



# Tropical Disease Research

## Progress 1997–98

### Fourteenth Programme Report





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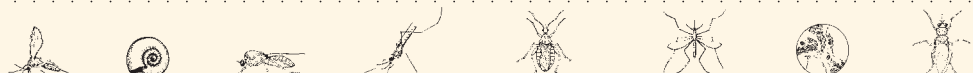
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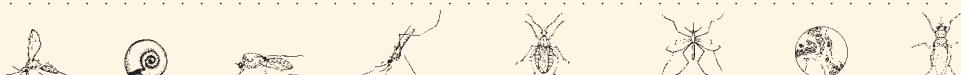
Front cover: Malaria. Coloured freeze-fractured transmission electron micrograph of a section through a red blood cell infected with malarial parasites.

Back cover: Mosquito. Coloured scanning electron micrograph of a malaria causing mosquito, *Anopheles sp.*



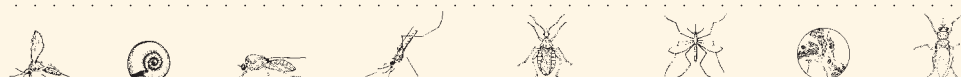
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# FOREWORD

DAVID L. HEYMANN – EXECUTIVE DIRECTOR, COMMUNICABLE DISEASES, WHO

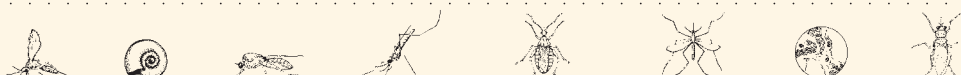
TDR BECAME PART OF THE WHO CLUSTER OF COMMUNICABLE DISEASES (CDS) programmes in July 1998, giving us an opportunity to re-evaluate and optimize research and development activities in both WHO and TDR.

We are enthusiastic about the prospect of integrating the wealth of experience and broad spectrum of activities of TDR into the new structure and working practices of WHO, and see this as a very positive move. TDR can now work very closely with other research activities in the department of CDS known as *Communicable Diseases Research and Development (CRD) including TDR*. This is a great synergy, with advantages for both WHO and TDR.

From the vantage point of the wider communicable diseases cluster, TDR can now examine with its co-sponsors its mandate to see if, assuming funding becomes available, a broadened mandate is a possible way forward. TDR's vision since its inception has been to develop tools for preventing, diagnosing and treating its target diseases, and to support training of scientists and improvement of research facilities in developing countries. This has been a solid and effective vision. In the restructured WHO this vision will be maintained, but because we live in changing times with changing needs, we now have the possibility of broadening the vision.

The synergy of TDR in CDS will permit more efficiencies. We were very happy in CDS when Carlos Morel graciously accepted to become Director of both the wider CRD and TDR. This means that research can now come to the forefront in areas such as vaccines, fixed drug combinations, new drugs and simplified diagnostic tests. In this setting, research and development on diseases such as tuberculosis, dengue and others can flourish and be addressed more comprehensively as TDR's experience in research and development of drugs and vaccines is expanded. At the same time, a staff member with responsibility for the diagnostics initiatives of tuberculosis and sexually transmitted diseases was transferred to TDR. Given TDR's renewed emphasis on diagnostics, having this expertise in TDR means that its diagnostics initiative for 'orphan' diseases has now become even stronger.

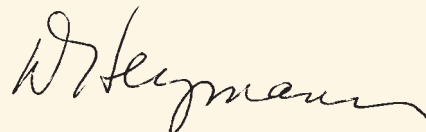
In the restructured WHO, vertical programmes such as the Control of Tropical Diseases and Global Tuberculosis Programme have become integrated in a functional matrix. This will permit the research arm of WHO (an expanded TDR) to work more smoothly and productively with these other programmes. At the same time, the management functions of TDR have been assumed by the Management Support Unit within CDS, so far freeing a quarter of a million dollars for technical staff and activities in TDR.



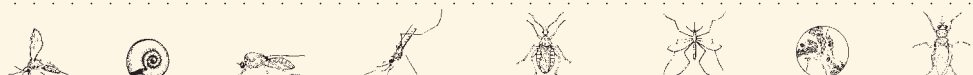
TDR has a vital role to play within the restructured WHO. WHO can benefit from and utilize the results of a quarter of a century of institution/individual training – expertise that can be adapted and applied to a broader range of targets. The new Roll Back Malaria (RBM) Project, also housed in the CDS cluster, permits clear interaction with TDR – RBM consisting of a secretariat that will mobilize and unify the international community working to control malaria, while at the same time subcontracting the necessary research and development activities to TDR. Links from TDR have been established with the Health Technology and Pharmaceuticals (HTP) cluster in WHO, through which TDR may eventually coordinate much of the vaccine research, from clinical trials to the registration stage, while the HTP cluster undertakes coordination of post-licensing policy research. This represents a powerful synergy which will, among other things, reduce the number of Steering Committees and ensure minimal duplication.

TDR also has a vital role to play in WHO's relationship with sectors other than health. TDR's expertise and experience in dealing with the private sector, which is a pillar of Dr Brundtland's vision, will be invaluable. Following the round table discussion between the Director General and key partners from the international pharmaceutical industry, we are very pleased that TDR is coordinating one of the working groups set up as a result of this meeting. The New Medicines for Malaria Venture (MMV), designed to produce one new malaria drug every five years, is also an excellent example of public/private partnerships working towards common goals, and TDR has a vital role to play in developing this venture within the Roll Back Malaria Project. TDR also has valuable experience to share in other areas as well, including its work with governments and NGOs in field research.

As we enter a new millenium, we are successfully preventing and controlling many of the important communicable diseases – but we must continue to develop more effective tools for their prevention and control. At the same time we must be on guard for other communicable diseases that may emerge. We look forward to working together in the restructured WHO to ensure the new and improved tools needed to maintain the momentum towards health for all.



David L. Heymann  
Executive Director, Communicable Diseases  
World Health Organization



# THE NEW WHO AND THE FUTURE OF TDR

.....  
CARLOS M. MOREL – DIRECTOR, TDR

*'How can current and future challenges be met? Research is crucial... This is a call for talented young scientists from many branches of knowledge to reach out to improve world health and for science policy-makers in governments, agencies, foundations, and industry to underwrite their mission... This call is not only for scientists and policy-makers in the industrialized world but also, and perhaps more importantly, for those in the developing world. The potential, passion, and perception of scientists close to the major problems of world health need to be tapped...'*

Gro Harlem Brundtland (1998)  
Reaching out for world health  
*Science*, 280:2027

## Introduction

The accession of Dr Gro Harlem Brundtland as the new Director General of the World Health Organization on July 21st 1998 inaugurated a new era in our Organization – and inevitably, in TDR. TDR's former Director – my colleague and friend Tore Godal, a key person in the shaping of the new WHO – was asked on 'day one' to start organizing a top priority of the new WHO, the Roll Back Malaria Cabinet Project. This left to me the honour of becoming the new Director of TDR and the responsibility of guiding this most successful Special Programme into the future.

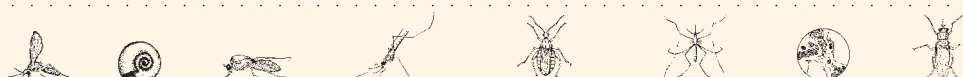
What is our vision of the future of TDR? How will TDR function in the new WHO?

The shaping of *'the new TDR, in the new WHO'* will involve several players including the Standing Committee, the Scientific and Technical Advisory Committee, the Joint Coordinating Board, and the scientific and donor communities. It should arise from a broad discussion involving all these actors and from the comprehensive analysis of the 'old' TDR carried out by the Third External Review Committee. It will have to take into account the restructuring of WHO and, most important, the health situation of the countries where tropical diseases are endemic.

Surely a complex and crucial task, which is just starting: November 1998 saw the formal approval of WHO's new structure into clusters and departments, and the publication of the report of the Third External Review Committee\*:

- WHO reform has located TDR in the Communicable Diseases cluster (CDS), in the *Research and Development Department (including TDR)*. In my view a very good start – for the first time we have an 'R&D Department' in the WHO structure, stressing the

\* Document TDR/JCB(21)/98.5, available on request from TDR





importance of R&D in the fight against disease. But this also raises a question and a challenge: what does this mean? Will TDR become the R&D branch of the whole CDS cluster? Will it broaden its disease portfolio? Will it become fully integrated into the regular WHO structure and lose its characteristics of a Special Programme? What will be the costs involved?

- The Third External Review Committee tells us in its report not to follow the old dogma ‘*if it is not broken, do not fix it*’. TDR is surely not broken, but the Committee made superb suggestions on how to improve even more a very successful Programme. Should we accept all of them? Are they still valid in the context of the new WHO structure? How, where and when to start? And again, what are the costs involved?

It is therefore our task to profit from this very special moment in the life of TDR and drive it to a new era, maximizing the opportunities and minimizing the challenges and difficulties ahead. For this purpose we will draw up a Strategic Plan to present to the next meeting of TDR’s Joint Coordinating Board in June 99. Although the elaboration of such a Plan is just beginning, we think it useful to address here some of its major issues.

### ***TDR should continue to exist as a Special Programme dedicated to research, development and training***

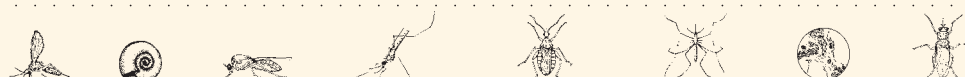
Research and development, particularly basic and strategic research, are long-term endeavours for which no one can guarantee success with 100% certainty. This kind of activity should preferably not be funded by WHO’s regular budget, which should be directed to those activities Member States identify as needing more urgent action. In the current global health situation it would be extremely difficult to allocate regular funds in, for example, a 20-year long malaria vaccine development project, when existing technology, if properly applied, can already save millions of human lives.

Similarly, training with the aim of creating self-reliance of the developing endemic countries is also a long-term goal. As in the old Chinese proverb, it’s about teaching how to fish – not just providing fish to hungry people.

Fortunately, there are donors and contributors who understand that investing in health R&D and in capacity building in developing countries is to speculate on the global future, and that new knowledge and human development can make an even greater impact in health than today’s existing tools. These are the same contributors who created and shaped TDR 24 years ago, and whose long-term vision has kept it alive and successful since then. We will count on their continuing commitment, and we will fight to keep TDR as a (very) Special Programme.

### ***TDR should re-evaluate its disease portfolio***

The fact that TDR is now located in the Communicable Diseases cluster and also that some of the ‘TDR diseases’ are heading to elimination as public health problems – in part because of the very work of TDR – makes inevitable the reopening of the discussions on the ‘ideal’ TDR disease portfolio.



Should other diseases be taken on board? Should TDR ‘discard’ some of its family of diseases? This will be one of the main issues of future discussions, and any decision will have to take into account the resources available, the need to have a focused Programme and the needs of the populations of the developing endemic countries.

### *TDR should explore new ways of collaboration*

The restructuring of WHO provides new opportunities to strengthen the collaboration with the CDS departments involved in disease prevention, control and eradication or elimination. In addition, TDR should interact closer with other clusters in WHO, such as Health Technology and Pharmaceuticals, in the development of new drugs, vaccines and diagnostics.

TDR has emerged as an important funding body for tropical diseases, with a significant influence in the policy-making of the field. The immensity of the tasks it addresses, however, makes it imperative to nurture new collaborations and seek cost-sharing mechanisms in order to become still more efficient. A special emphasis will be put on interaction with the private sector, a key player in the discovery and development of new drugs and vaccines.

### *TDR should aim at a stronger presence in the developing countries*

The countries that bear the heaviest burdens of endemic tropical diseases should receive special attention from TDR. Specific strategies should be developed to ensure appropriate financial support to strengthen institutions and collaborating networks/centres that could have national and regional impact.

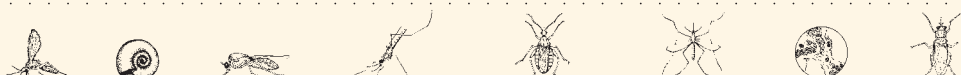
### *New tools and capacity building: the classical priorities are still there*

The Third External Review endorsed the original mission of TDR, recommending high emphasis on the development of new and improved tools for disease control and a renovated and focused strategy to increase the effectiveness of capacity development efforts, particularly in the least developed countries. The challenge that TDR’s founding fathers and mothers put forward 24 years ago is therefore well and alive: **do the best science in the world, while at the same time strengthening the most impoverished countries – and do this miracle counting only on a ‘catalytic’ budget.**

This is the immensity of our challenge, which some would name an impossible dream or an utopia. As dreams and utopias have the power of moving the world, I call on all TDR partners and staff to grasp the opportunities ahead to help make this dream come true.



Carlos M. Morel  
Director, TDR



## LOOKING BACK

TORÉ GODAL – DIRECTOR, TDR, 1986–1998

*Toré Godal reflects on the highlights of the 1997–98 biennium in the context of TDR's first 24 years.*

### *Tools and strategies for winning the battle against four diseases*

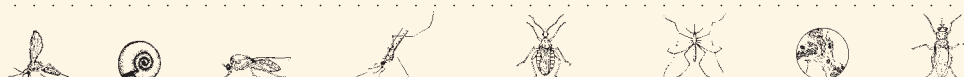
The most important outcome of TDR's 24 years of work is that tools and strategies have been developed for elimination of four of the eight diseases in TDR's portfolio. Although it's true to say that TDR had a role to play in this picture, such an optimistic outlook could not have taken shape were it not for the strength of all the other players in the arena. The partners working with TDR have been quite varied.

In the case of leprosy for instance, non-governmental organizations (NGOs), leprosy missions, the International Federation of Antileprosy Associations (ILEP), and Save the Children Fund have been particularly strong partners with TDR. In Chagas disease, the disease endemic countries have played the major role, along with the WHO office for the Americas (AMRO). In onchocerciasis, particular partners have been the World Bank, government development assistance agencies, the Onchocerciasis Control Programme (OCP), and the African Programme for Onchocerciasis Control (APOC), along with Merck & Co. Inc. from the private sector. And in lymphatic filariasis, the partners have been a combination of private sector, governments and development agencies. Constant partners have been WHO's former programme for Control of Tropical Diseases, and numerous scientists, governments and patients suffering from TDR diseases.

Just how much TDR has had to do with the proposed elimination was illustrated by the Third External Review reporting in 1998, which looked in depth at three tools developed by TDR.

For leprosy, multidrug therapy (MDT) is the cornerstone of elimination efforts. The External Review noted that TDR's contribution to the development of MDT had been critical. TDR had shown that drug resistance was a real and growing problem, and that a combination of drugs could cure the disease more quickly and effectively, therefore more cheaply, than single drugs. Crucial to the rapid adoption and subsequent implementation by countries, however, were the actions of the other partners in the story – WHO's Leprosy Unit and Programme for the Elimination of Leprosy; the Nippon Foundation through its branch for leprosy, the Sasakawa Health Foundation; and NGOs.

For Chagas disease, TDR helped in the development of a fumigant canister which householders can use to ensure their houses are kept free of vector insects between



conventional rounds of insecticide spraying. TDR's involvement was catalytic – it played a significant role in developing, testing and improving the canister, but otherwise local governments and industries, particularly in Argentina, were the leading players.

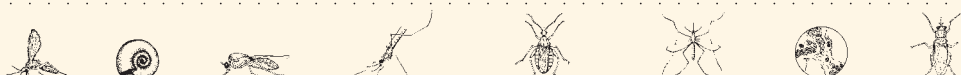
For onchocerciasis, the development of the drug ivermectin was key to plans for elimination of the disease in the many areas where vector control is not effective. In addition to TDR, major actors in this story were the pharmaceutical company Merck and Co. Inc. which developed the drug, OCP, and APOC. TDR was involved in clinical trials and provided Merck and Co. Inc. with an international network of experts and institutions for multicountry trials; helped argue the case for Merck and Co. Inc. to make the drug available free of charge; demonstrated that ivermectin was effective and safe for mass treatment; helped OCP achieve its goals and prepared the technical basis for APOC; and has continued to play a leading role in establishing and evaluating Community Directed Treatment.

Lymphatic filariasis is the fourth disease in TDR's portfolio that is heading towards elimination. Key to controlling this disease is mass drug administration with annual, single-dose treatment of all eligible members of high-risk communities. Partners include the drug companies Merck and Co. Inc. and SmithKline Beecham, the non-medical private sector, WHO's former Control of Tropical Diseases programme, and health services and communities in endemic countries.

### *The cutting edge of science brought to tropical diseases*

TDR has, over its lifetime, helped bring the cutting edge of science to tropical diseases. The most important factor in this has been bringing leading scientists from other areas into the field of tropical diseases. Key roles were played by such people as Barry Bloom, of Harvard School of Public Health, who helped identify talented researchers in the field; Louis Miller, US National Institutes of Health, the intellect behind TDR's molecular entomology drive, who brought people working on the fruit fly (*Drosophila*), such as Fotis Kafatos of the European Molecular Laboratory (in Heidelberg), into the field of mosquito research; and Ron Davis, Stanford University School of Medicine, who inspired the genome initiative.

TDR-sponsored research on molecular entomology, parasite genomes, and vaccines is particularly pioneering. Molecular entomology studies supported by TDR have been breaking completely new ground in the understanding of parasite/vector interactions, and genetic modification of the mosquito has been shown to be feasible. Mapping and sequencing of the genomes of five parasites (responsible for leishmaniasis, Chagas disease, African trypanosomiasis, lymphatic filariasis and schistosomiasis) has been proceeding fast and the current biennium saw many discussions about the post-genome agenda and use of genome information for developing drugs and vaccines. Pioneering vaccine work has been supported by TDR for 18 years; and during 1997–98, TDR moved to the vanguard of drug discovery and development through expansion of its Product Research and Development (PRD) component, which now has a professional team more in line with that in industry. Many people, from both public and private sectors, are collaborating with the new TDR/PRD team. In malaria for instance, TDR has been deeply involved in discussions concerning the New Medicines for Malaria



Venture (MMV), a partnership between public and private sectors which comes under the umbrella of WHO's new Roll Back Malaria initiative and aims to develop one new antimalarial drug every five years.

### ***Applied Field Research made more relevant to disease control***

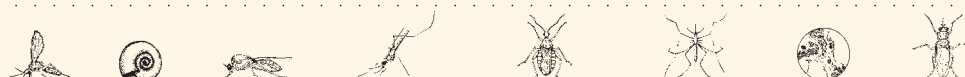
In recent years, field research in TDR has become very applied and geared to improving the prospects for control. There has been a drive to put research results into action. A crucial factor in this has been the change from an investigator-driven approach to a proactive, highly prioritized approach, where substantial amounts of money are invested in a few areas with very clear goals and outcomes as, for instance, in the development and delivery of ivermectin. TDR has acted very much as an amplifier in bringing results from the point of observation to the point of policy through carrying out multicentre, multicountry studies. The observation that insecticide-treated bednets can reduce childhood mortality, for example, was amplified by TDR in four mega-trials, costing US\$ 5 million, before becoming a priority intervention for malaria. The International Development Research Centre (IDRC), Canada, has been a strong partner in work on bednets.

Work in malaria provides a good example of the applied nature of Applied Field Research (AFR) in TDR. Many of the tools that TDR has helped develop for malaria control under its AFR programme (e.g. insecticide-treated bednets, rectal suppositories, simple packaging of antimalarials) are being implemented through the Roll Back Malaria (RBM) project of WHO, initiated by the new Director-General. Other aspects of TDR that have inspired RBM include its networking approach – RBM will depend on a dozen or so 'resource networks' to provide help for countries and monitor all interventions. Through the networking approach, true international networks can be formed without consideration of geographical boundaries. Thus the RBM Project has been built very much, both conceptually and practically, on TDR, and TDR will remain a cornerstone for its success – continued research for new tools (vaccines, drugs and vector control methods) will be essential for the long-term success of the Project. RBM is therefore a clear illustration of the transformation of TDR research results into action.

### ***Investment in capacity strengthening has paid off – a cadre of scientists is now present***

The RBM Project is also building on capacity created by TDR in endemic countries. TDR has been investing in capacity strengthening for 24 years, and the results of this are becoming increasingly apparent. The strategy has been a combination of investing in institutions and investing in talented people on a competitive basis.

A literature search for the years 1992–96, carried out as part of the Third External Review, found that TDR was acknowledged as a source of funds more often than any other funding body in papers published on six of the eight target diseases. Citation frequency, used as a rough measure of scientists' impacts in their fields, showed that more than 85% of TDR funded papers were cited at least once, and that frequently cited papers were not confined to authors from the North – the maximum number of

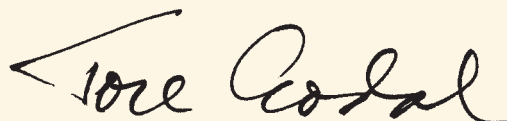




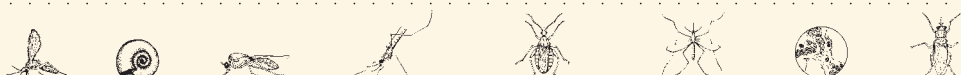
citations for any one TDR funded paper was 43, and this was a paper written by researchers in Tanzania. Of the 24 most highly cited papers in all subject areas, some 42% were authored by people from developing countries – a significant percentage when considered in the light of the well-known bias towards authors from industrialized countries.

Other evidence that capacity is available became apparent through the Multilateral Initiative on Malaria in Africa (MIM), an exciting initiative which began in 1997 in Dakar, Senegal, in which African scientists have been involved right from the start. The immediate goal is to facilitate collaboration between governments, control programmes, scientists and supporting agencies; TDR's particular role is to help assess scientific needs and take responsibility for strengthening the research capacities of malaria endemic countries in Africa. The number of excellent proposals for grants received from Africa indicates that investment by TDR and others in capacity strengthening in Africa is paying off.

And so to the future. Today TDR is playing a leading role in the new WHO, and responding to changing needs such as the post-genome agenda. My best wishes are with TDR in the years ahead.



Tore Godal





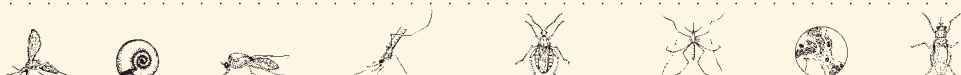
## PROGRAMME MANAGEMENT – CHANGES AND CHALLENGES

The 1997–98 biennium has been one of fundamental change in TDR's programme management functions. At the end of 1998, these functions are dramatically different to those it had just one year before. The new leadership at WHO has taken a fresh look at the Organization's administrative procedures, in particular the often cumbersome and lengthy personnel, financial and budgetary processes, which sorely needed rethinking.

As a Special Programme, TDR had circumvented many, but not all, of the bureaucratic stumbling blocks that WHO's regular programmes encountered daily. For some time, TDR has had its own administrative structure which included professionals in management, finance and budget, personnel, communications, information systems and external relations. Having its management decentralized to the programme level has undoubtedly been beneficial in accelerating resource allocation to TDR's grant holders in developing countries, and in following-up grant making and the progress of research trainees. TDR has been able to design special tailor-made information systems, including mechanisms that facilitate careful monitoring of financial expenditures and budget reporting. These have enhanced TDR's credibility as a transparent, well-run programme that is a sound donor investment. Ongoing links with TDR's co-sponsors and other contributing partners have been possible with a full-time external relations officer, and TDR's public profile has been well served by its communications/media team.

The incoming WHO administration, aware of TDR's adaptable structure, has sought to replicate it across the Organization. Since July 1998, WHO has undergone a complete reorganization that began by decentralizing programme management to nine WHO clusters, setting up self-sufficient Management Support Units (MSUs) within each. The hope is that the MSUs will contribute significantly to the timeliness and flexibility that has characterized the TDR programme.

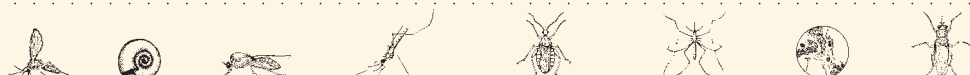
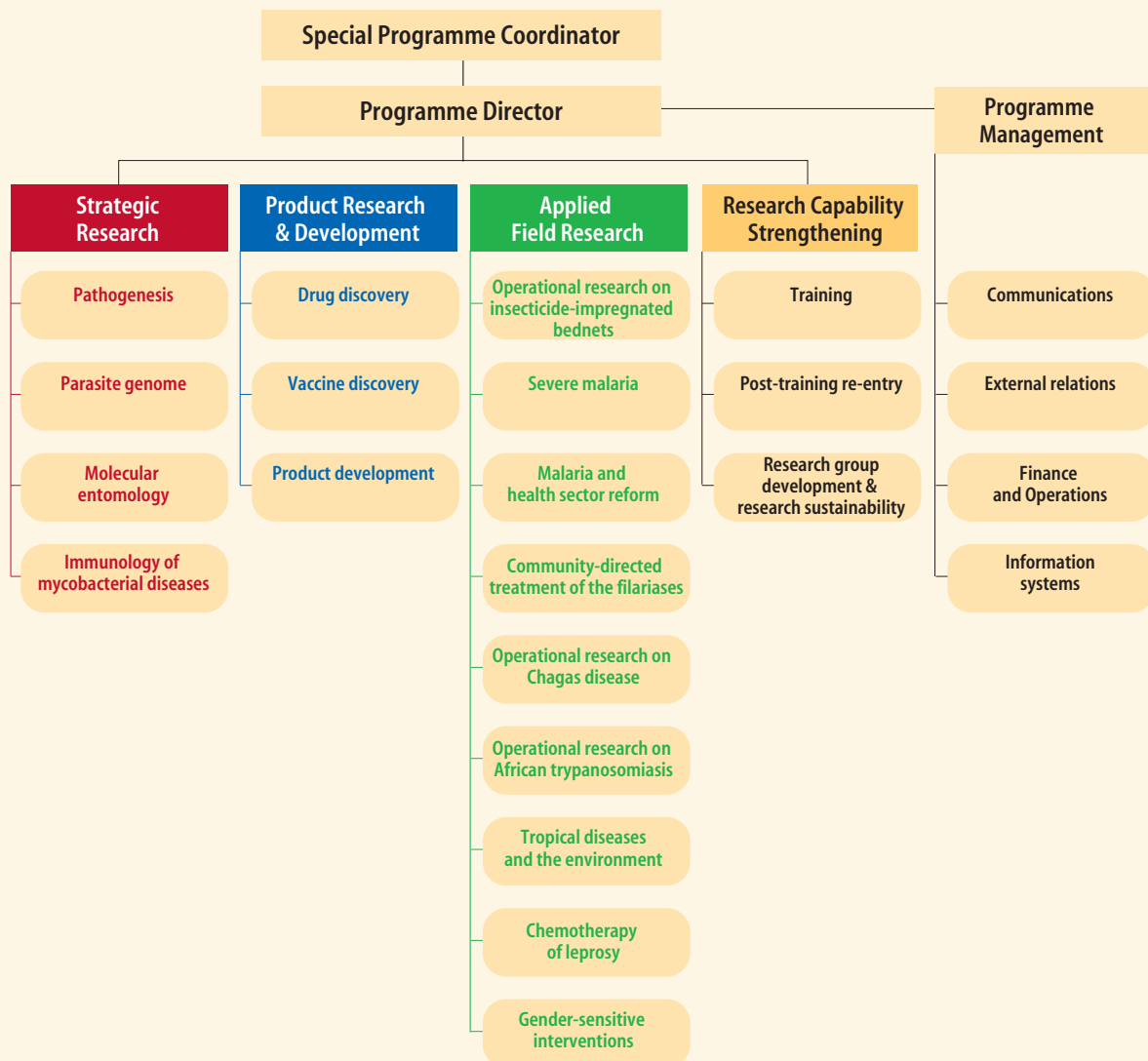
As a result, many of TDR's management functions have now been devolved to the CDS cluster MSU, in particular, personnel, finance and budgeting. TDR will continue to maintain its supplies area, thus facilitating ordering of equipment vital to TDR's grant holders. In addition, the Activity Management System, which has been promoted widely within the Organization, will allow each TDR unit to review its own financial situation against pre-approved work plans with clear objectives, targets and progress indicators. TDR's external relations and information systems will be likewise absorbed into the MSU, but the reassignment of TDR's staff responsible for these areas, and the appointment of specific individuals within the MSU to deal with TDR's needs, will allow human resources to be maximized.



What will TDR gain from an MSU structure? As yet, this question has no clear answer. The new restructuring might be viewed as decentralization for most WHO programmes, but for TDR it undoubtedly represents the centralization of management functions. The challenge for the MSU is to ensure that the service it provides to TDR is as good, if not better, than what TDR had in the past.

It is hoped that by devolving TDR's programme management to the MSU, cost-savings will be made. Staff that TDR previously had to hire to enhance management capability might now be funded by additional sources, instead of wholly by TDR. The funds saved could then be used to strengthen TDR's technical units by bringing in additional top biomedical and social scientists to support disease endemic countries in their efforts to address the problem of tropical diseases.

### *TDR: Organization (June 1998)*



# RESEARCH CAPABILITY STRENGTHENING

The Research Capability Strengthening (RCS) component of TDR is exclusively engaged in enhancing research capacity related to the health problems of TDR target diseases in endemic countries. However, all components of TDR are involved in RCS activities as far as their programmes permit. Whenever opportunities arise, RCS introduces capacity building elements (training, partnership, linkage, infrastructure development, improved communication) within projects funded by other components of TDR.

Although RCS philosophy has not changed during the biennium, new strategies have been developed to address the changing needs of different institutions and research groups in target disease endemic countries (DECs) and reflect the shifting priorities for TDR diseases. Today the major thrusts in RCS are on:

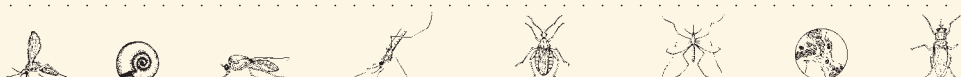
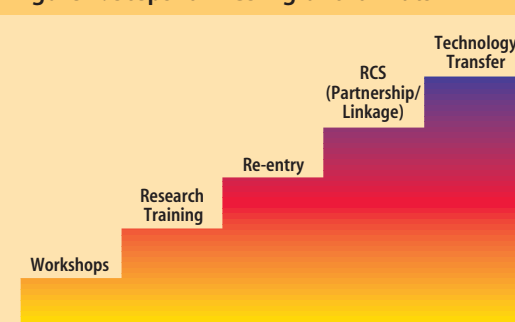
- establishing strong research groups in DECs
- sustaining and utilizing research capacity where already established
- developing human resources through formal and in-service training
- transferring technology and know-how to developing countries
- focusing on countries with least developed research capacity (LDRC)
- promoting Internet connectivity for DEC scientists.

## *Steps in the RCS process*

The steps in RCS are shown in Figure 1. There are RCS opportunities for researchers at all stages in their careers and in all DECs, particularly the least developed nations, ranging from short-term training in workshops to technology transfer activities in countries with good research capacity. Moving from left to right of Figure 1, from lower to higher levels of research capacity, TDR focuses more on young scientists from DECs who have recently completed training.

Short-term group learning activities (workshops) are used to provide needed training on a variety of topics for different outcomes. Some examples of recent workshops are given in Box 1. Workshops are organized when the need for specific training becomes apparent, e.g. because it is a TDR priority or because it is repeatedly requested. For instance, the 'small grant initiative' experience with WHO's former programme for Control of Tropical Diseases and the WHO Regional Office for the Eastern Mediterranean (EMRO), led to

**Figure 1: Steps for RCS – grant formats**





## Box 1: Selected workshops

### *Qualitative methods*

#### **Swiss Tropical Institute Field Station, N'Djamena, Chad**

Through a competitive process, 16 participants including many previous TDR research training award recipients, were invited to a 3-week Francophone training course on qualitative methods, organized jointly with the Swiss Tropical Institute, Basel, Switzerland. Over the first half of the course, participants were introduced to the basic concepts of qualitative research, including semi-structured and in-depth interviews, key informants, focus groups, role play, and the use of statistical software programmes for qualitative research. Over the final period, participants designed and carried out a field project, implementing in practice what they had learned in theory. The course was important in providing a group of young, active researchers with a new avenue of research. In addition to the training, two TDR publications were produced on the training methods and course outcome, complementing similar publications of a previous English language course held in Ifakara, Tanzania, 1994.

### *Protocol Development – Community Directed Treatment (ComDT) for the Filariases*

#### **National Institute of Communicable Diseases, Delhi, India**

As part of the global elimination strategy for lymphatic filariasis, TDR's Applied Field Research programme, in collaboration with RCS, organized a meeting of investigators in July 1998 to design a research protocol to assess the effectiveness of ComDT. Seven teams, each consisting of at least two people (1 epidemiologist and 1 social scientist) from three countries in Asia (India [x3], Myanmar, Viet Nam) and two countries in Africa (Ghana, Kenya) designed a common protocol that is currently being implemented in these countries. The participatory approach to protocol design increased the investigators' commitment to successfully carrying out the research, and permits the results of the different study sites to be compared and contrasted. The seven projects are on schedule; final results are expected in late 1999. A similar meeting for ComDT-onchocerciasis was held in Ouagadougou, Burkina Faso, in 1997.

### *Geographic Information Systems (GIS) for schistosomiasis*

#### **Jiangsu Institute of Parasitic Diseases, Wuxi, PR China**

With a rapidly developing GIS capacity in China, TDR supported a training course for Chinese researchers from the schistosomiasis endemic provinces. This course was a follow-up to a previous regional course in Guatemala (1996). The facilitators were led by an experienced group from the US Centers for Disease Control and Prevention (CDC), and included scientists from the National Aeronautics and Space Administration (NASA), the University of Louisiana, the Danish Bilharziasis Laboratories, and Environmental Systems Resource Institute (ESRI) who, in addition, donated the GIS software. The course brought together leading Chinese schistosomiasis researchers interested in improving their use of GIS tech-

nology in the prediction and control of schistosomiasis. This is particularly important considering the extensive flooding that occurred in China in 1998.

### *Good Clinical Practice (GCP) and regulatory issues for clinical trials*

#### **Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand**

To promote the practice of GCP and conducting clinical trials for registration, TDR, in collaboration with the Faculty of Tropical Medicine, carried out a 2-week training course on the planning and conduct of GCP trials. Half of the 20 participants came from neighbouring countries and territories – Laos, Viet Nam, Malaysia, Myanmar and Hong Kong. Participants were selected on the basis of current or imminent involvement in TDR-funded clinical trials. As such, the training was invaluable in raising awareness of the high clinical standards necessary for completing trials that may be used for regulatory purposes.

### *Research data analysis methods*

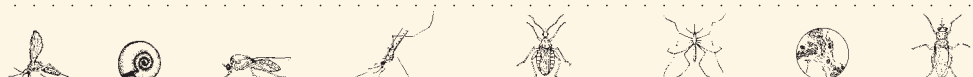
#### **University of Casablanca, Morocco**

For the past five years, TDR, the WHO Office for the Eastern Mediterranean Region (EMRO), and the former WHO Division for Control of Tropical Diseases, have co-sponsored a 'small grant initiative' to support research projects in the eastern Mediterranean region. The primary objective of the programme is to fund research of relevance to local, national or regional disease control programmes; a secondary objective is to raise awareness and interest in tropical disease research in a region not widely involved with TDR. The course used data from TDR-funded projects to upgrade the abilities of 14 researchers – currently engaged in TDR-funded work – in project design, data management and analysis, and presentation of results. In addition, the course presented an opportunity to identify young researchers with an interest and talent for research, who would then be encouraged to apply for more competitive TDR grants.

### *Research methods for the Integrated Control of Vector Borne Diseases (ICOVED) programme*

#### **Dhaka, Bangladesh**

The World Bank funded ICOVED project supports five clinical, entomological, and intervention studies related to vector borne diseases (malaria, leishmaniasis, filariasis, dengue). TDR has contributed to the programme, coordinated by the Ministry of Health and the WHO Country Representative's Office, through technical expertise and short-term training – in protocol development, interim analysis, and data analysis – at a number of stages in the projects. These short courses contributed to the researchers' knowledge and ability to complete their projects. Results of the studies, which are potentially important for disease control programmes and formation or refinement of policies for control and treatment of tropical diseases, will be presented to the appropriate government departments. One participating group, the malaria research group from Chittagong, subsequently successfully applied to TDR for a 3-year Research Capability Strengthening grant, and is currently preparing research for the TDR Task Force on severe malaria.



awareness that the quality of research proposals and final reports from the region needed enhancing, which led to a workshop in Morocco (see Box 1) on data analysis and report writing for Principal Investigators and other young scientists whose projects had been refused funding because of poor presentation although the topic was important for the region. Another workshop held at the Noguchi Memorial Institute in Ghana was designed to train clinical trial monitors from countries where it is hoped to carry out clinical trials of drugs and vaccines under development by TDR.

## *Capability strengthening grants available*

### *Research Training Grants*

Workshops such as those illustrated in Box 1 provide good opportunities for identifying potential 'champions' around whom research groups can later be developed, through mechanisms such as formal research training grants – the next RCS 'step' in TDR. Research training grants are meant to increase the research capability of qualified young researchers, including medical practitioners not previously involved in research. Calls for Applications are widely distributed once a year, through the offices of the WHO Representatives in countries and through the Internet. About 40–50 candidates are selected, mostly for Ph.D. degrees.

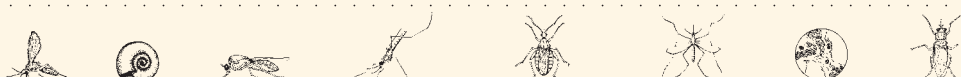
### *Re-entry Grants*

Re-entry Grants (REGs) constitute a further step for researchers, and are provided for young scientists returning to their home institutions following training. The aim is to help establish research environments in their home countries and prepare nuclei with critical masses which can compete successfully for funding from various sources, and to sustain research activity. At present, TDR funds REGs only for two years, but this process is in need of review because, although it might be possible for some young scientists in advanced developing countries to establish themselves in two years, it may require a longer period in other less advanced DEC.

### *RCS Grants*

The next level of grant is the Research Capability Strengthening (RCS) grant. With this type of grant, the level of funding, number of projects within each grant, and balance between training and level of infrastructure is flexible, allowing them to be adapted to local needs. There is now a generic application form for these grants and a highly competitive process is used for selecting projects for funding, based on scientific merit of the project and the potential increase in capacity to do research at the end of the grant, facilitated by linkages between different institutions with complementary expertise. The relevance of the proposed work to the workplans of TDR Task Forces and Steering Committees is also taken into consideration. Each proposal is ultimately evaluated according to its final impact on research and the ability to sustain research activities on topics relevant to health problems related to TDR target diseases after the project has been completed.

The RCS grant format unifies the previous multiple Institutional Strengthening grant formats.



## Technology Transfer

The final step in RCS is technology transfer (TT). Here, TDR experience has so far been rather limited. Examples to date include the upgrade of facilities for production of clinical-grade material for human vaccine trials at the Hong Kong Institute of Biotechnology (HKIB), in collaboration with the US National Institutes of Health. The Thailand/TDR programme (T2) has focused on strengthening national capacity in the areas of drug discovery and development, as well as on improving Good Laboratory Practice (GLP) and Good Clinical Practice (GCP). Training in combinatorial screening technology has been the focus of an RCS supported project in India. BIOBRAS in Brazil has received support to produce clinical-grade crude whole cell *Leishmania* antigen for human vaccine trials and develop tests to meet requirements for national registration should the crude antigen prove to be protective. Support and training in the principles of Good Manufacturing Practice (GMP) production and regulatory procedures have been given by TDR to the Pasteur Institute, Tehran, Iran, for production of recombinant leishmanial antigens. In the Philippines, TDR has supported successful efforts to establish a biologicals section of the national regulatory agency. In Colombia, the regulatory authorities (INVIMA) have been strengthened in GMP and quality control of peptide vaccine production, in preparation for possible regulatory activities related to synthetic malaria vaccine production at the Instituto de Inmunología in Bogota. TT in TDR takes a focused approach and is based on a true partnership strategy involving mutual commitment (including financial) from all parties.

## Trends in RCS funding

The funding for each RCS area is shown below:

	Training	REG	RCS	RCS/R&D*	MIM**/TDR
1997 (US\$ 5.8 million)	43%	8%	29%	20%	
1998 (US\$ 5.5 million)	39%	11%	37%	13%	(US\$ 2.3 million)

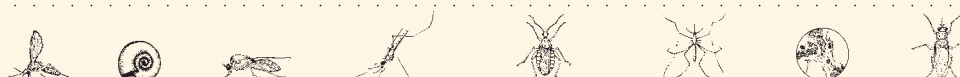
\* RCS funds to support training/R&D activities within approved R&D proposals

\*\* RCS activities of the Multilateral Initiative on Malaria in Africa, under the Task Force on Malaria Research Capability Strengthening in Africa

A total of 116 research training grants were awarded during the 1997–98 reporting period, including 26 Master's, 71 Ph.D., 6 post-doctoral grants and 13 short-term, non-degree training opportunities, for a total of US\$ 4.3 million. During this period, there were three Calls for Applications for research training grants, whereas normally there are only two. Figure 2 shows the present status and the changes in training for Least Developed Country (LDC – as defined by the United Nations) applicants, local funding, and gender balance in the previous six years.

Also, during 1997–98, a total of 25 new RCS proposals based on the principles of group development, partnership and regional linkage (US\$ 915 000), 18 Re-entry Grants (US\$ 394 000), and 24 renewals of ongoing grants (US\$ 776 220) were approved.

In 1998, the Research Strengthening Group was also responsible for reviewing 18 investigator-initiated proposals in Applied Field Research that did not fall within the Task Force workplans. Six were approved and funded (US\$ 167 900) from the AFR budget.



## RCS grant review

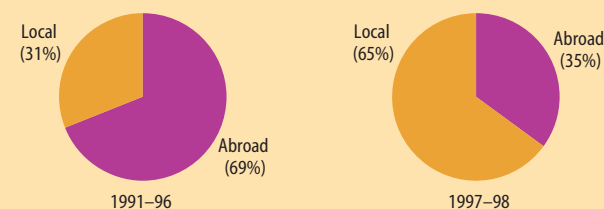
An extensive review of the impact and productivity of RCS grants was conducted during the Third External Review of TDR\*.

This review found that TDR 3+2-year (i.e. initially for three years, with the possibility of extension for an extra two years), non-competitive grants had contributed to establishing a core of scientists around whom to build research teams in DEC. The grants had been awarded specifically to countries with least developed research capacity (LDRC), and were focused on staff development, training opportunities, upgradation of general facilities in order to allow more effective involvement in TDR research and training activities, and, particularly, on integration with control activities. LDRC institutions involved in building research teams include: Institute of Malaria Prevention and Treatment, Yunnan, China; Epidemiology and Disease Control Division, Ministry of Health, Nepal; University of Papua New Guinea; Hainan Institute of Tropical Diseases, China; Ste Croix Hospital and Public Health Centre, Haiti; Makerere University, Kampala, Uganda; Regional Centre for Development & Health (CREDESA), Benin; National School of Medicine and Pharmacy, Mali; and Tropical Medicine Research Institute, Sudan.

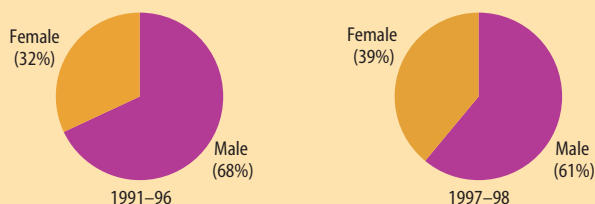
Programme-based grants awarded to institutions to establish or strengthen a focused line of research and/or training were found to have opened new areas of research in disciplines for which methods were not well established, such as socio-economic research (Chulalongkorn University, Thailand; Shandong Medical University, China; University of Ghana; Central University of Venezuela) and applied field research in malaria

Figure 2 . Changes in RCS

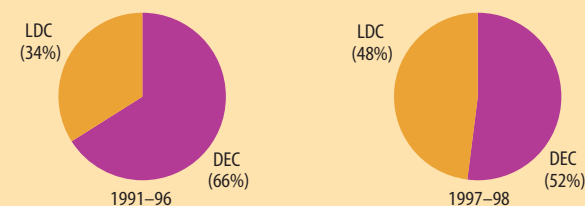
### Local training



### Training gender balance



### Training of LDC applicants

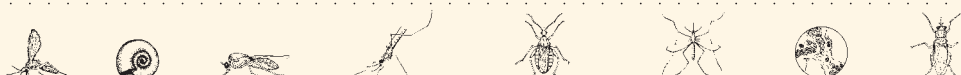


LDC = least developed country  
DEC = disease endemic country



Sudan. A lab technician at work in the Tropical Medicine Research Institute, which now houses a highly capable team of researchers.

\*Document TDR/JCB(21)/98.5, available on request from TDR



### Box 2. MIM/TDR Task Force for Malaria RCS in Africa

This Task Force, coordinated by TDR, represents a collaboration between a number of agencies for promoting capacity building activities in the context of the Multilateral Initiative for Malaria in Africa (MIM); it was established to strengthen capacity through supporting research in malaria endemic countries in Africa.

The objective of the Malaria RCS grants is to strengthen core African research groups in developing effective tools for malaria control and in improving relevant health policy strategies. Proposals are submitted and coordinated by African scientists working in research groups in Africa; each proposal includes at least two African research partner institutions (one established and one emerging) and at least one non-African partner, which can be an international institution in Africa.

Sixty-three proposals involving 40 countries and 161 partner institutions/research groups were reviewed in February 1998. Fifteen projects involving 20 African and 5 European countries and the USA were recommended for funding; 14 Ph.D. and 6 M.Sc. research training grants were approved in connection with the funded projects; and support was recommended for 12 additional investigators to improve their proposals. The proposals were wide ranging and covered the clinical and molecular basis of drug resistance, drug policy, epidemiology of immune response, evaluation of natural products, epidemiology of parasite diversity, pathogenesis, and insecticide resistance.

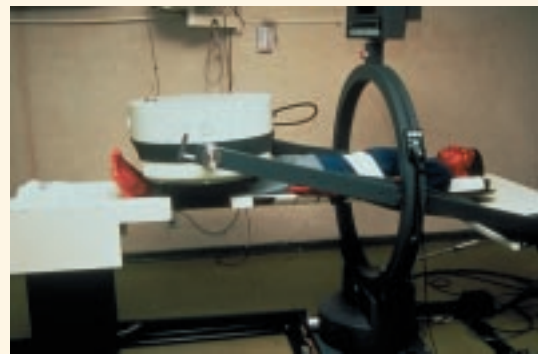
(Myanmar Ministry of Health; University of Yaoundé, Cameroon; Blair Research Laboratory, Harare, Zimbabwe; Instituto Juan Cesar Garcia, Quito, Ecuador; Fundación FES, Colombia). The majority of programme-based grants have addressed research questions directly related to disease control issues.

Ongoing partnership grants established to consolidate north-south or south-south collaboration between institutions with complementary expertise were found to have been an outstanding success (Malaria Unit, University of Colombo, Sri Lanka; Malaria Research and Training Center, Mali; Centro de Investigación de Paludismo, Tapachula, Mexico; Research Institute for Tropical Medicine, Manila, Philippines; Instituto de Investigaciones Biotecnológicas, Buenos Aires, Argentina; Centro de Pesquisas René Rachou-

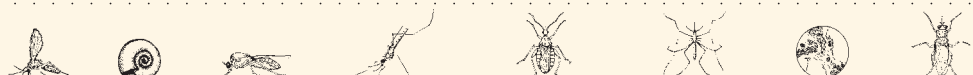
FIOCRUZ, Belo Horizonte, Brazil; Centro de Pesquisas Ageu Magalhães-FIOCRUZ, Recife, Brazil). These institution-strengthening grants are most prestigious and competitive, and scientific productivity has proved to be exceptional in that seven of the partnership grants generated 143 publications in international, peer-reviewed journals. Capacity building included advanced training of significant numbers of graduate students (11 M.Sc. and 48 Ph.D.) without any additional cost to TDR, and the grants were found to have been effective in integrating R&D with RCS objectives at the highest scientific level. The same principle of partnership is now being promoted by the MIM/TDR Task Force for Malaria RCS in Africa (Box 2).

The flexible and dynamic approach taken in employing a variety of institutional grant formats has allowed research groups at all scientific levels to participate in the TDR research programme. By and large, RCS grants have enabled DEC research groups to assume increasing scientific responsibility at national, regional and international levels, and have contributed to the translation of research results into national disease control programmes and activities.

Clearly, RCS is a long-term goal, and any impact must be evaluated as such. True qualitative evaluation does not have simple parameters, and criteria for qualitative evaluation of RCS activities in TDR are constantly being sought. The number of publications stemming from grants obtained from TDR, or from other sources following completion



Brazil. In Recife, collaborating institutions are conducting pioneering work in lymphatic filariasis, making innovative use of the latest technology.





of TDR/RCS grants, and the number of students trained, new degrees gained, and courses offered are all useful indicators. However, the ultimate goal is to see that these efforts lead to improvement in the control of the targeted diseases; and as many other factors are involved in disease control, the impact of RCS must be seen in the context of all factors.

To streamline activities and enhance the proactive approach, the RCS area of TDR has been reorganized and now has focal points for training and re-entry (post-training and establishment of new research groups), RCS-grants (institutional linkage, networking, partnership, transfer of technology), and establishment of electronic communications (see Box 3).

## Conclusions

TDC has focused its activities to clearly identify and close the gaps in different disciplines in target countries, particularly LDC's. Criteria for selection of target countries are based on their potential, health problems and socio-political status. Interested bilateral donor countries, funding agencies and NGOs are called upon to establish partnerships in infrastructure building for research.

The challenges and key objectives for 1998–99 are to:

- develop a strategic funding approach based on the varied regional needs and opportunities
- integrate RCS for malaria within global initiatives (the Roll Back Malaria initiative of WHO, the Multilateral Initiative on Malaria in Africa)
- monitor RCS development based on scientific output
- intensify support for selected countries with least developed research capacity
- strengthen capacity in disease endemic countries to meet R&D needs including GLP, GMP and GCP
- increase training and training opportunities in DEC's, with focus on LDC's
- expand Internet connectivity in Africa
- develop and sustain research through partnerships between established and emerging groups.

### Box 3. Connecting the Unconnected: the drive for e-mail connectivity of TDR scientists

In October 1997, 30% of grantees registered in the TDR database for Africa had e-mail access. A year later, the figure had jumped to 50%.

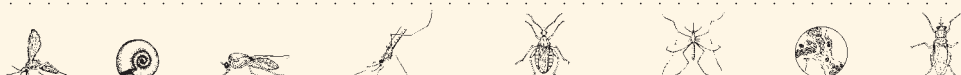
TDR's Research Strengthening Group has supported a number of initiatives for Internet connectivity in the past, including the use of micro-satellite groundstations in selected institutions in Africa and Asia, and allowed flexibility in budget reallocation of TDR grants in developing countries to support connection.

The ongoing efforts by the TDR/RCS secretariat include a modest but proactive TDR-ISP initiative whereby a number of TDR scientists (20 in 1998) in Africa were approached directly by local/regional Internet Service Providers (ISPs) to facilitate, where feasible, a quick link to e-mail or full Internet service.

Such an approach has become feasible because the number of ISPs in developing countries is on the rise and competition among ISPs in major cities has become intense. A challenge remains, however, in securing full Internet connectivity for scientists working in rural sites.

TDR is collaborating closely with other research funding agencies in connectivity/networking activities under the framework of the MIM (Multilateral Initiative on Malaria in Africa) Communications Working Group, in a multi-agency effort led by the US National Library of Medicine.

TDR continues to promote Internet use for science, with a particular focus on least developed countries.





## APPLIED FIELD RESEARCH

Applied Field Research is aimed at improving the use of existing and newly-developed tools or methods through field testing their efficacy, acceptability and cost-effectiveness. It is research in disease endemic countries that seeks to provide practical evidence that is relevant and useful to disease surveillance and control. Applied Field Research generates specific recommendations for addressing social, environmental, economic and policy constraints relevant to public health and infectious tropical diseases. It has very clear goals and outcomes and provides results that can be used by control programmes.

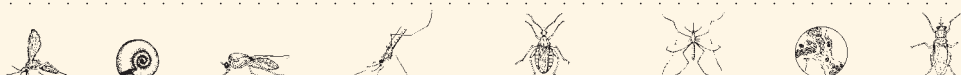
In TDR, most Applied Field Research (AFR) activities are managed by Task Forces constituted of multidisciplinary groups of outside experts in tropical disease research, prevention and control. Each Task Force reviews and funds research proposals, monitors progress and identifies areas where research capacity building is needed. Task Forces have a three year time period within which to accomplish set objectives, so the list of Task Forces is continually evolving. During the 1997–98 biennium, the following Task Forces or Steering Committees have existed – some are recent additions and others have concluded activities:

- Operational research on insecticide-impregnated bednets
- Severe malaria
- Malaria and health sector reform
- Research on drug resistance and policies
- Community directed treatment of the filariases
- Operational research on Chagas disease
- Operational research on African trypanosomiasis
- Chemotherapy of leprosy
- Tropical diseases and the environment
- Gender sensitive interventions

The following is a review of recent work on the various themes covered by one or more of these Task Forces. Many of the themes concern existing treatment – TDR has taken a variety of approaches towards making this more simple, effective, safe and convenient. Implementation of bednets, development of techniques for rapid assessment of disease burden in communities and individuals, assessment of social and economic costs, and vector control were some other foci of AFR during 1997–98.



*Measuring height to determine dose of ivermectin in Community Directed Treatment.*



## *Simplification of treatment*

It may not be easy to take or to fully comply with a treatment regimen. For instance, treatment may be long, as is multidrug therapy (MDT) for **leprosy** (6 months to 2 years). WHO has been advocating the use of combinations of drugs for the treatment of leprosy since 1982 and this has resulted in a dramatic reduction in prevalence of leprosy, from 5.4 million registered patients in 1985, to around 0.8 million in October 1998. Although MDT is incomparably easier on the patient than the earlier treatment of dapsone alone (which was often for life), and undoubtedly of great significance in the near elimination of leprosy, it nevertheless involves a patient in taking a total of three different drugs in four different dosages for 24 months (for multibacillary disease –

considered the more severe form of leprosy). This understandably makes MDT difficult to implement. It also presents an ordeal for patients, as leprosy is still associated with stigmatization – the longer the course of treatment the greater the stigmatization; 10–20% of patients do not complete the course.

As a result of studies completed during the biennium, the 24-month MDT regimen has been reduced to 12 months. This recommendation is based on field and experimental studies which showed that cases which fail or relapse after using the shorter regimen can be treated again with the same regimen. Reduction of treatment in this way does not significantly compromise the effectiveness of MDT, and represents

a considerable reduction in burden for both patient and health services. In this way, more patients are being reached through progressive simplification of treatment regimens.

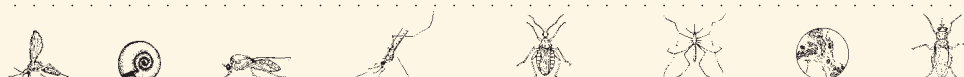
For paucibacillary (PB) patients, treatment is also being minimized. In PB cases with only one skin lesion, the lesion usually heals spontaneously and there is minimal risk of nerve damage, so patients often find it difficult to accept a diagnosis of leprosy or to comply with treatment. TDR supported a large, multicentre clinical trial in which a single dose of three drugs (rifampicin, ofloxacin and minocycline – ROM) was compared with the standard six-month course of two-drug MDT for single-lesion paucibacillary leprosy (monthly rifampicin and daily dapsone). A total of nearly 1500 adult and child patients were recruited into the trial, carried out at 9 sites in India. Final results in 1997 showed that a single dose of ROM is acceptable and cost-effective for treatment of single skin lesion paucibacillary leprosy.

## *Optimization of treatment for maximum impact*

In **lymphatic filariasis**, work on optimization of treatment has continued for a number of years. The initial two-drug treatment regimen – ivermectin in combination with diethylcarbamazine (DEC) – which had been shown to be significantly more effective than treatment with either drug alone, has now been joined by two other regimens found to be equally effective for long-term reduction of microfilaraemia. These are albendazole + ivermectin and albendazole + DEC, regimens that will form the mainstay of the new WHO Global Programme to Eliminate Lymphatic Filariasis.



*Sudan. A health worker explains to a leprosy patient exactly how MDT tablets should be taken.*



Community trials begun earlier (1992–94) at three sites (two sites in India and one site in Papua New Guinea) have evaluated various single-dose, once-yearly treatment regimens with DEC and/or ivermectin on the transmission of Bancroftian filariasis. The latest results show that all treatment regimens produce major reductions in transmission, but the two-drug combination of DEC and ivermectin is most effective. Reduction in transmission after three rounds of treatment was in the range 84–99% for the two-drug regimen. These studies are scheduled to continue until 2000. Even though this specific two-drug regimen will not be used in countries' elimination programmes, the studies will be continued to demonstrate the feasibility of interrupting transmission of lymphatic filariasis using such once-yearly, community-wide treatments. Results will be utilized in helping to refine predictions of the long-term impact of different treatment strategies as determined by the computer simulation model LYMFASIM. Other studies are directly testing the predictions of this model on disease transmission following chemotherapy using field data collected from studies in India and Tanzania.

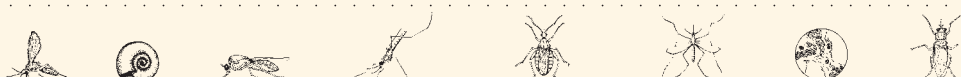
For **onchocercal skin disease**, a double-blind, placebo-controlled trial of different ivermectin treatment regimens (three-monthly, six-monthly and annual) at four sites (in Uganda, Ghana and two sites in Nigeria) was completed in February 1997. A decline of 40–50% in the prevalence of severe itching after ivermectin treatment (with all treatment regimens) as compared to placebo was apparent, and this decline was sustained for up to 12 months after the first treatment. There was also a statistically significant but small decline in the prevalence of reactive skin lesions in the ivermectin groups as compared to placebo, mainly as a result of a decline in early-stage skin lesions. Because of the demonstrated immediate effect of ivermectin treatment, in addition to the long-term preventive effect of repeated treatment, the researchers strongly recommended that use of ivermectin for the control of onchocercal skin disease be intensified. An in-depth, blinded analysis of serial skin photographs taken during the trial, which is yet to be completed, will provide further information about the effect of ivermectin treatment on skin lesions.

## *New treatment*

Preparatory work for large-scale, community-based trials of artesunate suppositories for coverage of children with **severe malaria** until they reach hospital has been carried out. The role and efficacy of this new therapy is being assessed in areas where no effective alternative treatment exists for patients who cannot take drugs by mouth – to cover them until they improve to the extent that they can take oral treatment. Sites for the trials have been identified in Africa and Asia, and when the trials begin, they are expected to verify the clinical benefit of the drug in the conditions of use and resolve any uncertainty regarding its safety. Final stages of the development of artesunate suppositories continue under Product Research and Development.

## *New drug regimens*

Even though MDT regimens for **leprosy** have been minimized, there is still room for improvement. This is why there is an emphasis on incorporating new drugs into MDT regimens, which may lead to increased effectiveness and shortened duration of treatment. One new drug is ofloxacin. A large-scale, double-blind, multicentre field trial is





ongoing at 15 sites in Asia, Africa and South America, to test the efficacy, safety and acceptability of ofloxacin-containing MDT regimens in comparison with the standard WHO recommended MDT regimen. Treatment of the nearly 3500 patients in the trial was completed in 1996; the patients are now being followed up for 5–7 years to detect relapses, if any. Short-term results have indicated that side effects and incidence of lepra reactions including nerve damage are minimal with all regimens. As well, patient compliance with the full course of treatment was seen to be excellent.

In the continuing search for better drugs for treatment of leprosy, a new study has been initiated comparing the activities of HMR 3647, clarithromycin, moxifloxacin, ofloxacin, rifapentine and rifampin against *Mycobacterium leprae* in a laboratory system.

## *Home treatment*

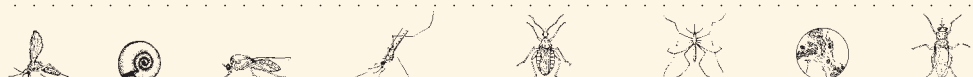
Although for some of the TDR diseases better drugs *per se* are needed, for most TDR diseases simply reaching the home and the community better with existing treatment can itself achieve much for disease control. In **uncomplicated malaria**, recent TDR-sponsored studies have shown that self-treatment at home using over-the-counter purchases is the dominant means of treatment, so there has been a push by TDR to explore feasible and effective ways to improve home management of the disease. This includes easy packaging of drugs, provision of clear information, involving and properly informing community members, and improving referral practices for severely ill children. Pilot studies using all or some of these approaches are ongoing in Ghana, Nigeria and Uganda on populations of over 10 000 people. The goal is to increase by 50% the proportion of pre-school children who receive early, appropriate home treatment for fevers. Results of the pilot studies will be available in mid-2000, after which large evaluation studies will examine the impact of improved home management of childhood fevers on mortality and morbidity.

## *Convenient packaging of drugs*

Drug packaging studies have shown that appropriate packaging can improve compliance with a full course of treatment for **malaria**, and have indicated that the prevalence of severe disease can be diminished if treatment is completed early in the course of infection. In Ghana, district-level studies showed that 82% of patients complied with the full course of packaged chloroquine tablets, compared to 65.2% in control communities; 54.3% and 32.5% respectively complied with chloroquine syrup doses. Packaging also reduced the under- or over-prescription of drugs by providers and the costs to patients. In South-East Asia, studies in six countries with multidrug resistance emphasized the importance of simple dose-packaging and simple advice in improving compliance and reducing recrudescence.

## *Clear information about treatment*

In Ghana, training and provision of information to communities, patients and drug sellers was shown to reduce the level of under- and over-supply of antimalarials, and the use of injectable and other non-essential forms of **malaria** treatment. In Kenya, 14 shopkeepers serving a community of 5000 were trained to offer information when



selling antimalarials and antipyretics (which reduce fever). The ensuing evaluation in nearly 600 childhood fever cases showed that the number of fever cases receiving treatment with chloroquine increased from 2% to 49%, and that the number treated with an adequate amount of chloroquine increased from 4% to 75%. This intervention is now being scaled up to district level.

### *Treatment for out-of-school children*

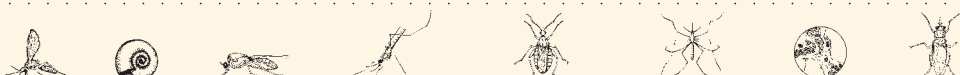
In **schistosomiasis**, TDR has placed emphasis on reaching out-of-school children. In Egypt, schistosomiasis is a major health problem in school-age children and earlier studies indicated that children who do not attend school regularly are more likely to be infected with the disease, and to be more heavily infected, than children who do attend school regularly. Although the Egyptian Ministry of Health and Population runs a school-based treatment programme for schistosomiasis in which health unit staff visit schools twice a year and selectively treat the children, studies have now shown that 15.8–61.6% of school-age children miss this treatment because of not being enrolled in school. Delivering interventions through schools should be cost-effective as an increasing proportion of children attend school. However, because it may be difficult to reach all children this way, a study was carried out to see how far treatment after screening could be extended to out-of-school children and to compare the costs of selective treatment (treating only infected children) with mass treatment (treatment of all children without prior screening). A very high proportion of out-of-school children (88.5%) were shown to be willing to make a visit to school in order to receive treatment, and cost-effectiveness analysis showed that mass chemotherapy is more efficient than selective treatment (due to financial and time costs). The study indicated that, if this intervention were to be implemented, only a relatively small number of children would miss treatment.

### *Involvement of the community in delivery of treatment*

In the **filariases**, earlier AFR studies indicated that Community Directed Treatment (ComDT) is a feasible method for mass treatment of populations affected by both onchocerciasis and lymphatic filariasis. The process of ComDT encourages communities to take control of their own treatment – to collect the drug from supply points, treat all eligible members, refer cases of severe adverse reaction, and report. Such a system is possible in the case of treatment of the filariases because the treatment is simple, consisting of a single dose taken just once a year.

In **onchocerciasis**, a multicountry study which began in 1997 in Ghana, Mali, Nigeria, Togo and Uganda, supported by TDR and the Onchocerciasis Control Programme, has an emphasis on seeing how ComDT works in real life, on how to integrate it with the health services and how the reporting system can be improved. In **lymphatic filariasis**, there are similar studies – a multicountry study under way in Ghana, Kenya, Myanmar, Viet Nam and India, is comparing a system of ComDT (with ivermectin or DEC) which involves the health services to varying extents with a system of mass treatment delivered by the health services alone. The two studies run in parallel and, where possible, support activities are organized for both studies together.

Results from the first phase of the studies were analysed in two workshops during the biennium. Attitudes to ComDT were generally found to be favourable. Roles for the



health services in ComDT include supervising, ensuring drugs are available, monitoring, and managing serious side-effects. District level authorities need to be involved and the functions of peripheral health services redefined, but although there is a will to do this, facilities are often insufficient – e.g. outreach services may be constrained by transport problems. Protocols and plans for the second phase of the studies, wherein two rounds of treatment will be given at 6-monthly intervals, were developed. Most results will be in by the end of 1999. It is hoped that the studies will open up opportunities for communities to look after their own health and take on board other diseases and other situations.

## *Drug resistance*

Stopping or delaying the development of drug resistance is essential in the fight against **malaria**. Now TDR has developed, in collaboration with other researchers and agencies, notably the Wellcome Trust, an integrated approach to the problem. This includes identifying markers of resistance for early detection and mapping of the extent of the problem, and predicting epidemics; optimizing treatment with drug combination regimens using chloroquine or sulphadoxine/pyrimethamine (the most affordable drugs for developing countries), or amodiaquine in combination with artesunate, and determining their effects on delaying resistance; improving the understanding of how antimalarial drug policies are formulated; improving the uptake of research results; and defining the criteria for changing policies, e.g. first- to second-line drugs. Work using combination therapy with artesunate for multidrug resistant *Plasmodium falciparum* infections in Thailand indicates that combination therapy with artesunate has the potential for halting the progress of drug resistance and increasing the longevity of existing drugs.

Drug resistance may also occur in the other TDR diseases – antimony resistance may be found in leishmaniasis for example, and perhaps praziquantel resistance in schistosomiasis. Even where drug resistance has not yet been observed, the situations are being closely monitored. In **onchocerciasis**, although it's thought unlikely that high level resistance to ivermectin will occur in the short term, techniques to detect resistance at the genetic level are being developed (under Product Research and Development). In **leprosy**, the most important component of the MDT regimen is rifampicin, and rifampicin-resistant *M. leprae* were identified when the drug was used alone as monotherapy. Recently however, it has been shown that the daily combination of dapsone and clofazimine is capable of eliminating any rifampicin-resistant mutants in an untreated MB leprosy patient within 3–6 months. So far, MDT has prevented the occurrence of drug resistance, but since rifampicin is a key drug in the MDT programme, resistance to it is being carefully monitored. The use of new drug regimens for treatment of leprosy will also help avoid the emergence of drug resistance problems.

## *Prevention of mortality with bednets*

Earlier TDR studies showed that insecticide-treated mosquito nets and curtains can significantly reduce all-cause childhood mortality in Africa (around 20%), and now emphasis is on effectively implementing, promoting and sustaining bednets in the long term.

'Dip-it-yourself' kits for treating nets with insecticides in the household have been developed by manufacturers of insecticides. A driving force behind this was that com-



munal 'dipping days' or community treatment centres which needed to be visited by net users failed to produce high rates of re-treatment of nets and were seen to be inadequate. A set of instructions for safe and effective use of the kits, even where literacy is low, has been tested thoroughly in urban and rural communities and adopted by two social marketing projects in Tanzania. 'Dipping-it-yourself' has now come to be recognized as the way most nets will be treated in the future, and most major pesticide producers have developed formulations and packaging adapted to this usage.

An area of concern is the very real threat of development of insecticide resistance to the pyrethroids used to treat the nets. Studies in two countries in areas where vectors are known to be resistant to pyrethroids, Benin and Côte d'Ivoire, have given encouraging results in that it seems likely that treated nets and curtains will remain effective for malaria prevention even in areas where pyrethroid resistance is already present. Treated nets still strongly deterred mosquitos from entering a room – whether they were resistant or not; and since resistant mosquitos spent a longer time in contact with treated netting they picked up a higher dose of insecticide and were killed almost as efficiently as susceptible mosquitos.



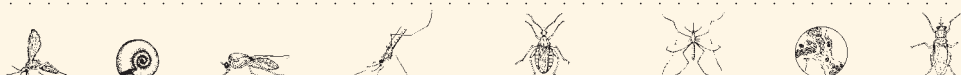
Ghana. Easy-to-use dipping kits allow bednets to be treated with insecticide at home, using simple equipment, whenever it is convenient.

## Rapid assessment

Before control programmes based on mass treatment can begin, the geographical distribution of a disease across a country or region must be determined and the particular communities to treat must be identified, priority being based on level of endemicity. New methods to rapidly assess the situation have been developed for onchocerciasis and lymphatic filariasis.

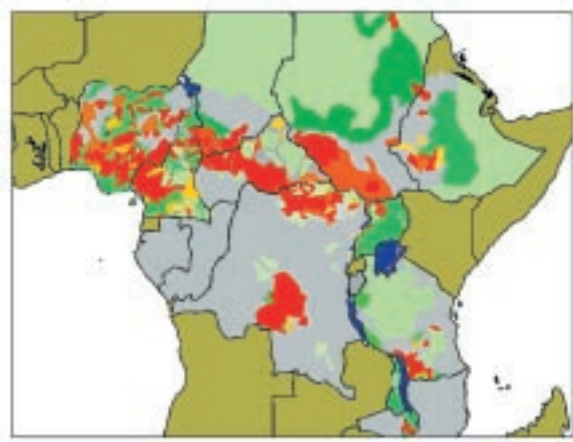
For **lymphatic filariasis**, TDR research has been helping to develop a simplified method for determining geographical distribution based on surveying limited samples of communities, as in Rapid Epidemiological Mapping of Onchocerciasis (REMO). Research to define the method was carried out in Ghana, India and Myanmar, and gave rise to a method for Rapid Geographical Assessment of Filariasis (RAGFIL) based on small numbers of villages selected using a grid sampling strategy. RAGFIL is now being tested in a multicountry study in Ghana, India, Myanmar and Tanzania.

For **onchocerciasis**, the established method for determining geographical distribution is REMO, which was developed by TDR and is now used by the African Programme for Onchocerciasis Control (APOC). REMO activities continued throughout 1997–98 in APOC countries: of the 19 countries, REMO and geographical information system (GIS) activities have been completed in 9 countries, partially completed in 6 countries, and have not yet begun in 4 countries. The results show a vast belt of hyperendemic onchocerciasis in the centre of Africa, ranging from south-east Nigeria,





Map 1. GIS – Onchocerciasis in central Africa



Prepared by HealthMap/CDS, in collaboration with TDR for APOC, December 1998.  
Base maps from the African Data Sampler, World Resources Institute.

through Cameroon, Chad, Central African Republic, Congo and south Sudan to north-east Uganda (see Map 1).

Other TDR-sponsored research is helping to develop simplified methods to replace the cumbersome, time-consuming, expensive, intrusive, night-time blood sampling currently used to assess the disease in communities. Two simplified Rapid Assessment Procedures (RAPs) for identifying communities were evaluated in a multicountry study:

*Physical examination for hydroceles.* There was good correlation between the prevalence of hydroceles and the prevalence of microfilaraemia and, after special training, local health workers were shown to be capable of clinically examining patients for chronic disease.

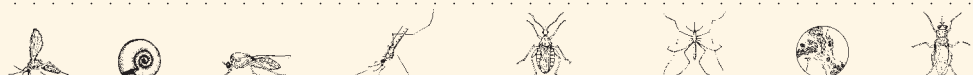
*Rapid Assessment Questionnaire.* In this method, key informants in each village (e.g. school teachers, village elders, women's leaders, traditional healers, health workers) answered questionnaires (either directly with health workers or indirectly through a delivery system involving Primary Health Care centres) about the prevalence of disease in the community. Results showed the Questionnaire to be a very promising tool because of its rapidity and cost-effectiveness ratio, but in need of further development and testing.

After analysis of results of the trial at a workshop in July 1997, two rapid geographical assessment methods were designed: one based on RAPs for hydroceles in a small sample of villages selected using a 50 km grid sampling strategy, and the other based on the use of questionnaires to be sent to key informants in a larger, but still circumscribed, sample of villages. These two RAPs can replace night-time blood surveys in assessing the presence and severity of lymphatic filariasis in a community and can help determine which communities to target with mass treatment.

## Diagnosis

The traditional test for diagnosis of **lymphatic filariasis** infection in individuals is to detect microfilariae in blood samples collected at night time (microfilariae appear in peak numbers in the peripheral blood between 22:00hrs and 04:00hrs). Recently, tests for detecting filarial antigens, which can be used at any time of the day, have become available. Two commercially developed antigen-detection assays have been field tested as part of TDR-sponsored multicountry studies; one is suitable for use in the field and the other for use in the laboratory.

The field assay – an ICT test (immunochromatographic whole blood test) – appears to work well in rapid card test format and is highly sensitive and specific for Bancroftian filariasis when immediate results are required, as in screening in the field or clinic; it worked well in the multicountry study – with a sensitivity and specificity of 99% in seven out of eight sites. However, in the eighth site – in South India – although specificity



was as high as in the other sites, sensitivity was only about 70%, and the test is being re-examined in this location.

A Rapid Assessment Kit, comprising all the equipment and documentation necessary for 50 tests as well as educational materials, has been developed for the ICT test and is currently being field tested by TDR in eight countries (Fiji, Ghana, Haiti, India, Kenya, Myanmar, Papua New Guinea and Tanzania). The cost of this assay is its main drawback (around US\$ 1 per test). However, the sensitivity and specificity of the test and the fact that it can replace the collection of blood samples at night time, may make it a valuable tool for rapid assessment and for evaluating the impact of control on the parasite reservoir. Because of its cost, the test is not likely to be used on whole communities, but on samples of communities and individuals who will be tested before and after treatment.

There is a great need for a simple and inexpensive test to detect **African trypanosomiasis** infection. The CIATT (Card Indirect Agglutination Test for Trypanosomiasis, an antigen detection test) was evaluated for specificity in a multicentre trial carried out in laboratories in Ghana, Côte d'Ivoire, Tanzania and Uganda. The CIATT is rapid and simple to perform; it was shown in earlier TDR studies to be specific and have potential for assessing cure following treatment. Results indicate that the CIATT gives reproducible results, can be used to diagnose both *Trypanosoma brucei gambiense* and *T. b. rhodesiense* infections, and gives similar results to other diagnostic tests such as the CATT and the polymerase chain reaction (PCR).

For early diagnosis of congenital **Chagas disease**, the PCR was found to be more sensitive than microscopic observation or immunofluorescence in a study in Paraguay, and, in another study, the IgG (immunoglobulin G) anti-SAPA (shed acute phase antigen) reagent was shown to have a specificity of 100% and a sensitivity of 67%. The prevalence of congenital infection was found to be up to 10% in endemic areas of Bolivia, and even up to 9.5% in urban Santa Cruz, demonstrating that Chagas is not necessarily a rural disease and that health centres should be alert to diagnosing it in pregnant women and their offspring.



Uganda. The CATT test (above) and new CIATT test will allow better, more rapid surveillance of individuals in affected communities.

## Prediction of epidemics

In many areas, **malaria** is likely to produce epidemics, and it is necessary to identify the geographical areas most at risk from these on a yearly basis. However, this has not, so far, been possible for malaria in Africa. Addressing this, TDR is supporting, through the Multilateral Initiative on Malaria in Africa (MIM) Task Force, the Pan-African collaboration on Mapping Malaria Risk in Africa (MARA), which is developing climate-based models to predict risk of malaria transmission on the basis of rainfall and temperature.





## Vector control

Aerial spraying of insecticides to destroy the breeding sites of the blackfly vector is used as the basis for **onchocerciasis** control in West Africa, in Onchocerciasis Control Programme (OCP) countries. In African Programme for Onchocerciasis Control (APOC) countries, where riverine vegetation is dense and aerial spraying cannot be carried out, the basis for control is treatment with ivermectin. However, a few foci in the APOC countries have now been identified as suitable for vector control with insecticides. These are the Itwara and Mpamba-Nkusi foci in Uganda, the Tukuyu focus in Tanzania, and Bioko Island in Equatorial Guinea (feasibility in this focus was confirmed in a TDR study). Elimination operations based on insecticide use began in these foci before the end of 1998.

In **Chagas disease**, traditional insecticides (e.g. pyrethroids) and slow-release insecticidal paints were shown to be the most effective and efficient combination of control measures in two studies – one in Nicaragua and one in Colombia.

The genetic profiles of vectors of Chagas disease in rural areas of Guatemala (*Triatoma dimidiata*) and in Colombia (*Rhodnius prolixus*) are being determined to help evalu-



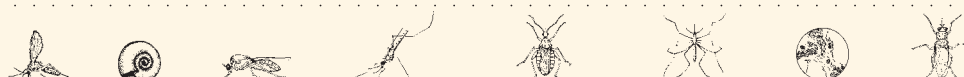
Colombia. *Rhodnius prolixus*, vector of Chagas disease in the country, offers a new challenge for vector control activities.

ate the efficacy of insecticides – differentiation of domiciliated from sylvatic vectors is necessary for control purposes. Initial results from Colombia, using the random amplified polymorphic DNA (RAPD) technique, indicate that there are two profiles of *R. prolixus*: one for vectors of sylvatic origin and one for domiciliated vectors.

To evaluate the impact of control programmes and the efficacy of insecticides, there is a need to monitor the effect of climatic conditions. The impact of microclimatic variables such as temperature, illumination and humidity on vector biology are being studied on *T. infestans* in Argentina. All these variables seem to affect the rate of hatching from eggs – an important parameter in vector population growth; temperature also affects the laying of eggs.

With regard to insecticide resistance, one centre in Argentina and one in Brazil have started to monitor the insecticide susceptibility of vectors following a common protocol developed at a meeting in 1994 held at the Center for Studies on Pests and Insecticides (CIPEIN), Buenos Aires, and sponsored by TDR. These two centres will serve the provincial and state control programmes of the different endemic countries who will regularly send vector samples to be tested for signs of insecticide resistance.

TDR has sponsored epidemiological studies to evaluate the impact of vector control measures in Paraguay and Chile. Results indicate that incidence of infection of **Chagas disease** in children less than five years of age has decreased by over 70% and 90% respectively, indicating that the transmission of the disease has been interrupted to the same extent.



## *Economic and social costs*

Earlier TDR studies showed that many **onchocerciasis** endemic communities, particularly those with low rates of onchocercal blindness, have a significant burden of onchocercal skin disease. Determination of the economic and social costs of this burden was the objective of a multicountry study, undertaken in Ethiopia, Sudan and Nigeria and completed during the biennium, on the economic impact of onchocercal skin disease. Some interesting measurements of the effect of onchocercal skin disease on labour input, and of severe reactive skin disease in the household on school attendance by children, were obtained.

As far as financial costs to the individual are concerned, people with onchocercal skin disease (OSD) spend US\$ 20 more each year (15% of their annual income) on health-related expenditures than people without OSD. Time costs are also substantial: people with severe OSD made significantly more visits to health care facilities and spent more time seeking health care.

As for social costs, where the head-of-household had OSD, children were twice as likely to drop out of school compared to other children of the same age from the same community. The relationship was especially strong among girls, who were 2.6 times as likely to drop out of school if the head-of-household had OSD than if the head-of-household did not have OSD. Given that OSD is very prevalent in endemic areas, it can be concluded that onchocerciasis is an important obstacle to child development.



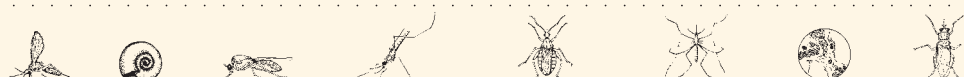
*Nigeria. Even when children do attend school, the severe itching of OSD reduces attention span and restricts educational performance.*

## *Malaria and anaemia*

Malaria is a major contributor to severe anaemia, and TDR-supported trials showed that in malaria endemic areas, daily oral iron supplementation throughout the first year of life prevented about 30% of severe anaemia episodes with no increased risk of clinical malaria. Where malaria chemoprophylaxis was given to infants weekly for 48 weeks from the age of two months, the rate of severe anaemia was reduced by between 61% (single episodes) and 64% (multiple episodes), with no increased risk of severe episodes following this prophylaxis. Thus, policies are now supported for iron supplementation of children in endemic areas to help prevent anaemia, and studies are ongoing to ascertain whether iron supplementation or intermittent treatment is more effective in protecting infants at risk from severe anaemia during their first year.

## *Tropical diseases and the environment*

Economic activities or policies that change land use, such as the creation of water dams, irrigation schemes and commercial tree plantations, and deforestation or refore-



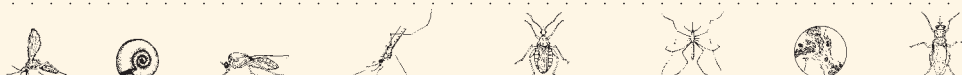
estation activities, can unintentionally result in changes in risk for vector borne diseases. This is because transmission of the diseases is influenced in a complex way by climatic conditions and by the immunity, mobility and behaviour of human populations. Consequently, understanding, quantifying and predicting the magnitude of the effects of changes in land use on tropical diseases is essential, and the Environment Task Force, which is co-managed with the International Service for National Agricultural Research, has concentrated on quantifying the benefits and costs of development projects in order to set the health gains and losses in context. In this way it is hoped to influence funders of major development projects to include assessment of health impact in routine appraisals of their projects, and guidelines are being developed to show how relevant improvements can be made in design and/or implementation of development projects. Studies under the Task Force have shown that 17-fold increases in malaria can result from development projects, and that decreases in leishmaniasis in coffee plantations can result from the use of new varieties of coffee bush.

## *Gender studies*

In TDR, the Gender Task Force (1994–97) began with a specific focus on women's health and tropical diseases. This approach has evolved in the newly constituted Task Force on Gender Sensitive Interventions (1998–2001). Because tropical diseases affect both sexes, the present Task Force supports research that examines how the interaction between biological and psycho-social factors can differentially influence men's and women's:

- exposure to tropical diseases;
- access to health services for early detection and to medication for treatment;
- access to and control over other resources that enable recovery, including attitudes towards health care;
- distribution of responsibilities and rewards in the health system, and how these affect the provision and quality of care.

To examine the interrelationships between gender and tropical diseases, the Task Force on Gender Sensitive Interventions is supporting 'demonstration research projects' in order to develop methods for, and ascertain the 'value added' by, incorporating a gender perspective in disease prevention and control programmes. The projects are focused on a number of TDR diseases in different parts of the world. To facilitate the process, TDR sponsored a proposal development workshop to strengthen the capacity of selected researchers to incorporate a gender approach into research design, methods and data analysis. TDR will continue to pursue a mainstreaming approach to gender, acknowledging that the main objective is for all AFR research to incorporate a gender perspective so as to increase the likelihood of prevention, early detection and appropriate treatment and improve health equity.



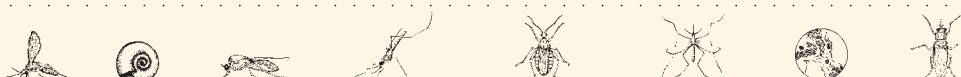
## PRODUCT RESEARCH AND DEVELOPMENT

The mission of Product Research and Development is the discovery and development of drugs and vaccines for the TDR target diseases. Drugs, although often inadequate or compromised by resistance, are still the mainstay for control of these diseases as no vaccine is yet registered for any parasitic disease. Due to the increased costs of developing and registering pharmaceutical products, together with the prospect of inadequate commercial returns, the pharmaceutical industry has largely withdrawn from the area of tropical parasitic diseases. To address this issue there was a considerable expansion of Product Research and Development (PRD) in 1997–98, coupled with a move from a disease-oriented administrative and management structure to a function-oriented matrix structure consisting of Drug Discovery Research, Vaccine Discovery Research and Product Development. Thanks to this change and to staff retirements, the PRD group in TDR (TDP) has had the opportunity of building a new team of professionals, many with previous experience of industry and/or the product discovery and development process. As well as drugs and vaccines, interest in the development of appropriate diagnostics is being rekindled in TDP.

In its work on drugs, TDP is involved in the many steps from discovery, through development, to registration of specific compounds and ensuring the drugs are available to those in need. **Drug Discovery Research** is managed through the recommendations of a Steering Committee together with logistical support from the secretariat.

The first step in drug discovery is to identify biological targets. With the expanding knowledge of parasite biochemistry it is now possible to identify important parasite enzymes and processes at the molecular level which might be suitable targets for drug action. Chemical compounds can then be identified that block the functioning of these targets and thus inhibit parasite growth. The initial lead compounds may result from high-throughput screening of many thousands of compounds, or may result from a more rational approach if structural information on the target is available. High-throughput screening is becoming more and more widely used in the pharmaceutical industry and TDP is accessing these systems for some of its biological targets.

Lead antiparasitic compounds can also be discovered by screening in more conventional parasite test systems, both in culture and in animal models. TDP supports the acquisition and testing of synthetic compounds and natural products in its lead discovery programme. Parasite test systems are critical in the further improvement of chemical leads and in the characterization and selection of compounds for clinical development. An aspect of drug discovery that is often underestimated by people outside the field is the parameters involved in the process. Lead compounds must be amenable to chemical





manipulation and, once identified, need to be optimized through an iterative process of testing and chemical synthesis until an active series of compounds has been identified. This chemistry may also need to take into account pharmacokinetics and toxicity parameters and ease of synthesis. The next step is to select the optimal compounds to undergo preclinical development studies; selection is made on the overall 'robustness' of the molecules for development.

Preclinical work focuses on two main areas: chemical development, including formulation studies and development of analytical procedures; and pharmacokinetics, especially toxicology studies in animals. Once preclinical work is completed successfully, a candidate drug will enter clinical trials, in which there are several stages. Further non-clinical development work on process chemistry, formulation and toxicology will continue during this period. While Drug Discovery Research is managed by recommendation through a Steering Committee, the development of individual candidate drugs is managed by separate **product development** teams. The process overall is monitored by the professionals from TDR and the pharmaceutical industry which make up an R&D committee and meet monthly to assess the portfolio. There is an emphasis on rapid progression of compounds from Drug Discovery Research into Product Development, and, should a molecule appear unsuitable for further development, terminating studies quickly. Special attention is paid to intellectual property rights in order to optimize the likelihood of obtaining pharmaceutical industry partners.

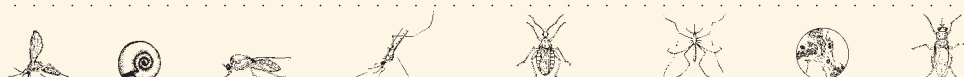
As for drug discovery research, **Vaccine Discovery Research** is also managed by a Steering Committee. Vaccines are being developed for malaria, leishmaniasis and schistosomiasis. They promise to be cost-effective interventions. In the last decade there has been considerable progress in understanding immune mechanisms to parasitic diseases,



*Iran. A potential vaccine for cutaneous leishmaniasis is undergoing field trials in schoolchildren.*

identifying vaccine candidate antigens and their genes, developing formulations with novel adjuvants, and testing the vaccines in animal models. Because of the complexity of parasitic diseases and the costs of vaccine development however, relatively few candidate vaccines have yet progressed to clinical or field trials. Parasite genome projects are giving rise to a multitude of new information on genes and their products, allowing new antigens to be identified; however successful development of parasite vaccines will require extensive collaboration with industry, leading scientists, institutions and disease endemic countries.

Vaccine Discovery Research is involved with identification of candidate antigens, evaluation of optimal combinations of antigens, and evaluation of various formulations and delivery methods including adjuvants, DNA vaccines, synthetic peptides, recombinant proteins and attenuated bacterial or viral carriers. Many of today's novel adjuvants (neutral substances which enhance the body's immune response to antigens) are promising, but to date, the only licensed adjuvant for disease control use is alum. However, pharmaceutical companies are now involved in the development of novel adjuvants and a number of new adjuvants are currently in clinical testing. The best antigen/adjuvant combinations are selected for development, with management, as for drugs, by separate **product development** teams. The overall vaccine discovery and



development process is, as for Drug Discovery, monitored by professionals from TDR and the pharmaceutical industry who make up an R&D committee and meet monthly to assess the development portfolio.

The following is a review of the current status of TDR-sponsored Product Research and Development activities in the various diseases.

## *Malaria*

In malaria, the need for new drugs is particularly urgent owing to increasing acquired drug resistance of the parasites. New antimalarials for treatment of uncomplicated disease need to be orally active and inexpensive to produce.

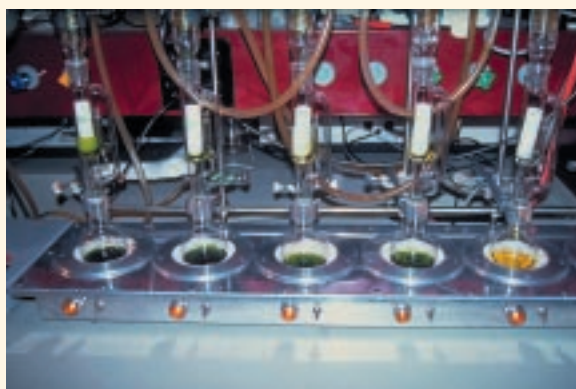
In **Drug Discovery Research**, several types of molecular drug target are under investigation. One target is haemoglobin degradation and biochemistry of the digestive vacuole in the malaria parasite. It is estimated that up to 80% of haemoglobin in an infected red blood cell is metabolized during the cell's infection by the parasite, and several protease enzymes involved in this metabolism are now recognized as potential drug targets. Attention is also focused on the haem molecule released during the metabolism of haemoglobin, which is thought to be the molecular target for action of chloroquine and endoperoxide antimalarials.

Nucleic acid metabolism is another focus of attention. The folate pathway is essential to the parasite, and two important classes of antimalarial (dihydrofolate reductase inhibitors and sulfa drugs) inhibit this pathway. It is hoped to develop better inhibitors that will overcome resistance to known drugs and to explore inhibitors for enzymes of the pathway other than those blocked by current antimalarials.

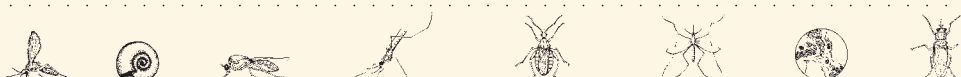
Transport processes, such as nucleoside transport and choline uptake, are also of interest, as is the apicoplast, a recently discovered organelle within the parasite which is essential for its growth. The apicoplast is thought to be the target for action of antibiotics (e.g. tetracycline) sometimes used in the treatment of malaria. A source of inspiration for the identification of new drug targets is the genomic information arising from the malaria genome project, funded through the Wellcome Trust, Burroughs-Wellcome Foundation, National Institutes of Health and the US Department of Defense.

For many of these molecular targets, assays are being developed and industrial partners sought for high-throughput screening of chemical libraries. In lead discovery work, TDP is also supporting several projects on screening of natural products, particularly from plants. Some very promising leads have been identified and attempts are being made to further improve these.

In lead optimization work, TDP currently has two major projects in malaria. One concerns research on the synthesis of more stable semi-synthetic derivatives of artemisinin and fully synthetic (second generation) endoperoxides. Some second generation endoperoxides have been identified with good activity in both culture systems and animal models and are being investigated in depth. The other major project is focused on choline uptake inhibitors. A series of highly active compounds



*Artemisinin is extracted from the plant Artemisia; it has served as a starting point for synthesis of several compounds that are in development and clinical use for malaria.*





has been developed based initially on choline analogues, and current efforts are aimed at enhancing the oral activity of these compounds.

In **clinical development**, there are four major drug development projects operating through TDR at the moment. The first involves the evaluation of a new formulation (suppositories) of artesunate, for treatment of severe childhood malaria. When malaria is severe and the child unable to take oral treatment, those living in inaccessible places may die before they reach hospital. Since 1996, TDR has been working to register artesunate suppositories for treatment of such patients until they reach hospital; the formulation has been developed for use at the periphery by persons with training in its delivery. Submission for registration of the drug in Europe and the USA for this specific purpose is expected by the end of 1999.

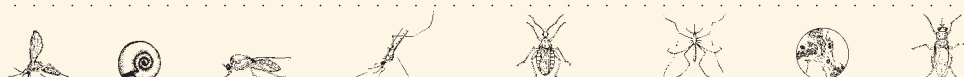
The second concerns an intramuscular formulation of arteether, developed with Artecef. Clinical development of this compound is now almost complete and the final parts of the clinical dossier should be submitted to the Dutch regulatory authorities by mid-1999.

For treatment of uncomplicated malaria in Africa, two compounds have reached late Phase II clinical development. These are oral formulations of pyronaridine and chlorproguanil-dapsone. The former is already marketed in China. Pyronaridine is seen as a potential low-cost replacement for the oral treatment of uncomplicated malaria in areas where there is resistance to commonly used antimalarials; data indicate that it is effective in cases of chloroquine resistance and is satisfactorily tolerated. Both oral and injectable formulations of the drug are on sale in China. However, the current Chinese dossier does not meet international regulatory standards, and TDP is engaged in efforts to develop a regulatory dossier for pyronaridine that would allow it to be used in Africa in areas where there is resistance to drugs such as chloroquine and sulphadoxine-pyrimethamine.

Chlorproguanil-dapsone is being developed as a fixed combination in joint partnership with the Department for International Development (DFID), UK, and SmithKline Beecham Tropicals. The idea is to develop an alternative to sulphadoxine-pyrimethamine using two molecules which are not cross resistant to, and which have much shorter half-lives than, sulphadoxine-pyrimethamine (Fansidar). Resistance to molecules with short half-lives should develop more slowly than that to longer-lived molecules. Thus, such a combination could not only be used as a replacement for sulphadoxine-pyrimethamine when resistance to it has developed, but also as a better alternative to chloroquine when this can no longer be used as first-line treatment. Because both components of the combination have been used before in humans, total residual development costs are estimated to be a modest <US\$ 3 million. Also, the necessary chemistry/pharmacy, preclinical and clinical development activities are being done in parallel, which should allow submission to regulatory authorities by as early as mid-2001.

In **Vaccine Discovery Research**, candidate vaccines are based on various antigens or combinations of antigens derived from different stages in the life cycle of the malaria parasite. Vaccines which target different antigens from different stages in the parasite's life cycle stand a better chance of being able to control the parasite in the host and eventually in the community.

Vaccines which target the *asexual blood stages* (merozoites) of the parasite prevent it from entering or developing in red blood cells. These asexual stages of the parasite are responsible for the symptoms of malaria, and a vaccine affecting them would have a



great impact on disease morbidity and death, although it would not necessarily prevent people from becoming infected.

About a dozen promising asexual blood stage candidate vaccines for *Plasmodium falciparum* malaria are in various stages of research and development. Those funded by TDR include MSP-1, MSP-2, MSP-5, EBA-175, RAP-1/RAP-2, SERA and MAEBL. Some of these candidates are quite advanced in the research pipeline, including MSP-1.19 and MSP-1.42, which are being expressed in baculovirus and two, EBA-175 and SERA, that are about to enter preclinical studies.

SPf66, a synthetic peptide 'cocktail' vaccine developed in Colombia, has now been field tested in a number of different settings in South America, south-east Asia and Africa, and has given mixed results. Recent results of a pivotal Phase III trial in Tanzanian children under one year old – the high-risk group – were disappointing. A new formulation of this vaccine, with a novel adjuvant QS-21, is now under Phase I clinical testing – initial results demonstrated an enhanced antibody response to the peptide. Additional Phase I dose-ranging trials are planned for 1999 by AQUILA, the commercial partner developing the vaccine with the developer, Dr M. Patarroyo, and WHO/TDR.

Apical membrane protein-1 (AMA-1) is a leading candidate vaccine under development in Australia, with long-term support from TDR. More recently, a commercial company – Vaccine Solutions – and USAID have joined the team. A Phase I trial is ongoing in Brisbane with the recombinant protein antigen formulated with Montanide ISA-720 as adjuvant. Phase II trials are scheduled for early 2000 in Papua New Guinea (PNG), where malaria epidemiology in children and mothers resembles that of many regions in Africa.

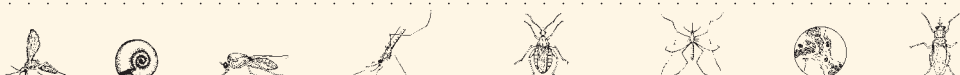
Combination B is a 3-component vaccine comprised of MSP-1, MSP-2, and RESA antigens together with Montanide ISA-720 as adjuvant. This combination vaccine has undergone Phase I and II trials in Australia and PNG, with support from outside the TDR programme. No protection was observed in a Phase I/IIa challenge assay using the semi-quantitative PCR assay system; results of the recently completed Phase IIb trial in PNG are expected soon.

A multi-stage, multi-component *P. falciparum* vaccine is being developed outside of TDR by a group in Atlanta, USA. It is based on epitopes (particular sites on antigens to which specific antibodies bind) identified from a study in Kenya. The synthetic vaccine gene contains 12 B cell, 6 T helper cell and 3 CTL epitopes, derived from 9 antigens (CS, SSP-2/TRAP, MSP-1, MSP-2, LSA-1, AMA-1, RAP-1, EBA-175 and Pfs27). The candidate 41kDa recombinant protein vaccine is expressed in baculovirus, and studies in rabbits have indicated that antibodies are induced which react significantly with the antigen and native parasites. They also showed functional activity in inhibition of invasion assays conducted *in vitro*. In 1999, there will be challenge studies in monkeys.

**Pre-erythrocytic stage vaccines** are designed to prevent the parasite's infective sporozoite stage from entering or developing within liver cells of an individual bitten by an



Tanzania. African children stand to benefit the most from an effective vaccine.



infected mosquito. This type of vaccine would prevent infection in non-immune individuals, thus averting the severe, life-threatening consequences of malaria.

The main focus so far has been on vaccines based on the circumsporozoite (CS) protein, of which the most promising is RTS,S, now in Phase IIb trials in The Gambia and Kenya. This vaccine is under development by SmithKline Beecham Biologicals and the Walter Reed Army Institute of Research (WRAIR). It consists of a recombinant DNA-produced CS-based antigen formulated in a novel adjuvant, SBAS2; it is complex and highly immunogenic. Phase I/IIa clinical trials (in Washington, DC) confirmed that the formulation is capable of inducing protective immunity in over 50% of volunteers challenged with five infected mosquitos. The duration of this observed protection remains an important issue. TDR has provided independent international monitors for the field trial in The Gambia. It has been requested to do the same for additional proposed field trials in Kenya, aimed at testing a new formulation of the vaccine which includes the liver stage antigen SSP-2/TRAP.

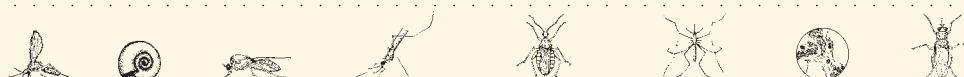
The second major CS-based candidate vaccine in clinical testing is a DNA version of the vaccine, under development by a group working at the US Department of Defense Naval Research Institute, in collaboration with Pasteur Mérieux Connaught and VICAL Corporation. In the Phase I studies to date, the safety profile was good, even at 2500 µg DNA per injection, and some impressive CTL responses were observed. On the other hand, as observed in other human DNA vaccine studies, antibody responses were disappointing. In addition to funding some of the previous basic development research for these DNA malaria vaccines, TDR has been requested to participate in the related capacity building aspects of preparing for eventual field trials of such DNA vaccines in Africa.

A third vaccine candidate based on the CS protein has been developed in the form of a synthetic Multiple Antigen Peptide (MAP), formulated with QS-21, another novel saponin-derived adjuvant. The vaccine, which has been developed by a group at New York University together with USAID support, is currently under Phase I/II trial in the USA.

And finally, a fourth CS protein-based synthetic peptide vaccine candidate under development at the University of Lausanne has just completed a Phase I trial in Switzerland. This 102 amino acid synthetic peptide vaccine is formulated with yet another novel adjuvant, Montanide ISA-720. Excellent results were obtained concerning the safety profile for the vaccine and for the antibody, CTL and cytokine responses measured, compared to alum.

**Transmission-blocking vaccines** are aimed at preventing the successful development of the parasite in the mosquito host. Such a vaccine would, if effective, help eliminate transmission of malaria in areas of low endemicity, but in areas of high transmission the vaccine would be used in combination with effective pre-erythrocytic and asexual blood stage antigens. A transmission-blocking vaccine would also contribute to controlling the emergence of drug resistant parasites and/or potential escape variants selected by partially effective pre-erythrocytic and asexual blood stage vaccines.

The leading *P. falciparum* candidate antigen currently under development by TDR and the National Institutes of Health (NIH) in the USA is Pfs-25. Formulated with alum as adjuvant, an initial Phase I trial with this antigen showed it to be safe and to induce antibodies, but they were not functional in a membrane feeding assay (an *in vitro*



system used to monitor inhibition of parasite development in the mosquito). In a second Phase I trial, using volunteers previously immunized with NYVAC-7, an attenuated vaccinia virus multi-stage vaccine containing 7 genes including the gene for Pfs-25, several volunteers did produce significant levels of transmission-blocking antibodies after one booster injection with Pfs-25, thus providing evidence that humans can be immunized to produce antibodies capable of blocking development of the sexual stages in the mosquito. The next major hurdle will be to test this vaccine candidate for transmission-blocking activity in semi-immune volunteers in malaria endemic countries.

A new, improved formulation of Pfs-25 plus alum is currently under development in a collaborative **technology transfer** project with the Hong Kong Institute for Biotechnology (HKIB). The HKIB participated in a TDR-sponsored technology transfer initiative, aimed at improving the capacity of relevant institutions in disease endemic countries to produce Good Manufacturing Practice grade clinical trials materials for use in Phase I/II trials. The three-year collaborative project between NIH, TDR and HKIB has been very successful. A Phase I trial of the new formulation of Pfs-25 is scheduled to start in Hong Kong in the second half of 1999.

In 1998 TDR sponsored a successful workshop in Bamako, Mali, which was aimed at setting the groundwork for initiating multicentre studies on the validation of the *in vitro* membrane feeding assay as a tool for use in the field for monitoring the development of transmission-blocking activity in human volunteers in malaria endemic countries. The results suggest that areas with intense but seasonal transmission might be feasible sites for testing transmission-blocking vaccines because of the high gametocytemic and mosquito infectivity rates and lack of pre-existing humoral-mediated transmission-blocking activity in adolescent volunteers.

## Schistosomiasis

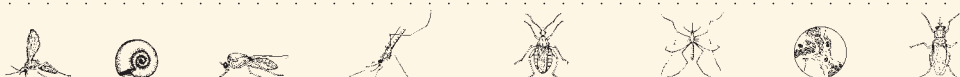
Control of schistosomiasis rests, in principle, on one drug, praziquantel, and the entire strategy would be in jeopardy should resistance to this drug emerge. Before long, we therefore might need new drugs. Today however, TDR efforts are focused on vaccines.

The majority of humans living in areas endemic for schistosomiasis acquire relatively good levels of immunity against schistosomiasis but the mechanisms which protect them from the disease are still largely unknown. TDR sponsors work on vaccines against *Schistosoma japonicum*, mainly found in China and the Philippines. The zoonotic nature of this species means that water buffalo and other large animals can be used as experimental hosts which might reflect the situation in man; a veterinary product could also have a positive transmission-blocking effect for human populations.

Scientists are encouraged by the fact that injection of attenuated cercariae (a free-living infectious form released from the snail vector) is capable of producing almost complete (sterile) immunity in experimental animals. However, rather than sterile immunity, reduction in risk of re-infection without stimulation of the egg-associated granuloma reaction is sought – since schistosomes do not replicate in the human



Male and female schistosome worms pair in the human body, and eggs are laid continuously, causing morbidity. However, the life cycle is not completed in the human host.





host, even a partially effective vaccine would reduce the development of morbidity. As transmission is not expected to be fully interrupted, natural infection may be counted on to boost vaccine-induced protection. The combination of chemotherapy followed by vaccination promises an effective intervention with long-term effect.

Currently TDR is monitoring the development and eventual field testing of four priority *S. mansoni* and one *S. haematobium* vaccine candidates. Parallel research efforts are being funded by TDR towards the development of a *S. japonicum* vaccine, and several antigens are at the stage of clinical testing in cattle, pigs and water buffalo. A *S. haematobium* glutathione-S-transferase vaccine has advanced to Phase I trials in France and, if successful, will be tested in Phase II/III field trials in a multicentre study in Africa. TDR has been requested to provide independent clinical monitors for the subsequent field trials in Senegal, Niger and Madagascar.

## *The filariases*

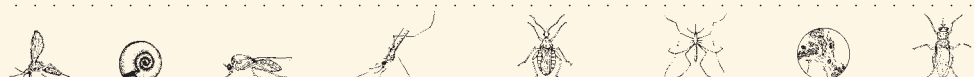
To cure filarial diseases, it is necessary to destroy the adult worms in a patient. Drugs which destroy immature worms are available, but as yet there are no safe drugs which kill adult worms (macrofilaricides). An ideal (safe and effective) macrofilaricide would need to: lessen progression of the disease and even cause regression of some symptoms; prevent recrudescences; reduce transmission; be safe for use in adults, including pregnant women, and in children, without direct medical supervision; be safe for use in patients receiving treatment for other parasitic infections; and be easy to use, guaranteeing good compliance.

Molecular targets in filarial worms were identified and discussed in 1998 at a meeting in TDR with scientists from industry and academia. Novel targets on which studies are being supported include glutathione-S-transferase, lipid modification enzymes, tubulin, and the endosymbiont *Wolbachia*. Studies involving TDR scientists suggest that killing the symbiotic bacteria that live inside the worm with antibiotics could be effective therapy especially if combined with existing drugs (e.g. ivermectin). The worms are weakened by killing the bacteria, and the adult worms produce fewer larvae.

There has also been rationalization of current screening systems for antifilarial compounds. A high capacity, parasitological *in vitro* screening system to bridge the gap between high-throughput molecular screening and high-cost, labour-intensive, efficacy and toxicity trials in animals has been evaluated. Also, the primary *in vivo* screening system has been rationalized in order to increase the number of compounds tested.

In preclinical trials, molecules chemically related to known anthelmintic or other antiparasite drugs are being evaluated, e.g. moxidectin, which is used in veterinary medicine. This has been found to have interesting macro- and microfilaricidal activity in animal models and studies are ongoing to see if it should be evaluated for human use. During 1997–98, and following preclinical trials in animal models, further work on the compound UMF 078 was terminated. In addition, lack of efficacy in a Phase II clinical study of amocarzine led to closure of that project also.

Efforts are being made to develop diagnostic markers for ivermectin resistance in TDP. Ivermectin resistance has developed in veterinary worms, and there is a fear this could happen for *Onchocerca volvulus*. At present, researchers are using nematode models to try to identify suitable genes to serve as diagnostic markers for the genotype/pheno-



type changes associated with resistance. The second phase of studies will be to validate the resulting observations using parasite material from humans. Finally, gene products will be isolated and a diagnostic tool for resistance designed.

## ***Leishmaniasis***

New drugs are needed for leishmaniasis because the standard treatments can only be given parenterally; the treatment courses are long, expensive, and may elicit severe adverse reactions; and key products such as the antimonials are being compromised by drug resistance. Compounds are being regularly tested against *Leishmania* through the Drug Discovery Research Screening Centres and new molecular target directed approaches are being sought for *Leishmania* drug discovery. A plant natural product is currently undergoing extensive preclinical testing.

One compound to be discovered as a result of these efforts is the orally active agent miltefosine, which is now in advanced clinical trials. Miltefosine is an anticancer drug that TDR has progressed to multicentre Phase II trials for visceral leishmaniasis. It is particularly active against intracellular *L. donovani* in animal models and, in a small dose-escalation clinical trial in India, was shown to be effective against visceral leishmaniasis.

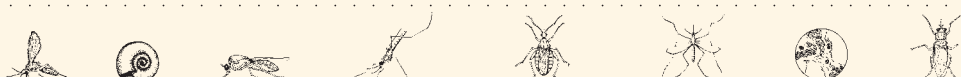
In a multicentre Phase II dose-finding study in three centres in India, the duration of treatment ranged from 4–6 weeks. To date the results are encouraging and suggest that oral miltefosine is well tolerated. At the 3-month follow-up, the drug had produced excellent parasitological cure rates at all doses tested; results of the 6-month follow-up will be available in 1999. Phase III trials of the compound against visceral leishmaniasis have been agreed for three centres in India.

Oral treatment for visceral leishmaniasis has long been sought, and hopes are high that miltefosine will be the first candidate in this respect.

Another drug in clinical trials is paromomycin, also known as aminosidine. This drug, in injectable form, has been used for bacterial and parasitic infections for more than 30 years and is now known to be active against *Leishmania*.

Two clinical studies conducted in Bihar, India, where there are high failure rates to the standard treatment of sodium stibogluconate (an antimonial), showed that injectable paromomycin could prove more effective than sodium stibogluconate and appears to be safe. In the studies, the safety and efficacy of paromomycin alone at three different dosage regimens (12, 16, 20mg/kg/day x 21 days) compared with sodium stibogluconate (20mg/kg/day x 28 days) was evaluated. Results from both studies indicate that injectable paromomycin at all dosage levels is more effective than sodium stibogluconate. In one study, paromomycin alone at 16 or 20mg/kg/day was statistically more effective than the antimonial alone, and, in the other study, paromomycin alone at 12mg/kg/day was statistically more effective than the antimonial alone. Data from both trials indicate that not only is paromomycin effective but it is safe – mild, dose-related oto-toxicity is the most common adverse reaction.

The Paromomycin Development Project for visceral leishmaniasis is a collaborative effort involving a number of public and private entities. The effort originated with TDR, and over the past 5 years, has produced a significant body of new data. These data, combined with historical data, form an almost complete regulatory dossier for





paromomycin for the treatment of visceral leishmaniasis. However, a new manufacturer of clinical trials material needs to be identified and definitive Phase III studies of paromomycin are yet to be carried out.

Injectable paromomycin has also been used, in combination with antimony, in a trial to treat mucocutaneous leishmaniasis in Peru. Preliminary results indicate that the combination is more effective than single-agent standard antimony. Previously, paromomycin alone proved insufficient for treatment of this disease in the same area.

Regarding **vaccine development**, scientists believe that a leishmaniasis vaccine will be feasible and cost-effective because long-lasting and strong immunity to reinfection follows recovery from leishmaniasis infection; *Leishmania* species share many dominant antigenic determinants; and protection with experimental vaccines and cross-protection has been shown in many experimental models. So far, most work has been on first-generation vaccines, which are constituted from live or attenuated promastigote stages of different species of *Leishmania* with BCG as adjuvant. A variety of these vaccines have now been tested in primate models and have undergone, or are undergoing, field trials for both cutaneous and visceral leishmaniasis. The trials are progressing well.

For cutaneous leishmaniasis in the Old World, field trials have been carried out in two different epidemiological regions of Iran using a vaccine composed of *L. major* promastigotes + BCG given in a single dose. Some protection was found, and work continues using the vaccine in multiple doses and with different adjuvants. For New World cutaneous leishmaniasis, Phase II/III trials in Brazil, Colombia, Ecuador and Venezuela have been carried out with vaccines composed of promastigotes of different species of *Leishmania* native to the continent, both with and without BCG.

For visceral leishmaniasis, testing of a first-generation vaccine was completed in 1998

in the langur monkey model developed by TDR; three injections of killed *L. major* whole cells + BCG (the same vaccine as used in Iran) produced protection. The vaccine is now in Phase III clinical trials for visceral leishmaniasis in Sudan where a large-scale epidemic of leishmaniasis with high fatality continues.

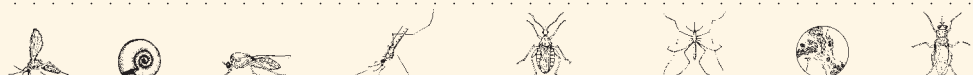
In most of the above trials, the vaccines have been constituted with BCG, but other studies have looked at first-generation vaccines constituted with other adjuvants. For cutaneous leishmaniasis, preliminary data using alum-precipitated killed *Leishmania* plus interleukin 12 (IL-12) or BCG indicate that the preparation with alum is far more immunogenic than the

one without alum; a preparation of alum and *L. major* is being developed for visceral leishmaniasis in Sudan. In Brazil, a preparation of merthiolate *L. amazonensis* (without BCG) was shown to be immunogenic in Phase II trials.

Second-generation vaccines against leishmaniasis, which are constituted from genetically attenuated parasites incapable of producing disease or from recombinant protein molecules or their corresponding DNA mixed together in a 'cocktail', are under



Sudan. A child undergoing a leishmanin skin test to determine whether she can take part in the visceral leishmaniasis vaccine trial.



development. Recombinant *Leishmania* antigens are currently being evaluated for possible inclusion in such a cocktail, and could enter preclinical testing by 2002.

In leishmaniasis there has also been work on diagnostics. A simple, inexpensive test is needed for diagnosis of visceral leishmaniasis under field conditions to replace the present invasive method of detection of parasites in spleen or bone marrow aspirates. TDR, in collaboration with WHO's former programme for Control of Tropical Diseases and other partners in Bangladesh, Kenya, and Sudan, performed a multicentre trial, completed in 1998, for evaluation of the Direct Agglutination Test (DAT). The test is easy to use and can be produced in the disease endemic countries; it was found to have high sensitivity and specificity if combined with clinical signs and symptoms of visceral leishmaniasis patients, but cannot be used as the sole diagnostic tool before starting treatment. It is planned to produce the DAT under Good Laboratory Practice at low cost in some disease endemic countries until cost-effective tests are developed.

## Chagas disease

There have been significant improvements in the control of Chagas disease by breaking the transmission of the disease through targeting the insect vectors. However, new drugs are still needed, especially to overcome the chronic form of the disease which still affects many millions of people. The Drug Discovery Research group regularly screens compounds for activity against Chagas disease and has identified several antifungal azoles that are undergoing detailed evaluation in animal models.

There has been further work on benznidazole. An efficacy trial of this drug for treatment of children during the early chronic phase of the disease was carried out in 1991–95. Five years of follow-up has shown that treatment with benznidazole produces significantly reduced risk of developing electrocardiographic lesions indicative of chronic pathology of the heart. This indicates the need for a policy concerning clinical management and treatment of children aged 7–12 years in the early chronic phase.

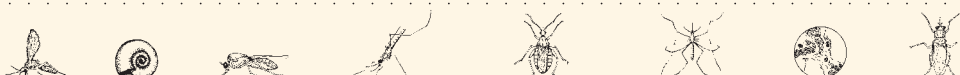
## African trypanosomiasis

In African trypanosomiasis, each of the drugs in use has its drawbacks. Pentamidine and suramin, used only for early-stage disease, both have serious side effects. Of the two drugs used for late-stage disease, melarsoprol is very toxic and even fatal while eflornithine is expensive and only affects *Trypanosoma brucei gambiense*. None of the African trypanocides can be given orally.

Drug Discovery Research funds several projects directed at exploiting potential novel drug targets such as trypanothione reductase and methionine recycling. A project to discover novel compounds with activity against late-stage disease, using animal models, is being supported at the Shanghai Institute of Pharmaceutical Industry, with logistic support and testing of compounds being coordinated through TDR.



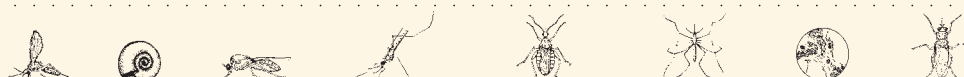
Uganda. Suramin and melarsoprol, mainline drugs for African trypanosomiasis. Both have serious drawbacks and new drugs are urgently needed.



Also, in connection with late-stage disease, a diamidine – CGP 40215 – is currently being assessed in vervet monkeys to determine its activity against late-stage disease and its ability to cross the blood-brain barrier. An industrial development partner is already available if this study shows promise.

Eflornithine is the least toxic of the two drugs available for treatment of late-stage disease but is expensive to produce. In efforts to reduce the costs of treatment with eflornithine, TDR sponsored trials to see if 7-day treatment, as opposed to the currently recommended 14-day regimen, would be appropriate. This would help reduce costs considerably. Final results of the trial showed that, for relapsing cases, the 7-day regimen can be recommended with the proviso that all treatments are closely followed up. For new cases however, the 14-day regimen continues to be recommended, and is highly effective except in Uganda (where collection and characterization of strains from north-western areas for drug sensitivity is now ongoing). Together, data from the studies show that the 7-day regimen is significantly less effective than the 14-day regimen and studies on drug combinations have been recommended – there is experimental evidence for synergism between eflornithine and melarsoprol (the other, more toxic, drug that is effective in late-stage sleeping sickness).

TDR is also looking at synthesizing eflornithine by a new route and as an oral formulation. Success here could quarter the cost of treating a patient.



## STRATEGIC RESEARCH

The aim of TDR's Strategic Research component is to develop new interventions for tropical diseases by building on advances made in the fields of molecular biology, immunology and gene technology. Developing tomorrow's tools from today's advances is a long process and the targets of Strategic Research are long term – some of them could take more than 15 years to reach but powerful tools developed in the process will facilitate long-lasting interventions. The work is directed by a single Steering Committee with each of the three areas – pathogenesis, parasite genome and molecular entomology – managed by a sub-committee. A fourth area of Strategic Research, the Immunology of Mycobacteria, which is concerned with leprosy, was managed during the biennium in collaboration with WHO's Global Programme for Vaccines and Immunization.

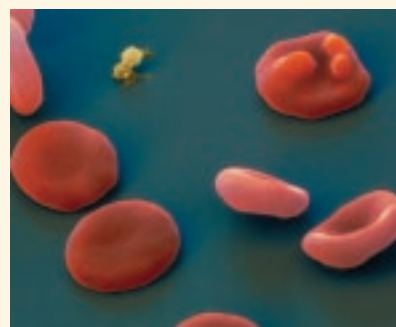
### *Pathogenesis*

The pathogenic effects of parasitic infections may be so subtle as to be unrecognizable, or they may be strikingly obvious, as for most of the TDR target diseases. Each has its own unique pathology – falciparum malaria is particularly associated with sequestration of infected red blood cells in the brain; schistosomiasis with pathology of the liver or urogenital organs; lymphatic filariasis with the deformities of elephantiasis; onchocerciasis with blindness and skin lesions; leishmaniasis with an array of different pathologies depending on whether the skin, mucous membranes or internal organs are involved; Chagas disease with the heart and/or intestines; African trypanosomiasis with lesions of the central nervous system; and leprosy with lesions of the peripheral nerve trunks, resulting in damage to the skin and distressing deformities. Research is helping to clarify some of the pathogenic mechanisms involved in disease etiology and progression. This work also contributes to the identification of potential drug and vaccine targets.

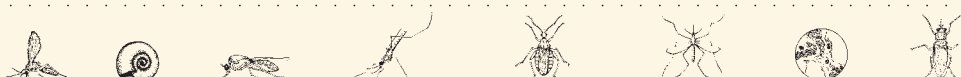
### *Interactions of host immune responses*

A great deal of morbidity is actually caused by the host's own defence system which, during its battle with invading infectious agents, often results in chronic inflammatory responses (see Box 1 for background information on immune response).

In **malaria**, the pathology associated with *Plasmodium falciparum* infection is mainly due to adherence of infected red blood cells, metabolic disturbances and organ dysfunction. Work has focused on the mechanism underlying the endothelial adherence and clumping together ('rosetting') of infected red cells, which involves the appearance



Red blood cells infected with *Plasmodium parasites* develop surface protusions involved with 'rosetting'.



### Box 1. Immune Response

Lymphocytes are white blood cells which react with antigens, initiating an immune response.

Some lymphocytes secrete antibodies (humoral response), while others, the T cells, are directly involved in cell-mediated immunity (CMI). The latter is important in protective responses against parasitic infections, but can at the same time also cause severe host tissue damage and sometimes death.

A series of molecules known as cytokines, which have very specific but also some non-specific effects, such as activating and/or attracting certain other immune cells, are synthesized and released following stimulation of a lymphocyte by a specific antigen.

Important cytokines with well-known effects include tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interferon- $\gamma$  (IFN- $\gamma$ ), and various interleukins (e.g. IL-2, IL-4, IL-5, IL-10 and IL-12).

Adjuvants are neutral substances which enhance the body's immune response to antigens.

of surface 'knobs' on infected red cells. Researchers have identified two parasite-derived 'adhesins' which are now under intensive study. A novel *in vitro* system shows how different adhesion molecules participate in a chain of events before the infected cell is firmly adhered to the blood vessel lining. In due course, the system may be used to analyse the effect of antimalarial drugs on cytoadherence.

In human malaria, pregnant women run an increased risk of clinical disease; the infection causes anaemia in the mother and the baby is commonly of low birth weight. After several pregnancies, there is increased resistance to placental malaria. In a series of studies in Kenya, resistance to placental malaria was shown to be associated with an increase in activated, memory lymphocytes, and

there are suggestions that susceptibility to placental malaria is due to failure to produce a strong interferon- $\gamma$  (IFN- $\gamma$ ) response.

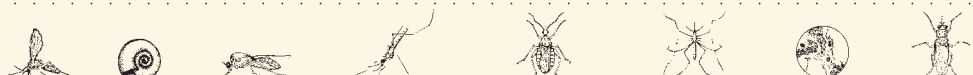
In intestinal **schistosomiasis**, pathology is particularly associated with the liver, where the majority of eggs are trapped. Secreted antigens initiate an immune reaction, surrounding the eggs by granulomas. Morbidity is directly related to the number of eggs in the liver and the resulting chronic inflammation leads to an increase in fibrous tissue (fibrosis). In severe cases the end result is portal hypertension which causes enlargement of the liver and spleen (hepatosplenic schistosomiasis) and swollen internal veins which may eventually burst and kill the patient.

The schistosome TNF- $\alpha$  receptor has been identified and this cytokine has been implicated in the induction of egg deposition by female worms, and it seems to be the key signal in granuloma formation. In patients infected with *Schistosoma mansoni*, a significant correlation has been found between TNF- $\alpha$  production and hepatosplenic schistosomiasis, and IL-4 also seems to be involved in the process. The roles of these cytokines in granuloma formation and egg production are being further evaluated by studying the responses of animals in which the genes for key signalling molecules have been removed ('knock-out' mice).

It has further been shown that stimulation by the *S. mansoni* egg antigen p40 suppresses granuloma formation in mice through reduced secretion of IFN- $\gamma$  from CD4+ cells, while increased secretion of this cytokine suppresses pathology. However, only a fraction of infected people develop severe morbidity and there are strong indications that there is genetic predisposition to severe schistosomiasis, linked to variations in two gene loci,



Hepatosplenic schistosomiasis is closely linked with TNF- $\alpha$  production.





*Sm1* and *Sm2*, the former located near the cytokine genes on chromosome 5 and the latter located in the vicinity of a gene regulating fibrosis. The combination of the schistosomicide praziquantel and  $\beta$ -aminopropionitrile, which inhibits collagen cross-linking, has been shown to reduce hepatic fibrosis and also to have an effect on post-treatment resistance in experimental mice. It has been demonstrated that the extent of repair of liver tissue after treatment depends on the stage of infection and the age of the patient. If treatment is given during the early stages of infection, or if the patient is young, liver damage may be repaired but otherwise removal of the parasite by treatment does not necessarily result in repair of scar tissue.

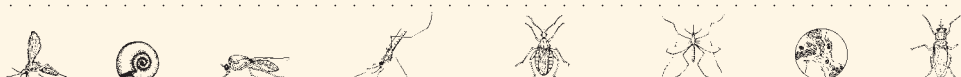
In **lymphatic filariasis**, there is no hard evidence to date that the immune response influences the clinical outcome of infection, although infection status does seem to be correlated to anti-filarial immune responsiveness. A long-term study in Haiti, where *Wuchereria bancrofti* is endemic, found no correlation between a child's antibody response and maternal infection status; thus familial clustering of infection is more likely related to the biting behaviour of the insect vector. This observation is being followed up by studying the relationship between mosquito distribution and biting preferences and the distribution of infection in children.

In **onchocerciasis**, there are two distinct strains of the parasite, only one of which is clearly correlated with blindness. It used to be thought that these strains were transmitted by different species of vector, but findings by the Onchocerciasis Control Programme (OCP) indicate that this is not the case. Scientists are currently trying to locate regions of the parasite genes that may be linked to the ability to produce blindness. Researchers in Cameroon have, through identification of polymorphic DNA sequences, been able to distinguish between four types of onchocerciasis – generalized, localized, asymptomatic, and blinding.

In experimental **leishmaniasis**, progression of the disease has been found to be associated with Th2 lymphocytes and IL-4 production, whereas resolution of infection is correlated with an inverse Th2/Th1 balance resulting in increased IFN- $\gamma$  production. A peptide present in saliva of the sandfly vector is known to enhance *Leishmania major* infection in mice and affect macrophage functions *in vitro*. The macrophages (host cells involved in CMI – see Box 1) take up significantly more parasites on addition of the peptide, and the peptide has also been shown to inhibit the ability of macrophages to kill intracellular parasites.

Chronic **Chagas disease** is particularly associated with heart dysfunction, and antibodies generated during infection may play a role in the development of this pathology by cross-reacting with certain heart cell receptors. Ways may be found of blocking this reaction, thus reducing severity of the disease. Some studies have shown that the severity of heart disease is directly correlated with the load of *Trypanosoma cruzi* in the heart, and inversely correlated with the number of CD8+ T cells producing IFN- $\gamma$ .

During both acute and chronic Chagas disease, inflammation provoked by the parasite is at least partly responsible for the pathology. IL-12 and TNF- $\alpha$  are key cytokines in the induction of CMI and are also important in regulating parasite growth and disease outcome in experimental models. A mucin-like protein, anchored to the membrane of the parasite, has been identified as the major molecule responsible for inducing the release of these inflammatory cytokines. IFN- $\gamma$  seems to prime macrophages first, before IL-12 and TNF- $\alpha$  are released, and to be partly responsible for inducing production of these cytokines.





In research on **African trypanosomiasis**, details are emerging about how infection with the parasite leads to the profound neurological changes in the host. The parasite, *Trypanosoma brucei*, releases a trypanosome lymphocyte triggering factor (TLTF) which brings about IFN- $\gamma$  production by CD8+ T lymphocytes, which in turn promotes parasite growth and also downregulates the host immune response. IFN- $\gamma$  may also be involved in the disruption of sleeping patterns and generalized muscular pain associated with the late stages of this disease. The link between expression of certain cytokine regulating factors and the induction of pro-inflammatory cytokines is being investigated – increased expression of TNF- $\alpha$  in the brain of infected rats correlates with selective degeneration of certain nerve fibres.

In **leprosy**, erythema nodosum leprosum and the reversal reaction, both based on immune responses against *Mycobacterium leprae*, are accompanied by nerve damage which is unique to the disease and can occur during or even after treatment. TDR-sponsored research has clearly shown the association of T cells with infected cells in these patients and identified the role of TNF- $\alpha$  in the pathogenesis of nerve damage.

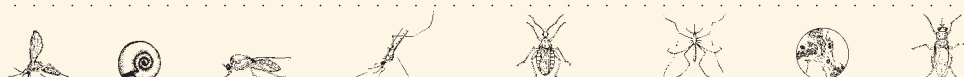
### **Drug and vaccine targets**

In the **malaria** parasite, drug and vaccine targets include nitric oxides and reactive oxygen intermediates. These compounds inhibit parasite development and have been shown to be induced *in vivo* following treatment with adjuvants. The *P. falciparum* enzymes choline kinase and choline phosphotransferase are being compared with those from human cells in the hope of locating differences which could be exploited. In addition, three new potent inhibitors of the surface protease responsible for secondary processing of *P. falciparum* merozoite surface protein (MSP-1) have been designed and synthesized. Two new mutations of the dihydrofolate reductase (DHFR) gene have been found in *P. falciparum* in Bolivia, where resistance to the combined antifolate drug pyrimethamine-sulphadoxine is widespread.

In **schistosomiasis**, there has been some work on drug resistance in the Senegal river basin, where unusually low cure rates using praziquantel have been found. However, resistance to praziquantel is a much contended issue and clear proofs are still lacking. In fact, it now looks more likely that the treatment failures observed are the results of particularly intense transmission.

With respect to **lymphatic filariasis**, two *Brugia* filarial antigens which could form the basis of a synthetic vaccine suitable for human use have been identified. One is a chitinase from the surface of *B. malayi* microfilariae (immature forms), which gives partial protection in experimental animals. The other is present in many stages of the parasite's life cycle and seems to be more effective than the first in reducing microfilaria levels and is also capable of reducing the host burden of adult worms.

In **leishmaniasis**, the parasites survive inside the very cells programmed to kill them – the macrophages – and there is evidence that cysteine proteases produced by the parasite are involved in this intracellular survival. Researchers have now found that transfection of a virulent *L. major* strain with a certain gene, which results in over-expression of cysteine proteases in the promastigote stage (the insect form), makes the strain avirulent, while over-expression of the corresponding amastigote-specific enzymes induces immunity against *L. major*. High levels of the cytokine IFN- $\gamma$  have been found in protected mice, while low levels have been seen in susceptible mice. It has further been shown that oral administra-



tion of the *Leishmania*-activated C kinase (LACK) antigen alone fails to induce specific T cell tolerization in the BALB/c mouse model, while partial tolerance to LACK and increased resistance to *L. major* is obtained when the antigen is coupled to cholera toxin B. So far it has not been possible to construct parasites without the gene for LACK – perhaps because the gene is so important for parasite metabolism.

Components of the mitogen-activated protein (MAP) kinase pathways, involved in intracellular differentiation in response to extracellular signals, are present in *L. major*. The gene (MRK1) for a related enzyme of the phosphorylation cascade has been further characterized and appears to be involved in a crucial signalling pathway (at least in promastigotes) that could conceivably be interfered with. In a novel mechanism of drug resistance, the multidrug resistance protein LeMDR1 has been shown to behave differently to the mammalian MDR, perhaps because it is located on the mitochondrial membrane rather than the plasma membrane as in mammals. This work may provide information for overcoming drug resistance and suggests the possibility of targeting drugs to the parasite mitochondrion as opposed to that of the host.

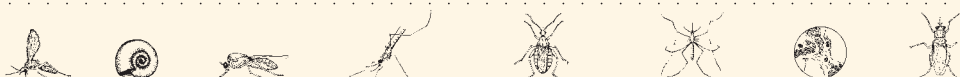
The involvement of free fatty acids in the differentiation from dividing non-infectious forms of the parasite to non-dividing infectious forms in the insect vector represents a critical step in the life cycle of *T. cruzi* which causes **Chagas disease**. Work on characterization of the signalling pathways involved may offer ways to block the step, while other studies aim to understand how the parasite regulates cell division. Two cdc2-related kinases from the parasite are being investigated and compared with the same enzyme in the host. Further, membrane compounds which anchor cell surface proteins of *T. cruzi* and other protozoan parasites are being investigated and preliminary results suggest that devising compounds to interfere with the synthesis of anchor molecules could be a suitable strategy.

Research on **African trypanosomiasis** has pointed to possible drug targets in the form of certain acidic compartments (acidocalcisomes) used to store calcium ions in *T. brucei*, the causative parasite. Biochemical pathways that are unique to trypanosomes include an inorganic *T. brucei* phosphate pathway and components of the RNA trans-splicing pathway, which have recently been identified. The structure of a capping enzyme important in the sequence of trans-splicing in particular seems to be mechanistically different from that in other eukaryotes and may be an ideal drug target. The genes for two phosphodiesterases which may play important roles in the life cycle have also been identified – these enzymes are a crucial part of the cAMP-signalling pathway, utilized by *T. brucei* in differentiation.

### Research models

Research on pathogenic mechanisms requires laboratory models, but a number of the TDR target diseases still lack suitable animal models which closely mimic the human host.

In **malaria**, researchers have finally succeeded in maintaining *P. falciparum* infected human red blood cells in severely compromised immunodeficient (SCID) mice. Using this system it has been confirmed that host macrophages help clear infected red blood cells in an antibody-dependent manner. In **schistosomiasis**, the lack of cell lines of any developmental stage of the parasite is a hindrance to antigen production and drug screening. Co-culture of sporocysts with embryo cells from the snail host was found to



result in some growth of sporocysts and production of daughter sporocysts. The development of schistosome cell lines is also being pursued through work on the *ras* gene which has recently been characterized, mutations of which result in continuous production of new cells.

## Parasite Genome

Deciphering the information contained in the genome of a variety of organisms has been possible since the 1970s. So far, 18 complete genomes from microorganisms have been decoded and a further 40 are in progress. The TDR parasite genome projects began in 1994 when five genome networks were established with the aim of encouraging and coordinating the worldwide efforts of scientists. The data for each parasite (*Schistosoma*, *Brugia*, *Leishmania*, *T. cruzi*, *T. brucei*) are pooled in databases which may be accessed via the Internet (see Box 2). Work on the malaria and leprosy genomes is not

currently supported by TDR since there are separate, independently supported, networks for these.

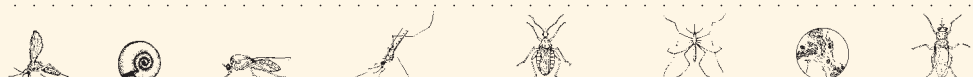
A main aim is to identify new genes (see Box 3 for explanation of terms). Finding parasite genes which are different from those of the host helps reveal the complex interactions between host and parasite and opens up ways of blocking or disrupting parasite development. Interference with genes crucial to a particular stage of a parasite's life cycle blocks its development and can be utilized in the battle against these infectious agents. New drugs, vaccines and diagnostic tests will result from the identification of new genes, while understanding the molecular basis of drug resistance will help create rational ways of dealing with this problem. Deciphering the information in any genome is a long process but con-

siderable progress has been made in all five projects covered by TDR's Parasite Genome Committee, and discussion of a post-genome agenda (functional genomics) is well under way. The rate of progress is different in the five genome networks because of the different sizes of the parasite genomes under study.

Of the five parasites, the schistosome genome probably represents the greatest challenge due to its relatively large size. Two species are being pursued – *S. mansoni* and *S. japonicum*. In the lymphatic filariasis genome project, the focus is on the genome of *B. malayi*. This project is facilitated by the near completion of genome sequencing of *Caenorhabditis elegans*, a free-living nematode closely related to the filarial parasites infecting humans. The *Leishmania* genome, with its relatively small size, is likely to be the first TDR target parasite to be completely sequenced. The aim is to have 45% of the genome sequenced by the year 2000 and 100% two years later. The Friedlin strain of *L. major* is the main focus. Genome work related to Chagas disease is complicated by the fact that *T. cruzi* strains vary considerably both in size and biological property. Research is focused on the CL-Brener strain. Despite considerable progress, it is still unclear how many chromosomes this strain contains – estimates vary from 20 to 42. Genome analy-

### Box 2 Genomes on the Web

Malaria (*Plasmodium*) genome project  
<http://www.wehi.edu.au/MalDB-www/who.html>  
 WHO/UNDP/World Bank *Schistosoma* Genome Network  
[http://www.nhm.ac.uk/hosted\\_sites/schisto/](http://www.nhm.ac.uk/hosted_sites/schisto/)  
 Filarial Genome Network  
<http://helios.bto.ed.ac.uk/mbx/fgn/filgen.html>  
*Leishmania* Genome Network  
<http://www.ebi.ac.uk/parasites/leish.html>  
*Trypanosoma cruzi* Genome Project  
<http://www.dbbm.fiocruz.br/genome/tcruzi/tcruzi.html>  
*Trypanosoma brucei* Genome Project  
<http://parsun1.path.cam.ac.uk>  
 MycDB: *Mycobacterium*  
<http://www.biochem.kth.se/MycDB.html>



sis of the parasite causing African trypanosomiasis is focused on a particular strain (TREU927/4) of *T. b. rhodesiense*. Although there is great variation in genome size between different stocks (as in *T. cruzi*), considerable progress has been made.

### Gene discovery

For *Schistosoma*, the focus has been on searching randomly for genes using the expressed sequence tag (EST) approach, and over 7000 ESTs have now been generated. More than 30% of all *S. mansoni* genes have already been found but considerably fewer have been isolated from *S. japonicum*. About 66% of the new sequences from *S. mansoni* and 75% from *S. japonicum* represent new genes. cDNA libraries have been constructed for all life-cycle stages. The future focus will be on gene 'families' responsible for producing important regulatory molecules which are vital for parasite survival.

Over 6000 *B. malayi* genes have been identified which is more than a third of those thought to exist. About half of the genes are similar to genes in other organisms. Over 16 000 ESTs have been generated, and cDNA libraries derived from each life-cycle stage have been constructed. Detailed sequence analysis and chromosome mapping of the most promising genes, including those for potential vaccine targets, is under way. Researchers are currently looking for genes from *B. malayi* and other nematodes which influence the hosts immune response, and for any genes which could be used to provide protective immunity.

DNA from the bacterium *Wolbachia* has been found in *B. malayi* genome libraries. This interesting discovery has led to a better understanding of the symbiotic relationship between *Brugia* and *Wolbachia*, which lives inside the nematode, and it has opened a new way for developing chemotherapeutic agents (as drugs which kill the endosymbiont may also kill the worm).

Over 2000 *L. major* ESTs have now been generated, of which 70% show no similarity to genes from other organisms, while the functions of the remaining 30% can be predicted. The complete sequence of chromosome number 1 is already available and this represents the first chromosome from a trypanosomatid parasite to be sequenced. Significant progress has also been made on sequencing chromosome number 3.

Nearly 7000 *T. cruzi* ESTs have been analysed from the epimastigote stage of the life cycle. Libraries of other stages in the life cycle are being prepared but progress is slowed down because several developmental stages are difficult to grow in the laboratory. Many genes have been identified and the whole sequence of chromosome number 3 is complete. The goal is to sequence 65% of the genome by the year 2000.

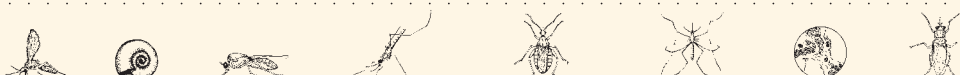
More than 3700 ESTs from *T. b. rhodesiense* have been isolated, of which 400 have been mapped to particular chromosomes. The focus is on identifying genes which are active at different stages in the life cycle. The first chromosome to be tackled is the smallest (number 1), for which funding has been obtained from external sources. Chro-

### Box 3. Gene Identification

One way to identify genes is to determine the exact **order** of the four different **bases**, or molecular building blocks, along the DNA backbone. Although this process produces comprehensive results, it is relatively slow and expensive. However, once the sequence is known, computer programmes can be used to predict the structure of the genome under study.

A quicker but less comprehensive way to identify genes is by '**gene hunting**'. Here, collections of cloned pieces of complementary DNA representing the active genes in the parasite are first created. These are known as **cDNA libraries**. Clones are then selected at random from the libraries, and short stretches of DNA from each clone are sequenced. Unique sequences within these stretches are identified and used as tags for the particular short stretch of DNA they represent – and hence are known as **expressed sequence tags (ESTs)**. The genes can then be isolated and overlapping sections of DNA – '**contigs**' – can be **mapped** to particular locations on the chromosome.

The process of introducing 'foreign' genes into a genome is known as **transfection**.



mosomes II (the next smallest) and IX (one of the easier ones to isolate) will follow.

### ***Chromosome mapping***

A simple physical map of the *Schistosoma* chromosomes is already being compiled. Collections of DNA pieces from chromosomes number 1 and 3 have been gathered and libraries of cloned DNA created from these chromosomes. Since these chromosomes are relatively large they can be observed using a simple light microscope enabling the location of several DNA markers and even that of small gene families to be determined. Mapping of the *Brugia* chromosomes is proceeding well and researchers hope to produce a medium-resolution map by the end of 1999.

The chromosomes of the *Leishmania* parasite are too small to be seen using a light microscope, and pulsed-field gel electrophoresis (PFGE) is used to separate and visualize them. This has been completed for the Friedlin strain of *L. major* and over 1000 clones of genomic DNA have been mapped. Contig maps of chromosomes number 3 and 4 of *T. cruzi* have been completed, while both genetic and physical maps of the chromosomes of *T. brucei*, the parasite of African trypanosomiasis, are being assembled. Chromosome number 1 has been completely mapped and the remaining ten chromosomes partially mapped. Chromosome number 1 varies the most and chromosome number 2 the least.

### ***Functional genomics***

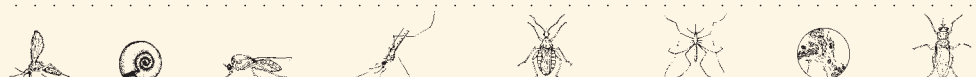
In the *Schistosoma* network, researchers are preparing to use state-of-the-art technology to create 'DNA chips' or gene arrays. With these they will be able to identify genes which are active at different stages of the life cycle, in male and female worms, in drug-resistant and drug-sensitive parasites, etc. Also researchers may look at mitochondrial genes where, for example, several proteins required for key metabolic pathways are produced.

Several of the more interesting *Brugia* genes identified are now being studied in more detail, for example, macrophage migration inhibition factor (MIF), which is expressed in all life-cycle stages and is thought to interact with macrophages in the patient, perhaps even being involved in reducing the host's immune response. A computer-based modelling project, to generate new drug and vaccine targets, has recently begun, as has a proteomics project (study of proteins).

The majority of *Leishmania* genes have little similarity to genes from other organisms, and it is proposed to determine their functions by using gene array technology to directly compare gene expression between different parasite populations (as in schistosomiasis). Proteins expressed from identified genes could be ideal targets for new drugs or vaccines. It is also proposed to randomly pool groups of ESTs and use them directly as DNA vaccines in experimental systems, an approach which can be used when the function of genes is not known.

In the *T. cruzi* gene project, the focus is still on sequencing. When studies on gene function begin in earnest, the intention is to concentrate first on genes which are essential for parasite survival, are developmentally regulated or are unique to the parasite.

In *T. brucei*, the main interest is on gene discovery and using this information to create new methods of controlling the disease. Future studies will include examination of regions of DNA in between the genes, which may reveal novel mechanisms of gene





regulation. *T. brucei* is known to use an unusual mechanism for processing RNA – this is a very attractive target for intervention.

### ***Leprosy***

Sequencing of the genome of the leprosy bacillus, *M. leprae*, has been initiated and catalysed by TDR, even though the majority of funds for the activity have come from outside. TDR's initial investment in genome mapping goes back to 1989; it proved a stimulus to in-depth analysis and eventually to genome sequencing. An international network of laboratories, developed by TDR, first performed small-scale sequencing. This was later improved with the help of other sponsors (such as the Heiser Program for Research in Leprosy and Tuberculosis of the New York Community Trust), so that, by the end of 1998, only a small number of gaps in the sequence – corresponding to less than 5% of the entire *M. leprae* genome – remained. These efforts served as a catalyst for the successful TB genome project.

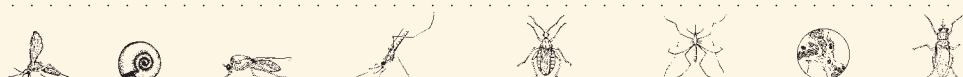
Sequence data are being used in TDR-sponsored research to develop a diagnostic skin test capable of determining whether or not an individual has been exposed to *M. leprae*, which will help us understand the transmission of the disease and its epidemiology, and is a step towards determining the feasibility of eradication. The skin test is based on the use of 193 peptide strands derived from *M. leprae* sequence data. Each strand consists of 15 peptides and has been tested in leprosy patients, leprosy contacts and controls from non-endemic areas. Five strands were shown to induce immune responses only in patients and their contacts but not in controls. However, only one of these was confirmed as specific to *M. leprae*. Work is now focused on finding specific peptides derived from *M. leprae* proteins.

### ***Molecular Entomology***

The ultimate aim of TDR-supported research in molecular entomology is to replace the natural vectors of malaria in the wild with populations of anopheline mosquitos that are unable to support the development of malaria parasites. The mosquitos would live in their normal environment but be unable to transmit malaria. Controlling malaria through manipulation of mosquito genes may sound futuristic but is beginning to look increasingly realistic. In 1991, 36 specialists were brought together by TDR, the Wellcome Trust and the MacArthur Foundation to discuss the most promising lines of research in this new approach to mosquito control. Three main areas of research were identified and work carried out so far in these areas is outlined below. It was estimated that the task would take 10-15 years to complete.

#### ***Identification of genes responsible for disrupting parasite development in the mosquito***

The human malaria parasite does not develop in all species of *Anopheles* mosquito. One line of research is to discover why refractory species of mosquito do not support the parasite's development. Refractory mosquitos use different mechanisms to kill the parasites. In one, the mosquito is able to burst or lyse the parasite, and in another, the parasite becomes encased in a black sheath of melanin. Some progress in this area has been made, and, if identified, the responsible genes could be transferred to susceptible



strains of mosquito. Three quantitative trait loci which account for encapsulation of a monkey malaria parasite have been identified in mosquitos, and a major gene locus for the lytic response has been found on chromosome number 3 of *An. gambiae*.

Strains of *An. stephensi* mosquitos which are susceptible to malaria have been found to contain 22 proteins in their midgut cells which are not found in refractory strains. Certain refractory strains of this mosquito have been found to limit parasite development through the release of nitric oxide, and increased expression of the nitric oxide synthase gene has been found to follow infection with *P. berghei* and *P. falciparum*. It has been observed that many parasites are killed in the mosquito haemocoel while trying to reach the salivary glands, and the mechanism for this is also being explored.

Another line of research is to identify the cues that the parasite recognizes and reacts to in susceptible mosquitos and which facilitate its development. The first step in the parasite life cycle in the mosquito is the activation of gametes. One of the triggers for this event is a molecule known as gametocyte-activating factor. Recently this molecule was identified – in *An. stephensi* – as xanthurenic acid, which is a by-product of the tryptophan metabolic pathway used to form pigments in the insect's eye.

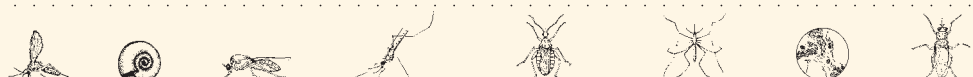
At each different stage of its development in the mosquito, the malaria parasite must select the correct cells to invade, and it does this by recognizing 'receptors' on the surfaces of the cells. Blocking the recognition of such receptors is a potential target point for interrupting the parasite life cycle. An SGS-1 protein has been identified in the salivary glands of an *Aedes* species (this species is refractory to malaria). This protein may well be a receptor and there is recent evidence for the existence of such a protein also in the important malaria vector *An. gambiae*. Possible receptors in the midgut cells are thought to be the enzymes V-ATPase and an aminopeptidase.

In addition to looking for ways to interrupt parasite development inside the mosquito, researchers are also looking for ways to stop the parasite infecting the mosquito in the first place. Two strategies are being used. In the first, an *Anopheles* species which feeds on humans indoors is being cross-bred with a species which prefers to feed on animals outdoors. By selective cross-breeding, researchers hope to introduce animal-preferring genes into the human-preferring species. The second strategy is to understand the mosquito's preference for humans and to design novel biological control strategies based on this knowledge. So far, proteins in the mosquito which are thought to be involved in recognizing human odours have been identified and two olfactory genes have been cloned and characterized.

### ***Development of genetic and molecular tools to insert selected genes into the mosquito genome***

Tools for carrying out transfection (introduction of genes into a genome) have been developed in insects such as the fruit fly *Drosophila* but are not yet perfected for use in mosquitos. Researchers have identified at least six families of insertion elements in the mosquito but have not yet managed to insert selected genes into the *Anopheles* genome. This has, however, been achieved in another species of mosquito, *Aedes*, responsible for the transmission of dengue virus.

Introduced genes should ideally contain DNA control regions capable of switching the genes on and off. So far, only control regions from other insect genes have been used but recently considerable progress has been made in identifying control DNA sequences



from mosquito genes. Some control regions from *Drosophila* have been shown to function in *An. gambiae* cell lines.

As well as developing procedures for transformation of mosquitos, there has to be an easy way of distinguishing mosquitos which contain the gene of interest from those which don't. An eye colour mutation has been used for this purpose in *Ae. aegypti*. Now a white eye gene has been identified in *An. gambiae* and mutant mosquitos lacking the gene have been produced although the technique needs improvement. Efforts are continuing with the production of mutants lacking the gene for tryptophan oxygenase, an enzyme responsible for synthesis of eye pigments. Some researchers have used marker genes from other organisms in *An. gambiae* and have obtained promising results, e.g. the jellyfish gene for Green Fluorescent Protein.

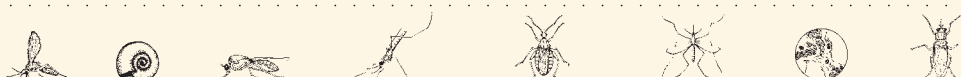
#### Box 4. Mosquitos on the Web

Genomic and biological information on anopheline mosquitos:

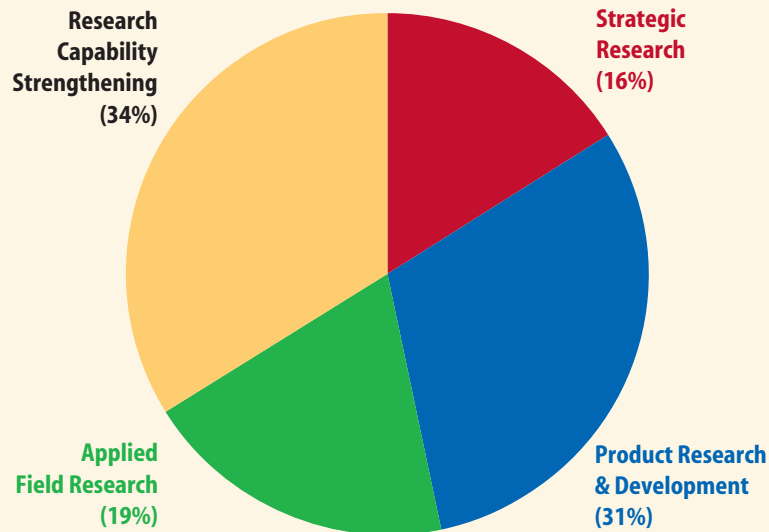
<http://konops.imbb.forth.gr/AnoDB>

#### *Development of methods to spread selected genes in wild mosquito populations*

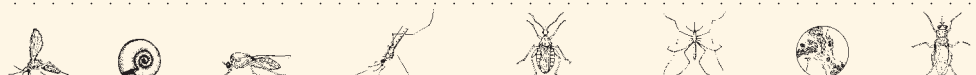
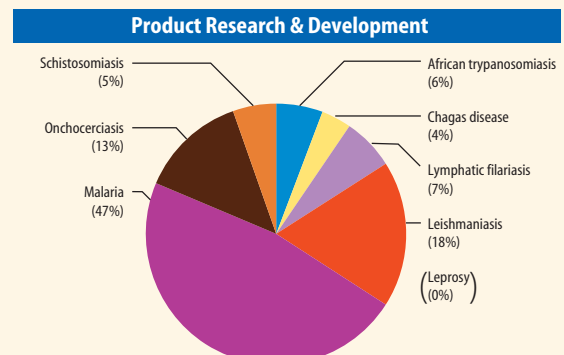
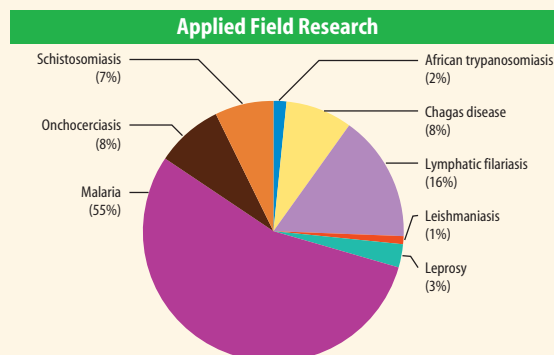
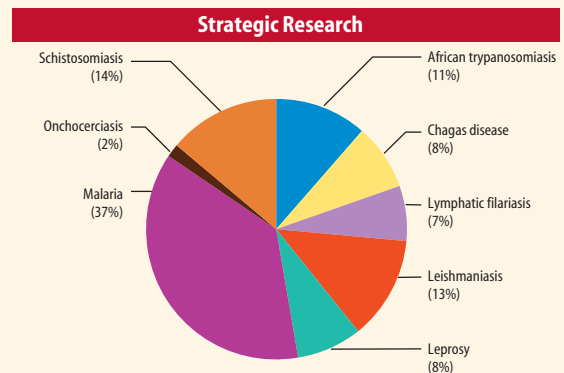
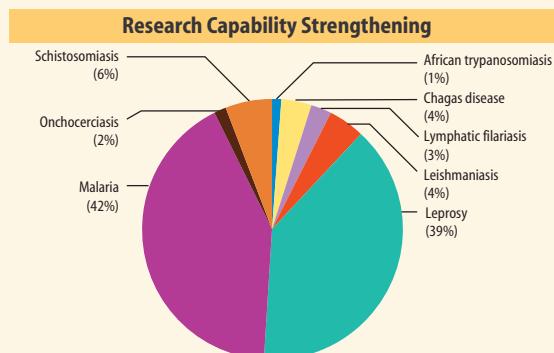
Early small-scale laboratory and computer modelling predictions suggest that creating modified mosquito populations is a feasible strategy for controlling the malaria vector, hence the ethical implications of releasing genetically modified mosquitos into the environment are now under consideration. This area is potentially the most problematic of the three main lines of molecular entomology research. A thorough understanding of mosquito population genetics is essential; scientists are studying the structure of natural populations of mosquitos to find out the amount of genetic variation between them and how individual species regulate the growth and fitness of their populations.



# BUDGET BY PROGRAMME AREA 1997–98

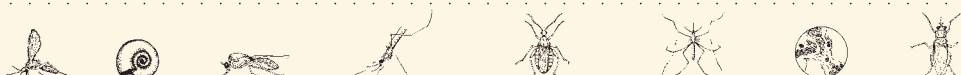


## BUDGET BY DISEASE



# SELECTED TDR PARTNERS (1997–98)

ACF Beheer	Marion Merrell Dow Pharmaceuticals Inc.
African Programme for Onchocerciasis Control	Merck and Co. Inc.
Aquila Bio Pharmaceuticals	National Institutes of Health
Asian Development Bank	NeXstar Inc.
Association of British Pharmaceutical Industries	Norwegian National Commission for UNESCO
Bayer A.G.	Novo Nordisk A/S
Biobras-Bioquímica do Brasil	Onchocerciasis Control Programme
British Leprosy Relief Association/International Federation of Anti-leprosy Associations	Organisation de Coordination et de Coopération pour la Lutte contre les Grandes Endémies
Burroughs Wellcome Company	Organisation de Coordination pour la Lutte contre les Endémies en Afrique
Child to Child Trust	Oswaldo Cruz Foundation, Brazil
Childwatch International	Partnership for Child Development
Cochrane Collaboration (Infectious Diseases Group), Liverpool School of Tropical Medicine	Pasteur Institute, Iran
Conselho Nacional de Desenvolvimento Científico e Tecnológico	Pasteur-Mérieux-Connaught
Council on Health Research for Development	Pfizer Limited
Cymbus Biotechnology Ltd	Pharmacia and Upjohn
Daiichi Pharmaceutical Co. Ltd.	Razi Vaccine and Serum Institute, Iran
Danish International Development Agency	Rhône-Poulenc Rorer Doma
E. Merck Pharma	Rockefeller Foundation
Edna McConnell Clark Foundation	Shanghai Institute of Pharmaceutical Industry
Entremed, Inc.	SmithKline Beecham Biologicals
European Commission	SmithKline Beecham Pharmaceuticals
F. Hoffmann – La Roche	South East Asian Ministers of Education Organization, Regional Tropical Medicine and Public Health Project
Glaxo Wellcome Research and Development Limited	Southern African NGO Network (SangoNet)
Hong Kong Institute for Biotechnology	Swedish Agency for Research Cooperation with Developing Countries
IHARABRAS S.A., Industrias Químicas	Swiss Tropical Institute
ILEX Oncology	United States Agency for International Development
Indian Institute of Chemical Technology	United States Army Medical Research and Development Command
Indian Council of Medical Research	United Nations Children's Fund
International Centre for Genetic Engineering and Biotechnology	University of Witwatersrand
International Development Research Council	Vaccine Solutions
International Federation of Pharmaceutical Manufacturers Associations	Walter Reed Army Institute for Research
International Livestock Research Institute	Wellcome Trust
Janssen Research Foundation	Zambon SpA
John D and Catherine T MacArthur Foundation	
Laboratories Gador	





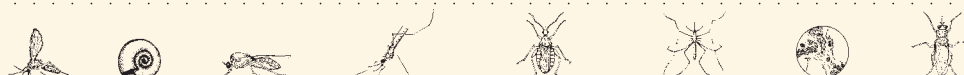
# FINANCIAL CONTRIBUTIONS

## UNDP/WORLD BANK/WHO SPECIAL PROGRAMME FOR RESEARCH AND TRAINING IN TROPICAL DISEASES (TDR)

(IN US\$ UP TO 31 DECEMBER 1998)

CONTRIBUTOR	1974–1993	1994	1995	1996	1997	1998	Notes
Total							
AFRICAN DEVELOPMENT BANK GROUP	1,850,000	405,461	0	0	0	0	
ARAB FUND FOR ECONOMIC AND SOCIAL DEVELOPMENT	162,813	167,392	0	0	0	0	
AUSTRALIA	6,803,817	564,410	1,144,524	1,207,153	696,240	588,150	
AUSTRIA	593,008	0	0	0	0	0	
BAHAMAS	500	0	0	0	0	0	
BAYER AG (FRG)	10,000	0	0	0	0	0	
BELGIUM	7,992,887	1,488,860	948,195	928,606	835,185	830,601	
BRAZIL	100,000	0	0	0	0	0	
CAMEROON	79,182	0	0	0	0	0	
CANADA	18,864,884	1,314,815	1,194,030	1,065,693	1,014,493	937,500	(a)
CHINA	600,000	55,000	55,000	55,000	55,000	55,000	
CUBA	24,589	0	2,500	0	0	2,000	
CYPRUS	239	0	0	0	0	0	
THE FORMER CZECHOSLOVAKIA	34,965	0	0	0	0	0	
DENMARK	47,454,363	2,668,823	0	5,778,337	2,483,307	2,485,028	
EDNA MCCONNELL CLARK FOUNDATION (USA)	650,000	250,000	125,000	0	0	0	
FINLAND	2,810,104	0	0	0	0	0	
FORD FOUNDATION (USA)	0	0	20,000	0	0		
FRANCE	5,214,822	542,907	0	266,152	0	166,945	
GERMANY	20,530,169	863,284	252,540	1,003,464	730,290	643,138	
GREECE	304,173	0	0	0	0	0	
GULBENKIAN FOUNDATION (POR)	25,000	0	0	0	0	0	
INDIA	422,573	0	25,280	23,055	50,481	0	
INTERNATIONAL DEVELOPMENT RESEARCH CENTRE (CAN)	2,407,909	572,984	229,647	72,595	306,531	61,768	(a)
INTERNATIONAL FEDERATION OF ANTI-LEPROSY ASSOCIATIONS	1,840,407	175,600	151,000	89,459	86,888	23,737	
IRAN (ISLAMIC REPUBLIC OF)	10,000	0	0	0	0	0	
IRAQ	5,000	0	0	0	0	0	
IRELAND	0	150,400	163,910	157,000	156,340	141,210	
ISRAEL	6,500	0	0	0	0	0	
ITALY	5,953,512	246,436	93,168	0	33,222	87,209	
JAPAN	1,270,000	300,000	300,000	350,000	300,000	150,000	
LEPROSY TRUST BOARD (NZ)	109,445	0	0	0	0	0	
LUXEMBOURG	759,358	258,359	294,423	385,480	418,060	475,007	
MACARTHUR FOUNDATION (USA)	5,900,000	0	100,000	154,000	190,000	36,000	

(a) Contribution in 1994 of US\$ 572,984 made by the International Development Research Centre, with funds made available by the Canadian International Development Agency



**UNDP/WORLD BANK/WHO  
SPECIAL PROGRAMME FOR RESEARCH AND TRAINING IN TROPICAL DISEASES (TDR)**

(IN US\$ UP TO 31 DECEMBER 1998)

CONTRIBUTOR	1974–1993	1994	1995	1996	1997	1998	Notes
	Total						
MALAYSIA	40,000	20,000	10,000	25,000	0	25,000	
MEXICO	125,953	13,000	13,000	0	20,000	10,000	
NETHERLANDS	18,992,835	2,289,427	2,216,878	1,755,996	1,772,434	1,793,018	
NEW ZEALAND	71,676	47,273	49,230	52,980	0	82,125	
NIGER	2,252	0	0	0	0	0	
NIGERIA	512,983	2,390	0	0	0	0	
NIPPON FOUNDATION (JPN) (formerly Japan Shipbuilding Industry Foundation)	5,540,767	200,000	200,000	100,000	100,000	100,000	
NORWAY	38,906,039	3,566,875	3,977,716	3,712,500	4,483,298	3,803,211	
OPEC FUND FOR INTERNATIONAL DEVELOPMENT	890,000	0	0	100,000	100,000	0	
OSWALDO CRUZ FOUNDATION (BRA)	150,000	25,000	25,000	75,000	0	75,000	
PAN AMERICAN HEALTH AND EDUCATION FOUNDATION (USA)	951,980	40,000	5,000	29,139	6,000	0	(b)
PORTUGAL	7,500	7,500	7,500	7,500	7,500	7,500	
ROCKEFELLER FOUNDATION (USA)	0	50,000	1,200,000	75,000	0	250,000	
ROMANIA	1,995	0	0	0	0	0	
SANOFI (FRA)	39,216	0	0	0	0	0	
SLOVAKIA	1,985	0	0	0	0	0	
SWEDEN	42,007,201	2,493,438	1,308,540	1,419,065	1,963,405	2,564,524	
SWITZERLAND	16,497,827	769,231	956,522	775,194	714,286	709,220	
THAILAND	76,570	19,763	20,243	19,920	0	40,494	
THRASHER RESEARCH FUND (USA)	50,000	0	0	0	0	0	
TURKEY	44,951	0	15,000	0	9,712	5,000	
UNITED KINGDOM	16,530,427	1,421,475	1,290,715	3,271,651	80,645	3,749,667	
UNITED STATES OF AMERICA	43,503,912	1,500,000	1,000,000	300,000	2,300,000	3,950,000	
UNITED NATIONS DEVELOPMENT PROGRAMME	36,689,708	2,000,000	3,565,500	2,226,200	988,180	1,000,000	
WELLCOME TRUST (UK)	25,000	0	0	0	0	0	
WORLD BANK	40,490,000	3,800,000	3,800,000 1,350,000	3,800,000	2,500,000	3,000,000	(c)
WORLD HEALTH ORGANIZATION	21,425,816	1,008,000	1,008,000	1,008,000	1,008,000	1,132,300	
AFRICAN PROGRAMME FOR ONCHOCERCIASIS CONTROL (APOC)	0	0	0	0	400,000	0	
WHO REGIONAL OFFICE FOR AFRICA	0	0	0	0	250,000	0	
MISCELLANEOUS	33,559	332	0	2,175	15,147	3,089	
<b>TOTAL CONTRIBUTIONS</b>	<b>416,400,371</b>	<b>29,298,435</b>	<b>27,118,061</b>	<b>30,291,314</b>	<b>24,074,644</b>	<b>28,983,441</b>	

(b) Contributions made possible by: The Upjohn Company, US\$ 5,000; the Wyatt Company, US\$ 15,000; Merck & Co. Inc., US\$ 986,980; and a private legacy, US\$ 25,139

(c) Contribution designated for Onchocerciasis Operational Research under the TDR Applied Field Research Component



