from the EST project (Melville, et al, 1998; El-Sayed, et al, 1995) and sequenced to ca. 7 X coverage. The end-sequences determined in the first phase of the project (section 2.2) allow the selection of BACs with minimum overlap for maximum efficiency. The progress on chromosome II has been similar to that on chromosome I, with similar problems in regions of clustered multicopy sequences. Chromosomes IV and VI are currently approximately 75% completed at TIGR. Preliminary (and therefore in parts inaccurate and incomplete) annotation is provided by the sequencing centres prior to completion. It is best to follow progress by regularly monitoring both sequencing centre websites (http://www.sanger.ac.uk/Projects/T-brucei/; http://www.tigr.org/tdb/mdb/tbldb/index.html).

The funds to complete chromosomes IX, X and XI were awarded to Barrell (Sanger Institute), Melville and the network in 2000. Chromosome X shotgun sequence is now available (http://ftp.sanger.ac.uk/pub/databases/T.brucei-sequences) and chromosomes IX and XI are in library preparation. The funds to complete chromosomes III, V, VII and VIII were awarded to El-Sayed at TIGR (collaborators Donelson, Ullu, Melville) in 2001. Each sequencing centre will therefore provide approximately 50% of the megabase chromosome DNA sequence. The projects run until 2004 and all the sequence is likely to be in the databases by 2003 at the latest; however it cannot be foreseen how long it will take to complete contiguation.

To make full use of the substantial investment in genome sequencing it is necessary to complete the task, to ensure there is complete information on all enzyme pathways. It is also absolutely imperative to invest substantial effort into bioinformatics to make the data accessible and informative, and to stimulate new ideas in the search for novel approaches to combat African trypanosomiasis.

Access to Data
The fields of genomics, databases and bioinformatics are dynamic and researchers may find it difficult to learn how best to access the most recent data, or where to find the most innovative new programmes for analysis. The field is developing rapidly and here we aim to provide an outline of where data may be found at different stages of the sequencing projects. Complementary DNA (cDNA) sequencing has been carried out in individual research laboratories and the sequences are made available via the database for expressed sequence tags (dbEST) at the National Center for Biotechnology Information (NCBI). All genomic sequence data are made available immediately via the websites at the respective sequencing centres (TIGR and Sanger Institute). These are single-pass sequences and, although no error correction or annotation are offered at this stage, this is an important resource for researchers who are looking for genes in T. brucei that have homologues in other organisms. The clustering data provided by the Sanger Institute are also generated automatically and any errors will be incorporated. Therefore, use of such data always requires verification by the researcher. Search engines are provided, allowing researchers to look for sequences with high similarity to the gene they seek (http://www.ebi.ac.uk/blast2/parasites.html; http://www.tigr.org/tdb/mdb/tbldb/seq_search.html). Data on significant similarities to genes in the databases are provided but researchers should note that new information becomes available in the central databases (European Molecular Biology Laboratory, EMBL/GENBANK/Database of Japan, DDBJ) all the time, and should check when the most recent BLAST searches were performed. At intervals, sequences are submitted in batches to the public databases at the European Bioinformatics Institute (EBI) and NCBI. Random genomic shotgun sequences and end-sequences of genomic clones are submitted to the database for genome survey sequences (dbGSS) (http://www.ncbi.nlm.nih.gov/dbGSS/index.html). Sequences of shotgun clones derived from whole BACs are submitted to the database for high-throughput genomic sequences (dbHTGS) (http://www.ncbi.nlm.nih.gov/dbHTGS/index.html) in three stages (at 3 X and 7 X coverage, and at completion). All these data will eventually be mirrored in the GENBANK, EMBL and DDBJ databases, ensuring that all available T. brucei sequences may be found in a single database. However, there are some advantages in looking at the data in the specialized genome data-
bases, as annotation is more extensive and sequence similarity assignments are provided.

On completion of a chromosome sequence, the most careful annotation is carried out. Great emphasis is laid on ensuring the sequence is accurate and contiguous (some areas of ambiguity may be tolerated in the final sequence, if too many resources are required to provide final clarification), and on trying to identify all open reading frames (ORFs). Researchers should be aware that, however careful the annotation, some protein coding genes may not be predicted from sequence analysis alone and a few of those predicted may not be transcribed. The analysis of the chromosome is published in a peer-reviewed journal and the sequence, with full annotation, can then be viewed with the Entrez browser at NCBI (http://www.ncbi.nlm.nih.gov/Entrez/Genome/). The sequencing centre responsible for the sequencing of the chromosome also places the same data on its website.

In an ideal world it would be possible to carry out searches of all data at a single site but the curation of such a database would necessitate delay in access to the data. Immediate access to raw, unfinished sequence is highly prized by a research community waiting impatiently for gene sequences, and the sequencing centres have responded to this need. This is only really possible via deposition on home websites prior to batch submission to NCBI. It is therefore important for researchers to consider which databases contain the most valuable data (e.g. the public genome databases because of their extensive annotation, or the sequencing centre databases because they may contain sequences not yet processed for transfer to NCBI) and, in some cases, to perform searches at several sites. Long-term curation of interrelated genomic and functional data is a different consideration (section 3.1).

**Summary and Predicted Timeline.**

The following sequence data are available in 2001:

- 513 EST sequences, providing approximately 2000+ unique genes.
- 96 474 GSS sequences (~45.87Mb), including 10 990 BAC and PI end-sequences.
- Clustering data of all GSS and EST sequences, with data on significant similarities.
- The complete sequence of chromosomes I and II (to be fully annotated in 2001).
- 75% of chromosomes IV and VI as complete BAC sequences.
- Chromosome X shotgun sequence (04/01); chromosomes IX and XI shotgun sequence to follow.

Predicted timeline to 2002-2004:

- 1 x sequencing of IX, X and XI-specific BACs.
- Chromosomes III, V, VII and VIII to be sequenced BAC by BAC and appearing in the databases piecemeal over the next two years.
- Contiguity and annotation of chromosomes III – XI over at least three years.

The timeline to complete sequencing is the most difficult to predict, and depends to some extent on the determination to complete subtelomeric regions, VSG arrays, etc.

**CURRENT STATUS OF FUNCTIONAL AND APPLIED GENOMICS PROJECTS**

The African trypanosome genome network has initiated discussions on the “post-genomic” agenda. This requires careful coordination and the involvement of appropriately skilled researchers. At the 1999 and 2000 network meetings, lists of priorities drawn up (http://parsun1.path.cam.ac.uk/network.htm) included microarrays for transcription analysis, techniques for gene knockouts, generation of mutants, RNAi and phenotype characterization, and use of the genetic map for positional cloning of genes (some to proceed in collaboration with other kinetoplastid genome networks). Recommendations of interest to this discussion include identification of trypanosome-specific genes essential for infection and development, characterization of molecular structures and parasite-specific metabolic pathways, elucidation of unique trypanosome-specific mechanisms of gene regulation and RNA processing, and analysis of the protein profile of the parasite to identify functionally important genes (proteomics). Perhaps one or more of these approaches could lead to the identification of novel drug targets, and/or vaccine candidates, but only if those researchers skilled in, and committed to, their development join the global collaboration and contribute to the post-genomic projects of the future.

**Sequence Analysis and Relational Databases**

The Wellcome Trust Functional Genomics Initiative has provided funds to create a trypanosome database (genome databases for *Schizosaccharomyces pombe*, *Leishmania major* and *T. brucei*) to Drs B Barrell, Sanger Institute; M Rajandream (*S. pombe*); M Carrington and S Melville (*T. brucei*). These databases will contain all genomic information, e.g. primary sequence annotation from the sequencing centres, secondary (ongoing) sequence annotation, references, expression data, protein characterization, knockout and RNAi phenotype analyses. They will be relational databases using SQL (structured query language) and hosted by the Sanger Institute. The *T. brucei* database will
be developed in close collaboration with TIGR, and is overseen by a management committee. Genome databases of this type are long-term projects, providing a single site to review ongoing genomic and functional genomic analyses long after sequencing of the reference strain is complete, and as such serve a different function to the rapid access sequence deposition on the sequencing centre websites or the parasite genome BLAST server (see 2.5). News on progress will be posted on relevant websites.

The importance of ensuring that this database is readily available to African scientists hardly needs explaining - they should not need to rely on collaborations with laboratories in Europe or the Americas to gain direct access to such comprehensive data. In the long-term, the enabling of molecular biological research on trypanosomes will require that African laboratories have secure Internet connections of wide band-width. Until such time as this is possible, we should consider providing CD versions of the database at regular intervals. This may be proposed to the database committee via Dr Melville, and will require some coordination.

Bioinformatics Projects
The field of bioinformatics is changing rapidly and the databases described here will not remain static. NCBI, EBI, the sequencing centres (and many others, including academic institutions) are actively developing better analysis tools, and it is necessary to watch their websites for innovations and developments.

In addition, there is certainly scope for specific bioinformatic analysis projects, either based on the examples from more advanced genome projects (yeast, *C. elegans*) or on novel approaches based on parasite-specific interests. For example, investigation of the components of metabolic enzyme pathways (requiring skilled biochemists, but also novel bioinformatics approaches to reduce the time required for the largely manual approach currently used); comparison of metabolic pathways to the homologous pathways in humans and livestock (again requiring biochemists and informaticians); searching for commonalities in the biochemistry of the kinetoplastid parasites, that differ from the mammalian host; analysis of targeted or global/single-pass comparative sequencing of other trypanosome strains and species.

Biological Characteristics of the Reference Strain
A minimally culture-adapted line of the original stock TREU927/4 has been generated with greater stability of variant antigen types (TREU927/4 GUTat 10.1). This grows to a higher density in culture (M Turner, personal communication). The karyotypes are identical. The P1 library is prepared from 927/4 and the BAC library from 10.1. The primary sequencing substrate is 10.1 procyclic genomic DNA.

Stock 927 has the smallest nuclear genome of all *T. b. brucei* or *rhodesiense* stocks examined so far with approximately 10 Mb less DNA (Melville, et al, 1998), reducing the amount of funding and sequencing required quite considerably. It is capable of completing the life cycle, indicating that all vital genes are present and it is likely that much of the extra DNA consists of expansion of multicopy DNA – gene families and repeats. Nevertheless we must always remember that trypanosomes are phenotypically variable, and that the basis of this variation must lie in the genome. It will require thought and innovation to find methods to compare the genotypes of phenotypically variable strains.

Both 927/4 and the derivative 10.1 may be transformed with foreign DNA at the procyclic and bloodstream-form life cycle stages. Transformation of the bloodstream-form is less efficient than in strain 427, but this is common to most strains that have not been replicated *in vitro* for long periods. A line of GUTat10.1 expressing the tetracycline (TET) repressor has also been produced and tested. This is useful for any experiment involving inducible expression or inducible ablation of transcription/translation, and this line is currently available from Professor Christine Clayton, Heidelberg (van Deursen, et al, 2001).

As stated in the introduction to this section, stock 927/4 is found to be ‘intermediate’ in its resistance to lysis by human serum (Turner, personal communication). The SRA (serum resistance-associated) gene has been found in the genome databases (Pays, personal communication), although it is not yet known if it is expressed in the correct form. Therefore, bearing in mind that *T. b. brucei* and *T. b. rhodesiense* may only be genotypic variants of a single species and that the basis of serum resistance may not lie exclusively in SRA expression, it should be assumed by all researchers using 927/4 and its derivatives as bloodstream forms in experiments that there may be human-infective cells in the population.

Transformation Technology
Transformation technology for genetic analysis of *T. brucei* is in routine use (Clayton, 1999). There are multiple vectors, several types of transformation markers (drug resistance, fluorescence) and a system of inducible expression. Foreign DNA inserts
into the genome by homologous recombination and this can be precisely targeted if the exact sequence of the target site is known or determined, as recombination occurs preferentially at sites of exact homology. Expression from episomal vectors is less successful. At Tri-Tryp 2000, there was some discussion of the need for technology development. There was a proposal that enhanced transformation efficiency of bloodstream forms is one requirement for high-throughput analysis (e.g. of gene knockouts or RNAi mutants) of the mammal-infective form, as transformation of the procyclic forms (which is more efficient) followed by transformation of the cells to bloodstream forms requires passage through the tsetse fly. Of course, the ability to culture other life cycle stages, e.g. metacyclic forms, would also represent a considerable improvement in the tools available for genetic analysis.

**Microarrays**

Some doubts have been expressed regarding the usefulness of expression analysis in the Kinetoplastidae. The observed polycistronic transcription suggests that control of gene expression is entirely post-transcriptional, a possibility supported by reports of protein products of genes within the same polycistrons that were found to be up/down-regulated at different life cycle stages. Nevertheless, there clearly is some life cycle stage-specific transcription and this is useful data for investigation of processes such as infectivity, etc. It is easy to obtain large amounts of cultured procyclic RNAs from many strains, but perhaps prudent to perform some experiments to compare cultured procycling to those extracted from tsetse flies. It is possible to obtain sufficient RNA from bloodstream forms replicating in laboratory rodents, although slow-growing (e.g. less virulent) strains can prove more problematic (but the rate of improvement in microarray techniques is still in the exponential phase). Unfortunately, it is very difficult to obtain sufficient DNA from epimastigotes or the infective metacyclic forms. The metacytic life cycle stage is arguably the most relevant for studies of infectivity, and for vaccine development. With the involvement of researchers with tsetse fly colonies, it may be possible to perform some careful experiments with metacytic DNA after full optimization of the array technology.

Still, for the examination of wild-type/reference strains, it remains necessary to carry out specific experiments that detect changes in levels of individual mRNAs after processing of polycistronic transcripts, and to compare these to the concomitant levels of the protein products. This will provide data on the relative importance of post-transcriptional and post-translational controls on gene expression, and will provide a firmer basis for the design of many experiments. For example, if transcription levels are found to be relevant in at least some cases, microarray technology could be very useful to compare transcription in strains of different clinical phenotype, or to examine mutant strains, to observe not only the ablation of the targeted transcript but also any “knock-on” effect on other genes. For now, the discriminative power of microarray technology in some respects remains unproven. It also remains an open question whether an array based on a single strain (927) is sufficient to detect all relevant differences; it is possible that strains carry environment-specific genes and that strain 927 lacks the genes of interest. Nevertheless, if arrays are made available for use by the community, such preliminary (“look-and-see”) investigations could be carried out at little cost. No doubt as production costs become less prohibitive, the number of strains from which arrays are available will increase.

The materials currently available for the creation of standard DNA-spot microarrays are the ESTs and GSS clones. The ESTs almost all derive from bloodstream forms. Two GSS libraries have been used: one with average inserts of 2 Kb and the other with inserts of 4 Kb. The GSS libraries may prove as useful as EST libraries for microarray analysis due to the high gene density in the genome, and they are certainly more representative of all the genes in the genome than are EST libraries. However, the clones may contain fragments of more than one gene, thus complicating full analysis of the dataset. The usefulness of GSS arrays is being investigated.

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An additional aim of the network is to develop standard operating procedures for microarray analysis and presentation of data in collaboration with other organizations, including the EBI, where they are establishing a public repository for microarray-based
gene expression data (http://www.ebi.ac.uk/array-express/). These organizations are, in turn, members of the Microarray Gene Expression Database Group (MGED), whose aims are to facilitate the adoption of standards for DNA-array experiment annotation and data representation, as well as to introduce standard experimental controls and data normalization methods (http://www.mged.org/).

Proteomics
Proteomics is a rapidly developing field that encompasses small-scale 2-D gel analysis to high-throughput techniques such as mass spectrometry that are not available to all. Analysis of the T. brucei proteome is ongoing in several laboratories (e.g. Wellcome News issue 26, Q1, 2001, p. 19). However, for a coordinated network approach to global proteomic analysis, it is necessary to develop standard operating procedures and common marker proteins before any proteomic data are presented on the web. This was discussed at some length at Tri-Tryp 2000, and the first step towards this goal in the T. brucei network is to combine proteomic and array analysis as discussed in section 3.5.

Novel DNA-Based Markers for Epidemiology, Diagnosis and/or Positional Cloning
DNA-based markers have become increasingly important in epidemiology and diagnosis in the last decade. The availability of genome sequence offers the capacity to identify a whole range of new markers (microsatellites, minisatellites, single nucleotide polymorphisms or SNPs, indels) at much greater efficiency than before. Previously, satellite markers were identified by cloning and hybridization of individual oligonucleotides, or by serendipity, whereas now they are identified using informatics. The identification of SNPs and indels requires sequence from different strains, but may provide the advantages of isoenzyme analysis – stability over millennia - at less cost in terms of money and researcher time. There is no doubt that numerous research groups are considering using the DNA sequence in this way, and we may see more papers appearing using recently identified polymorphic markers.

It seems, then, timely to suggest that these data could usefully be collated at a central site rather than leaving individual researchers to draw up their separate lists from the literature. This may also prove especially useful to scientists in institutions with less access to journals. This is not to subvert the use of publication, which is vital to all researchers, but rather to suggest that, on publication, all markers should be submitted to a central database and that this database should be available to all – on the web and, if necessary, on CD (see section 3.1). Such a database could include:

• unique marker name/identifier.
• type of marker.
• oligonucleotide primer sequences.
• polymerase chain reaction (PCR) parameters.
• size(s) of PCR product in genome project reference strain 927.
• brief description of population studied and results.
• list of allele variants in named isolates with details of isolate history.
• references.
• coordination with physical mapping/sequencing data to provide information on genomic location, etc.

Over time, sufficient information should accumulate to facilitate the choice of suitable markers for given areas and defined experiments, without extensive literature searching or unnecessary collaborations.

RNA Interference as a Method to Investigate Gene Function
A common approach to the investigation of gene function is to remove the gene from the genome (gene knockout) or to prevent its transcription and translation. Gene knockout requires two rounds of transformation and gene knockout, as each gene is present as diploid alleles. RNAi functions by transformation of a plasmid encoding a double-stranded RNA that is homologous to part of the transcript under study. By an unknown mechanism, the presence of the exogenous dsRNA prevents the translation of the endogenous mRNA (Ngo, et al, 1998). This requires only one round of transformation and is useful for the analysis of function of single-locus and multi-locus genes. To date, RNAi is most successful in procyclic forms, although many groups are now working to improve efficiency in bloodstream forms.

A group of UK researchers (M Field, Imperial College, et al) has recently been awarded a grant from the Wellcome Trust Functional Genomics Committee to begin analysis of gene function in T. brucei by RNAi. This will involve analysis of phenotype following systematic disruption of individual genes identified on the sequenced chromosomes I and II. It is the requirement of these types of grants that both the data and the biological resources are made available to the community. The data will be web-accessible and prepared in collaboration with the functional genomics database (see section 3.1). Again, it may be necessary to consider how this information may be accessed from areas with insecure access to the Internet. The DNA resources (con-
The mass of genomic sequence provides the primary data for discovery of new proteins and new metabolic pathways. Initially, we will be faced with thousands of novel genes for which we have no function. At this stage, bioinformatic analysis (of metabolic pathways, for example) and systematic (preferably high-throughput) analysis of gene function (as attempted in the RNAi analysis described in 3.8) will be important. We would expect that these kinds of analyses will provide new leads for biochemists to investigate further at a molecular level and/or for design of high-throughput combinatorial screening programmes. However, the latter will require funding for the considerable work involved in such analyses, even in the preliminary stages.

• At this stage, the retention of such work in the academic sector due to lack of interest from pharmaceutical companies could be exploited to ensure that such studies are coordinated, data shared, basic functional data all web-accessible, and effort is not wasted. (Although, future problems with intellectual property rights have to be considered.)

• The establishment of the genome network has provided one paradigm of how the research community can pull together to collaborate rather than compete. There are already well-established groups with an interest in increasing funding for the discovery of new drugs for diseases of the poor, e.g. TDR working groups, the Access to Medicine Campaign. Is it now possible to bring (sections of) these disparate groups together to discuss a new “network”?

• Currently in the UK there are possibilities to apply for funding for “functional genomics”, a fundamentally different activity to basic, hypothesis-driven research. Are funding agencies in other countries initiating such programmes? Is this a good moment to use these openings to consider an “applied genomics” application, aimed directly at drug discovery? If Wellcome Trust-based, this would involve UK researchers, but the Trust recognizes the need to involve international groups.

• It is possible that an early-stage drug would be more attractive to manufacturers if it were useful against several parasites, for example all kinetoplastids. Some effort should be put into bioinformatics comparison of kinetoplastid genomes as they become available. This could provide information on common parasite pathways that differ substantially from those found in humans and mammals.

The following observations need critical assessment:

• The mass of genomic sequence provides the primary data for discovery of new proteins and new metabolic pathways. Initially, we will be faced with thousands of novel genes for which we have no function. At this stage, bioinformatic analysis (of metabolic pathways, for example) and systematic (preferably high-throughput) analysis of gene function (as attempted in the RNAi analysis described in 3.8) will be important. We would expect that these kinds of analyses will provide new leads for biochemists to investigate further at a molecular level and/or for design of high-throughput combinatorial screening programmes. However, the latter will require funding for the considerable work involved in such analyses, even in the preliminary stages.

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Applied Genomics: Discovery of Novel Drug Targets

The authors are not experts in drug target validation and, to avoid charges of naivety, first wish to acknowledge the considerable work by leading groups of biochemists on parasite-specific pathways that may represent valid targets for design of drugs of greater specificity. No doubt such projects will be brought to the discussion table by other contributors. However, under the maxim that no single method guarantees the discovery of a better drug, and that all methods (rational design and global approaches) have potential for both success and false leads, we wish to pose the questions: is it necessary to expand our efforts now to increase the number of leads on the table? and how can we best use the limited funds available? This discussion needs to take place across a wide range of researchers with different skills. As observed by members of the pharmaceutical industry when faced with the mass of new genomic information, we may yet need further breaking down of the barriers between scientific disciplines (Browne and Thurlby, 1996).

“Until recently genes have been cloned and expressed usually as the result of targeted programmes of research, their biochemistry and pharmacology evaluated, high-throughput mechanism-based screens devised and leads identified for optimization by chemists ... In this traditional model of pharmaceutical research, gene identification had often been considered rate limiting. The paradigm has shifted.... We need to be able to predict which genes may be useful and why... there are potentially many, many more proteins to work with (and) the abundance of opportunities (must) impact on conventional research strategies” ... (extracted from Browne and Thurlby, 1996).
obvious how the availability of genome sequence and the discovery of novel genes and pathways may provide new approaches, given these results. The only high-throughput parasite vaccine testing project known to the authors is that of Professor J Blackwell, who is testing pooled DNA vaccines against leishmaniasis. All the resources for such a project applied to infection with T. brucei are available, but any assessment of applicability should involve a wider range of researchers with appropriate skills.

SHARING OF INFORMATION AND ACCESS TO DATA AND RESOURCES: SUMMARIES

The T. brucei Genome ‘Network’

It can seem difficult to obtain information from, or to know how to “join”, the genome network. This is not in any way due to exclusivity as such, but because it works as any other network – people who know each other and who have common interests come together in subgroups to seek funding. Inevitably this is also driven by the availability of suitable funding for functional/applied genomics in different countries – for example, the Wellcome Trust (UK) is currently the most forward planning in providing funds for global analyses, reducing its insistence on hypothesis-driven research etc., so, predictably, recent groupings have been UK-driven. For this reason it is very important that WHO continues to direct some funds towards helping those African scientists with an interest in this work to travel to network meetings, to make contacts, to set up collaborations and to talk about priorities. This latter point is very important: there are many very good biologists working on the fascinating basic biology of T. brucei, but their priorities may differ somewhat from those of the endemic countries and only the presence of outspoken scientists whose interests lie in controlling the disease will ensure that we do not lose sight of the necessity to direct funds towards disease-applied genomics.

Obtaining Information

Until now, information dissemination has been underfunded and very dependent on the commitment of certain individuals to spreading the word, maintaining websites, and being approachable and amenable to email requests for information and explanations. But it is increasingly recognized that collected information and properly run databases are becoming vital in our brave new world of collaborative “big science”, and as more resources are made available towards these aims, it should become easier to obtain up-to-date information on the progress of genome network-based projects. However, it is also inevitable that these information resources will be web-based. It is therefore vital to consider now how we can ensure that the entire research community has access to information. In the near future this may necessitate distribution of CDs, in which case how should this be coordinated? In the more distant future, WHO and those institutions who see a role for such web-based information must seek a path to improved institutional infrastructure and electronic resources.

Access to Unannotated and Annotated Sequence Data

All raw unannotated sequence data are made available immediately on the websites of the sequencing centres, and then in the public databases. However, access to annotated data is more problematic. The Sanger Institute is unable to annotate fully until close to completion of a chromosome, due to their shotgun approach. Their provision of preliminary annotation of chromosome I caused considerable confusion as researchers did not fully appreciate that the chromosome was discontinuous. TIGR is able to provide some annotation of completed BACs as they are finished, but this approach does not provide as many raw sequences as early on in the project. This problem is unresolved and subject to much discussion at network meetings. One interim solution is provided by the clustering and BLAST analysis of discontinuous sequence data (GSS, shotgun, ESTs) (http://www.sanger.ac.uk/Projects/T_bruc ei/Toolkit/blast_server.shtml; http://ftp.sanger.ac.uk/pub/databases/T.brucei_sequences/GSS/clusters/). Many researchers annotate the small regions of immediate interest to them, but for some scientists this requires some bioinformatics training. We hope that some of these problems will be addressed by the creation of the relational genome database.

Access to Comprehensive Genome Information in Relational Databases

As stated many times above, access to all databases currently under development will ultimately require secure access to the Internet, although provision of CDs may be an interim solution. In addition to the genome database to be hosted at the Sanger Centre (section 3.1), a separate Wellcome Trust-funded project (Melville, see 4.5) will also provide genome mapping data in a relational database that will be community-interactive and will facilitate some analyses before it is superceded by the complete genome sequence. Once again, this will be web-accessible and, while it is possible to provide copies on CDs, personnel resources are limited to providing full explanations and guidance on a website.

Obtaining DNA Resources for Use in Research Projects

All biological resources used by the genome net-
network are available to researchers on request, including the TREU927/4 stock and 10.1 derivative, high-density filters of genomic libraries, genomic DNA clones, cDNAs and karyotype blots (Melville, et al, 1998; http://parsun1.path.cam.ac.uk). The genome website provides addresses and email links. The genome network insists that hybridization and sequence data derived using its resources are returned to the database curator for inclusion in the genome database (http://parsun1.path.cam.ac.uk). These resources were provided with funding from WHO/TDR for five years. Since the beginning of 2001, the Wellcome Trust Functional Genomics Initiative has provided a four-year programme grant to expand this facility to assist the growth of functional genomics projects within the network (Melville, Cambridge).

Obtaining Reference and Mutant Strains for Use in Research Projects
As part of the above project, a collection of reference strains, transformed lines and mutants will be maintained and provided to researchers on request (Turner, Glasgow). Current funds are not sufficient to consider curating large field collections. The sharing of well-curated field collections may be of value in certain comparative genomic projects and could be offered up for discussion. Many institutions have good collections of primary or very limited passage isolates that could be harnessed as a valuable resource. WHO is supporting efforts to establish good quality databases in a number of places, including Kenya Trypanosomiasis Research Institute (KETRI).

INVolVEMENT OF SCIENTISTS FROM TRYpanosomiasis-ENDEMIC COUNTRIES
It is with some disappointment that we report the low level of participation of African institutions in the genome network to date. There may be a range of reasons for this: inappropriateness of the tasks to institutional aims or facilities, competition from other laboratories with access to materials and the facilities to perform high-throughput tasks with greater efficiency, disinterest in genomics given the more pressing problems of control of livestock infection and human epidemics or research on pathogenesis etc., or lack of sufficient contacts and encouragement. Maybe we need not be too disappointed that African laboratories did not play a central role in the process of sequence generation itself (other than the ESTs, the majority of which were generated in Kenya; and we should also note the employment of African scientists in European and US laboratories). But we should consider it a scandal if this tremendous resource, initiated by TDR, is not made available to the entire global research community and is not applied to the full problems of control and treatment of trypanosome infection. All scientists should have access to such data as will simplify hypothesis generation and experimentation, whether their interests lie in basic biology or in applied science. In addition to the promotion of good scientific experimentation, it seems likely at the current time that African scientists must help persuade international funding bodies and the scientific community to apply the data for the development of novel epidemiological tools, drugs and vaccines.

The area of genomics and applied genomics has expanded considerably over the past decade or so; the driving force in this being the development of bioinformatics tools that make access to the available data possible. Quite important also is the wide range of publicly accessible tools developed to make use of these data. Yet many African scientists who could use such information cannot, or do not, for many reasons. While this Scientific Working Group (SWG) will address the problem of African trypanosomiasis, there is no doubt that the opportunities and impediments are generic, and hence applicable to virtually all disease problems in endemic countries in Africa, and perhaps in most developing countries. Increasing the ability of African scientists to participate in the application of data derived from genome projects will stimulate research activities not only in this area, but also in a multidisciplinary manner, since such data have to be evaluated in processes that require broad-based competencies.

We propose here ways in which participation in Africa, outside of international institutions, can be developed and strengthened.

Bioinformatics (Present Competence and Training Needs)
Although there is some competence in bioinformatics within the science community in Africa, it is largely fragmented and inadequate. To quantify it would be virtually impossible. However, it is known that this capacity is mainly concentrated in international institutes, a situation that is inappropriate, and efforts should be made to extend this to universities and national research centres. One way of beginning to do this is to develop a series of training courses in bioinformatics, either continent-wide or regionally, to develop Africa’s capacity in bioinformatics. The value of this is two-fold:

- It will equip participants with knowledge on what information is available, where it is, how it can be accessed and utilized to develop tools and methods to address public health problems.
- It would begin to expand the human resource base in bioinformatics among African scientists, hence contributing to the expansion of the
research activities in African trypanosomiasis and other major disease situations. This would create an environment for continuous in-house training.

It is anticipated that such courses would bring together expertise from within Africa and elsewhere (in or outside the African trypanosomiasis community). Doing this would help build the necessary partnerships (within and across diseases) to sustain the knowledge base in bioinformatics. It may be possible to bring in experts in computing who have little biological sciences training but who are willing to use their expertise in biology.

Capacity building in bioinformatics can also be enhanced through providing funding support (by TDR and other donors) for Internet service provider (ISP) and access charges within projects. Addressed as a budget item in relevant proposals, this could include visits to laboratories with expertise in bioinformatics (north or south). We refer you also to the proposal from the Pathogenesis and Applied Genomics Committee for regional bioinformatics courses. If the funding is secure and consistent, these may go some way to meeting these aims.

Infrastructural Capacity
Worldwide, the capacity of personal computers (processor speed and storage space) continues to improve phenomenally. Africa is no exception. Electronic access to information is often limited due to poor communication infrastructure; good, fast and dedicated connections are few and far between, and often only reliable in international institutions. With proper training, however, good quality information can still be obtained by email via a telephone line, even where institutions have a very limited number of computer terminals with on-line access (even if only one terminal with limited time slots). Improvement of institutional capacity should continue to be an important objective; admittedly, maximal access to databases is only achievable in an environment that recognizes the value of bioinformatics.

TDR can help by giving small grants, perhaps about US$2000 annually, to national institutes to fund electronic access. Such funds would be used to pay ISP and connection charges to individual scientists within national institutes and universities. Such funding would not necessarily be linked to specific project funding, although applicants could be encouraged to budget for this within projects.

Institutional Linkages
African scientists who have undertaken some training in bioinformatics have experience that can be shared. However, there is not enough linkage with- in the continent for within-continent exchange of expertise. In some cases, such limitations exist even within countries. One reason for this is that the value of bioinformatics is not well recognized by managers of national institutions. Sharing of information across areas of disease interest is also not good, especially in African trypanosomiasis, where many institutes have a single disease mandate. Thus:

• there could be closer interaction between national and the international institutions where there is greater capacity to apply data derived from genome projects, to use the available resources more efficiently.
• there should be greater effort to promote broad multidisciplinary projects constituted in part by the use of applied genomics. Such linkages will enhance the use of available information from various genome projects to address different biological questions.

Retention of Trained Personnel
It may be prudent to say that no single agency can guarantee that good quality personnel are retained in research, and particularly in national systems within disease endemic countries. However, important contributions towards stemming the outflow of personnel can be made, even by a single stakeholder.

Perhaps the flight of personnel largely accounts for the paucity of good quality funding applications from Africa to address the problem of African trypanosomiasis. In the terms of reference for this SWG, TDR recognizes that “many well trained personnel in African trypanosomiasis have left the field for others, such as HIV/AIDS”. Personnel have indeed left in many directions, including to other countries, primarily in the north, to other research areas within Africa, or even out of science.

While it has been suggested that TDR could follow a strategy of choosing a few studies and the centres to carry them out, the success of this will depend on the retention of good quality personnel in the field. Within Africa, the SWG should consider:

• allowing African investigators working in their own countries to draw salaries or salary support from projects funded by TDR, since poor remuneration is a major impediment to creating a critical mass for research.
• promoting collaborative initiatives between countries in the south, where this is scientifically sound, hence expanding the collaborations that exist currently.
• encouraging collaborative projects (north-south; south-south; inter-institutional within countries) that have a bioinformatics (and bioinformatics
training) component in order to strengthen the capacity to apply genomics data to address public health problems.

FUNDING AND HUMAN RESOURCES: REQUIREMENTS AND OPPORTUNITIES
The genome sequence will be a valuable resource for the research community, but there is much to be done to turn its potential for new directions and new discoveries into reality. No researcher need feel there is no opportunity to become involved. However, involvement will depend heavily on having access to the available information and contact with other groups. One way to become involved in the genome network is to develop an interest group, investigate the resources available, then look for funding to use the genomic data for a purpose – such a group may then be “hooked in”. The RNAi group is one example, a drug discovery group may be another.

The next phase of the sequencing revolution will be “comparative genomics”. Technologies that aim to simplify the resequencing of different genotypes (strains) are in development. Although it hardly seems possible that we might be able to sequence more than one genome, only five years ago we didn’t believe we could sequence even one. Now is the time to identify those species and strains that would provide vital information to researchers if comparative sequence information were available.

Funding agencies are increasingly realizing the value of large collaborative groupings and high-throughput techniques. It is timely to consider the potential of novel applications of genomic methods to current problems, as the openings are there. The Wellcome Trust is in the forefront of such developments. The Trust also has an active international programme in (inter alia) tropical disease research (http://www.wellcome.ac.uk/en/1/biosgfintintfu-nunkcrg.html) and has recently indicated its desire to achieve a higher profile for this programme. Therefore, at this point in time, north-south collaborations may be seriously considered: both full inter-institute collaborations and relatively minor collaborations that facilitate technology transfer and the kind of training and contacts that enable scientists to develop an independent career.

Some projects mooted here are large and costly, requiring coordination and commitment. While TDR funds may not be sufficient to underpin coordinated applied genomics projects, it is important that working groups such as this take a lead role in defining the problems that need to be addressed, to ensure that the genomics revolution does not bypass those directly affected by trypanosomiasis.

RECOMMENDATIONS
• Improve institutional capacity in Africa to access data from genome projects by acquiring computing hardware suitable for bioinformatics (through projects or independently). In the long term, this will be vital for full involvement in molecular biological research on trypanosomes.
• Identify possibilities for adding value in the collation of data, for example in a central database for DNA-based markers for use in epidemiological studies.
• Identify areas of bioinformatic analysis that lack support, e.g. identification of metabolic pathways, comparison of pathways across the Kinetoplastidae. Identify the researchers required for such projects.
• Identify now the requirement for further genomic analysis and sequencing of other strains (e.g. gambiense and other clinical variants) and species (T. congolense, T. vivax). This may be complete genomic sequencing or shotgun sequencing to a given level (e.g. 3x, 7x, 10x) and could be based, once again, in high-throughput or local centres.
• Consider the techniques available for comparison of strains, including the possibility of comparative sequencing (e.g. of gambiense).
• Consider inclusion of genomics experts in ongoing drug development working groups.
• Ensure that the voices of African scientists and applied scientists are heard within the genome network. Bring a drug development working party into the network.
• Encourage the dissemination of information that may lead to the formation of new interest groups (where possible within existing groupings, e.g. TDR, the Programme Against African Trypanosomiasis (PAAT), Global Forum on Agricultural Research (GFAR), to discourage proliferation) to access the genomic resources for new projects.
• Consider current possibilities for funding of genomics-based projects leading to drug target identification and validation; in this context, reevaluate the potential for inclusion of scientists from trypanosomiasis-endemic countries.
• Facilitate training in bioinformatics to increase competency and promote the utilization of genomics data in Africa. We take note of the call by TDR for proposals in bioinformatics and applied genomics to develop research and training centres/networks in Africa, Asia and Latin America.
• Encourage the establishment of contacts between scientists/institutions (in Africa and elsewhere), through exchange visits, training courses and joint proposals, to maximize additive competencies.
• Improve the remuneration of African scientists working on TDR-supported projects in order to...
retain trained personnel, hence improving institutional and national research capacity.

- Promote collaboration between countries (South-South; North-South) to take advantage of shared experiences and of possibilities for shared funding.

Acknowledgements

Some of the text included here is extracted from:
Melville SE. Characterisation and sequencing of the African trypanosome genome. In: Guidelines and issues for the discovery and development of drugs against tropical parasitic diseases, Vial H, Fairlamb A, Ridley R, eds. World Health Organization (2001). We thank all our colleagues for discussion of the points presented here and especially the members of the genome network for their open sharing of information and progress.

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for example, see Wellcome News issue 26, Q1, 2001, p. 19.
Annex 7

INSTITUTIONAL CAPABILITY STRENGTHENING
INSTITUTIONAL DEVELOPMENT AND CAPACITY BUILDING IN COUNTRIES ENDEMIC FOR SLEEPING SICKNESS

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INTRODUCTION
This report is intended to stimulate discussion on the possible solutions available for improving research and training in, and strengthening institutions for, effective delivery of services in the 37 countries affected by trypanosomiasis and the tsetse vector.

The financial crisis that occurred in Africa in the 1980s and 1990s had an almost paralysing effect, leading to:
• lack of funds to maintain physical infrastructure, vehicles and equipment, or to replace outdated and broken-down equipment.
• decline or lack of funding, at both national and institutional levels, to carry out field surveillance and control of tsetse, and field and laboratory research in African animal trypanosomiasis and sleeping sickness.
• poor remuneration of staff, hence internal and external brain drain of trained manpower and, therefore, paucity of leadership.

Given this background, and considering that determined efforts made over the years to solve the problems have not had the desired impact, a more rational approach to research, training, and institutional development is indicated. Issues arising include: number and size of available research and training institutions; appropriateness or otherwise of any research and training undertaken; incentives for staff to remain on the job, e.g. career development, adequate remuneration and an enabling environment.

INSTITUTIONAL DEVELOPMENT
One of the recommendations made at the 1974 FAO Expert Consultation on Animal Production and Health Research in Copenhagen, Denmark, was to provide facilities for training research workers “in their own environment” and to strengthen existing national and regional institutes. Presently, most research institutes established pre-independence or immediately post-independence in Africa are in a deplorable state due to breakdown of physical infrastructure, equipment and vehicles, lack of funds for laboratory and field work, and severe depletion of research and field staff.

Rehabilitation and strengthening of existing national and regional institutions to carry out appropriate training, research and control work, must remain a priority. The first step would be to carry out an inventory of existing research and training institutions, and identify areas of immediate and long-term concern. This should assist in the identification of national and regional institutions in Africa which could be strengthened by the international community in collaboration with national institutions and governments. Linkage arrangements, between national and overseas institutions with donor support, need to be further explored, strengthened and extended.

TRAINING
In the past, training specifically addressed the problem of tsetse and trypanosomosis in the laboratory and field with a view to ensuring long-term commitment and contribution towards solving the problem. However, this training did not take fully into account some vital concerns, e.g. that African trypanosomiasis is just one of the many health problems Africans are exposed to, the solution of which demands a broad understanding and approach.

To retain the interest and commitment of personnel who have been trained, there is a need for career development. In recent times therefore, efforts have been directed at broadening of training. Yet much remains to be done, especially in light of the developments towards privatization of services, including in tsetse control. It is in this light, and in light of the competing demands by different sectors of the economy for limited funds, that institutional development and training in disease endemic countries should be viewed.

Four types of training are generally recognized:
• Training at sub-professional level, leading to the award of a diploma or certificate, e.g. animal health assistant, animal health technician.
• Training at professional level, leading to the award of professional degrees and diplomas.
• Training leading to postgraduate degrees and diplomas.
• Short-term training leading to specialization.

To these must be added the training of:
• auxiliary personnel, e.g. clinical officers and field assistants, who need short courses and/or in-service training for field work.
• field agents, whose activities are coming more and more into the limelight because of their closeness to the communities in community-based primary health care.
• contacts and farmers (general training).

For the first four types of training listed, a prerequisite for admission is formal education at secondary
school level, while on-the-job and in-service training are essential to develop and sharpen skills. In the case of training of auxiliaries, the requirement is that they should have attended a secondary school, but may have dropped out along the way. In the case of field agents, the ideal requirement is basic primary school education, emphasizing numeracy and enabling them to follow simple instructions and training and to be selected by the community whom they will serve.

**Training Needs**

The priority given to training of the different cadres will vary from country to country but will largely depend on:
- the prevalence of tsetse and trypanosomiasis.
- the impact of tsetse and trypanosomiasis on the national economy.
- the human, material and financial resources available for dealing with the problem.

While there is a great need for experienced professional laboratory and field staff, there is, at the present time, much greater demand for specialized middle-level personnel to carry out the arduous task of field and laboratory operations, and for auxiliary and field agents who are crucial to the success of community-based health care and field programmes.

**Training Institutions**

While there is no problem finding institutions to train personnel at professional and postgraduate levels, there are serious problems with training for middle-level specialized personnel. In the last few years, many national and regional training institutions which give emphasis to tsetse and trypanosomiasis have either been reduced in size or closed down completely because of funding difficulties. Yet this is the training area of great need. The number of trainees that can be deployed to serve in the field depends largely on the number and size of the training institutions available, and whereas concern has been expressed, it appears that no solution to sustaining the necessary institutions is in sight.

Although there are certain advantages of tying training in institutions to ongoing projects, e.g. with regard to the funding and training of personnel, the disadvantage is that the lifespan and funding of projects is uncertain. What is needed is a training institution with its own lifespan and funding, and with participation from ongoing projects.

What then are other viable options? Would a donor consider supporting two or more training institutions in Africa on a sustainable basis? Another suggestion, which at first may appear remote, is to explore whether there is a place for private initiative in training.

**STAFF RETENTION**

The problem of staff retention is a global one, not peculiar to the field of tsetse and trypanosomiasis. What makes the case of those in the field of tsetse and trypanosomiasis different, however, is that career advancement can be painfully slow and frustrating in the public sector. Among the causes of inability to retain staff are:
- slow career advancement and lack of career prospects.
- poor salary and working conditions.
- delay or non-payment of field allowances.
- lack of transport.
- lack of self-worth.
- few or no opportunities to take part in short-term and/or in-service training programmes, which were a major incentive in the past but which are now scarce due to lack of funds.

The low remuneration and lack of career prospects makes recruitment of good staff for training very difficult. Young, talented people tend to decline appointment unless they lack other job opportunities. Trained staff, on the other hand, either leave their employment in favour of more lucrative economic activities and/or political appointments totally unrelated to their training, or, because of the need to supplement their meagre pay through other unrelated economic activities, tend not to devote attention to the job.

**SUMMARY**

Training is needed at all levels, but more so at the middle personnel and auxiliary levels. It is important to address the career prospects and other benefits of trainees, e.g. short courses and in-service training that could encourage staff to remain in the job for which they are trained.

The issue of funding research and training institutions on a sustainable basis must be addressed, for which it may be necessary to review the present trend of guaranteeing funding of research projects for a period of 12 months only. The possibility of involving private initiative in training, and how this can be implemented without sacrificing quality, should be examined. South-south research and training should be encouraged. There is an urgent need to find out the present status of national research and training institutions with a view to advising national governments on the role of such institutions in tsetse and trypanosomiasis research and control.
In order to provide an enabling environment for field and laboratory research and training, institutional development needs to be given new impetus. A possible starting point would be to carry out an inventory of the existing research and training institutions to provide insight as to what type of strengthening may be needed. Linking national institutions and overseas institutions with donor support is a possible area for expansion.
Annex 8
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