

Tropical Disease Research

TDR

Seventh Programme Report
1 January 1983 - 31 December 1984



UNDP / WORLD BANK / WHO
Special Programme for Research and Training

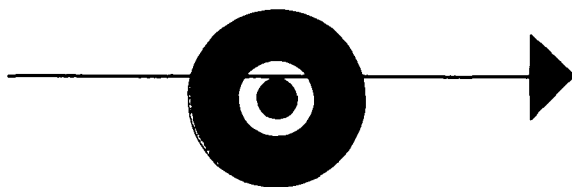
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UNDP/World Bank/WHO
Special Programme for Research and Training
in Tropical Diseases
Box 88, Geneva, Switzerland
Tel: (076) 814511 or 814142
RN: 06036 / ism 2082
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Special Programme for Research and Training
in Tropical Diseases

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France*

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Preface

The UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (TDR) is a goal-oriented research and training programme with two interdependent objectives:

- research and development to obtain new and improved tools for the control of major tropical diseases;
- strengthening of the research capabilities of the tropical countries.

The research is conducted on a global basis by multidisciplinary Scientific Working Groups; the training and institution-strengthening activities are limited to the tropical countries where the diseases are endemic.

The six diseases initially selected for attack are malaria, schistosomiasis, filariasis (including onchocerciasis), the trypanosomiasis (both African sleeping sickness and the American form called Chagas' disease), the leishmaniasis and leprosy. Scientific Working Groups are also active in "trans-disease" areas: biological control of vectors, epidemiology, and social and economic research.

The Seventh Programme Report describes work during 1983 and 1984. It provides a more descriptive and analytical account than previous Programme Reports and also shows how the great variety of TDR research topics and training activities — from gene splicing to geographical surveys and from individual training to international workshops and institutional networks — all interrelate to achieve the common goal of improving disease control and human welfare in tropical countries.

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Dr Adetokunbo O. Lucas, Director
Special Programme for Research and
Training in Tropical Diseases
World Health Organization
Geneva, Switzerland

Scientists interested in participating in TDR
are invited to write for further information to:
The Office of the Director,
Special Programme for Research and Training
in Tropical Diseases,
World Health Organization,
1211 Geneva 27, Switzerland

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1 Overview

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1 Overview

The Special Programme for Research and Training in Tropical Diseases (TDR) is now in its third stage of development. *Planning* dominated the first stage: goals were defined and plans drawn up. The second stage focused on *implementing plans*: research projects were funded and scientists throughout the world, working in many disciplines but often separately, were brought into a network through which they could channel their expertise into the achievement of common goals. At the same time, through grants to institutions and support for training and other activities, TDR began helping developing countries strengthen their research resources. The present, third stage in TDR's development is marked by *results*: usable products and technologies are emerging from work supported by the Programme.

Looking back, these stages can be viewed not as discrete entities but as parts of an interacting system: advances in research permit the definition of new goals; strengthened research facilities in tropical countries generate new resources; experience gained in developing one product can be used to develop others. The system has been shown to work and to be capable of transforming plans into products.

This report, which covers the two-year period 1983-84, presents tangible examples of progress made, through TDR support, in developing the tools needed to control the six major tropical diseases within the Programme's mandate—malaria, schistosomiasis, filariasis, the trypanosomiases, the leishmaniases and leprosy.

These tools, the result of TDR's efforts in mobilizing, stimulating and coordinating the activities of national academic institutions and the pharmaceutical industry throughout the world, have from the outset been designed to meet strict specifications. They must be: *effective*, in preventing and/or curing infection and disease in endemic areas; *safe*, so that they can be used without special skills or supervision; *simple*, so that they can be used under a wide variety of field conditions, particularly in a primary health care context; *practicable*, within the limits—economic, social and cultural—of local communities.

Some of the tools developed through TDR sup-

port are ready for use or are actually being used for disease control and treatment. Others are at an advanced stage of development: they have been shown to be effective but require refinement before they can be used. Yet others are still only promising leads derived from TDR-supported research.

Four main types of tools are being developed for the control of the six "target" diseases: *drugs*, to treat individual patients and help control diseases within a community; *vaccines*, for primary prevention and sometimes as adjuncts to chemotherapy; highly specific, sensitive *diagnostic methods*; and *new vector control techniques*, especially those based on innovative approaches.

Not all the developments described in this report stem originally from TDR-supported work. Nor have they all been supported exclusively by the Programme. But in many cases where original discoveries were made outside the Programme, their subsequent development—including the many stages of laboratory and field testing—has benefited from TDR support.

Chemotherapy and drug development

Drugs ready for or in use

Drug combinations for leprosy

For decades, monotherapy with dapsone had been the mainstay of leprosy treatment and control, until resistance developed to the drug. To assess the magnitude of the problem, the Programme has sponsored surveys on the prevalence of primary and secondary dapsone resistance.

Controlled clinical trials sponsored by the Programme have led to the formulation, by a WHO Study Group, of recommendations on new multi-drug treatment schedules comprising rifampicin, dapsone and clofazimine. These schedules render patients noninfectious in a relatively short time, substantially reduce the duration of treatment and diminish the risk of drug-resistant strains emerging. Although rifampicin is relatively expensive, the operational efficacy conferred on multidrug regimens

by this powerful bactericidal agent makes them highly cost-effective.

Mefloquine and mefloquine combinations

In collaboration with the Walter Reed Army Institute of Research (WRAIR) in the United States and with the Swiss pharmaceutical firm, Hoffmann-La Roche and Company, a new antimalarial drug, mefloquine, has been developed to the stage of registration for human use, initially only in adult males and nonpregnant females and in children over two years of age. Clinical trials conducted in Africa, Asia and Latin America have confirmed that the drug is not only well tolerated but also effective against strains of *Plasmodium falciparum* resistant to chloroquine and other drugs.

To reduce the risk of mefloquine-resistant parasite strains appearing and spreading, mefloquine will normally be used in combination with other drugs. TDR-supported projects have shown that the fixed combination of mefloquine with sulfadoxine and pyrimethamine is effective and well tolerated. Where this fixed combination is contraindicated, mefloquine should be given alone, followed by a dose of primaquine to prevent transmission of chloroquine-resistant strains. This approach has been successfully field tested by TDR and WHO's South-East Asia Regional Office.

In agreement with the drug company, mefloquine will only be sold in accordance with guidelines established by TDR and WHO experts. It will be available for sale in nonendemic countries for the use of short-term visitors to endemic areas. In endemic countries it will not be sold over the counter nor promoted publicly, but offered for sale through governments in areas where the parasite does not respond adequately to chloroquine.

Drugs at an advanced stage of development

Malaria

Several antimalarial compounds are now at an advanced stage of development, i.e. their antimalarial activity has been demonstrated in experimental animals and there is evidence that they would be effective in man. TDR is cooperating with Chinese scientists in the development of artemisinin and its derivatives. This compound, which was originally extracted from the traditional herbal remedy *Artemisia annua*, is effective against chloroquine-resistant parasite strains. Unrelated in chemical structure to any existing antimalarial compound, it will probably lead to the development of a new generation of drugs with a novel mode of action and therefore less risk of parasite crossresistance to other drugs.

Several other antimalarial compounds are now ready for trial in man and will be developed in collaboration with industry. One, halofantrine, will be developed in collaboration with WRAIR and a pharmaceutical company.

Within the next five years, at least three antimalarial drugs with novel chemical structures will probably become available for use. Malaria control programmes now relying on chloroquine and related compounds will have a variety of drugs available for prevention, treatment and control.

The leishmaniases

Treatment of the leishmaniases, especially of systemic forms, remains a challenge, since no satisfactory drug exists. Trials of allopurinol in patients with visceral leishmaniasis have shown promising results. Interest is currently focused on allopurinol riboside, the metabolite which seems to be the active principle of the compound and which is currently under trial in the treatment of the mucocutaneous form of the disease.

African trypanosomiases

Studies of *Trypanosoma brucei* metabolism have highlighted the key role of polyamine synthesis and, in particular, the enzyme ornithine decarboxylase. DL- α -difluoromethylornithine (DFMO), known to be a specific inhibitor of this enzyme, has proved effective in experimental infections and is currently being evaluated clinically, both alone and in combination with bleomycin.

Filariasis

In collaboration with the Onchocerciasis Chemotherapy Project, TDR has accorded highest priority to the development of new drugs, especially for the treatment of onchocerciasis. Currently available drugs are of limited efficacy: they have unpleasant side-effects and unless used under the careful supervision of an ophthalmologist, may cause permanent eye damage. In collaboration with industry, major advances have been made in the search for safe and effective drugs.

The most promising compound is ivermectin, a drug originally developed by industry for veterinary purposes and now undergoing clinical trials for the treatment of human onchocerciasis. WHO is collaborating with the manufacturer in the evaluation of this compound. Early results are encouraging: a single dose has an apparently strong microfilaricidal effect, and the limited evidence so far available suggests that ivermectin is better tolerated than existing drugs. If preliminary safety and efficacy findings are confirmed, ivermectin would represent a major

advance in the treatment of river blindness.

Other anti-onchocerciasis drugs are in the pipeline. One which would kill or permanently sterilize the adult onchocercal worm is still needed. In a study carried out in Mexico (in an institution receiving a TDR long-term support grant), parenteral flubendazole produced a marked, sustained fall in microfilarial counts in onchocerciasis patients, but the injection was painful. The drug is currently being reformulated before being further evaluated in man.

Two experimental compounds, CGP 6140 and CGP 20376, have been found to kill filarial worms in animal experiments. They are now ready to be submitted to Phase I clinical trials: CGP 6140 will first be tested against onchocerciasis and CGP 20376, against lymphatic filariasis.

Sterilization of Trypanosoma cruzi-infected blood

In some areas endemic for Chagas' disease, blood transfusion carries a relatively high risk of *T. cruzi* infection. Although stored blood can be sterilized with gentian violet, the resulting deep purple staining is often unacceptable. Moreover, gentian violet may have other, as yet unknown, effects on blood or on the transfusion recipient. A new screening technique developed by the Programme has led to the identification of 21 promising compounds for sterilizing blood. The list is being narrowed down to two compounds, one of which may turn out to be safe and effective.

Promising leads for new drugs

Screening

TDR gives support for the screening of compounds for activity against malaria, the trypanosomiasis, the leishmaniasis, filariasis and leprosy (Fig. 1.1). The pharmaceutical industry and academia submit to these screens compounds which in many cases are selected on the basis of structure-activity relationships and other leads.

Rational drug development

Comparative biochemistry is providing useful leads for the development of new drugs. Studies on the purine metabolism of *Leishmania*, for example, have led to the identification of allopurinol as a potentially useful drug for the treatment of leishmaniasis. Attempts are being made to identify and exploit other metabolic differences between the parasite and the host.

Re-examination of existing drugs

The Programme has systematically re-examined drugs being used in the treatment of the six tropical

diseases. Findings from these studies are leading to more effective, safer drug use and providing leads for the development of new drugs. New data on drug distribution and metabolism are enabling empirically established drug dosage schedules to be designed on a rational basis. New regimens, for example, have been devised for pentavalent antimony compounds in the treatment of visceral leishmaniasis and new formulations and dose schedules are being developed for organic arsenic compounds in the treatment of African trypanosomiasis. Although chloroquine's mode of action and the mechanism of the malaria parasite's resistance to chloroquine remain perplexing problems, significant progress has been made in the study of primaquine metabolism.

Vaccines and immunotherapy

Rapid progress has been made in the development of vaccines against leprosy and malaria.

Vaccines at an advanced stage of development

Leprosy vaccine

A candidate vaccine against leprosy, based on killed armadillo-derived *Mycobacterium leprae*, induced cell-mediated immunity and was well tolerated in a Phase I trial in volunteers from a nonendemic area. Phase II clinical trials are now in progress in endemic areas. Significant advances have also been made in cloning *M. leprae* genes and should lead to the development of second-generation leprosy vaccines based on well-defined antigens produced by genetic engineering techniques.

Malaria vaccines

Work towards malaria vaccines has advanced on several fronts. Well-defined candidate antigens have been tested in experimental animals and are showing promising results. Research is most advanced on a sporozoite vaccine. Studies conducted within and outside the Programme have led to the chemical characterization of the circumsporozoite antigen, and the biologically active sites of the molecule are being identified. A sporozoite vaccine will in all probability be tested in man within the next few years. In collaboration with the United States Agency for International Development (USAID) and other agencies, the Programme has stimulated the interest of the pharmaceutical industry in the next phases of malaria vaccine development. One company is committed to developing, in collaboration with TDR, a polyvalent vaccine (directed against sporozoites, merozoites and gametes).

Promising leads

The leishmaniases

Significant progress has been made in probing, with a view to future vaccine development, the immune mechanisms involved in leishmanial infections. Further work will focus on the identification of candidate antigens that might be capable of conferring protective immunity.

Other diseases

Host-parasite relationships are now better understood for African trypanosomiases, Chagas' disease, schistosomiasis and filariasis, but more work needs to be done before the feasibility of developing vaccines against these infections can be assessed.

Diagnostic tests

Simple, sensitive, highly specific tests are being

developed for clinical and epidemiological use.

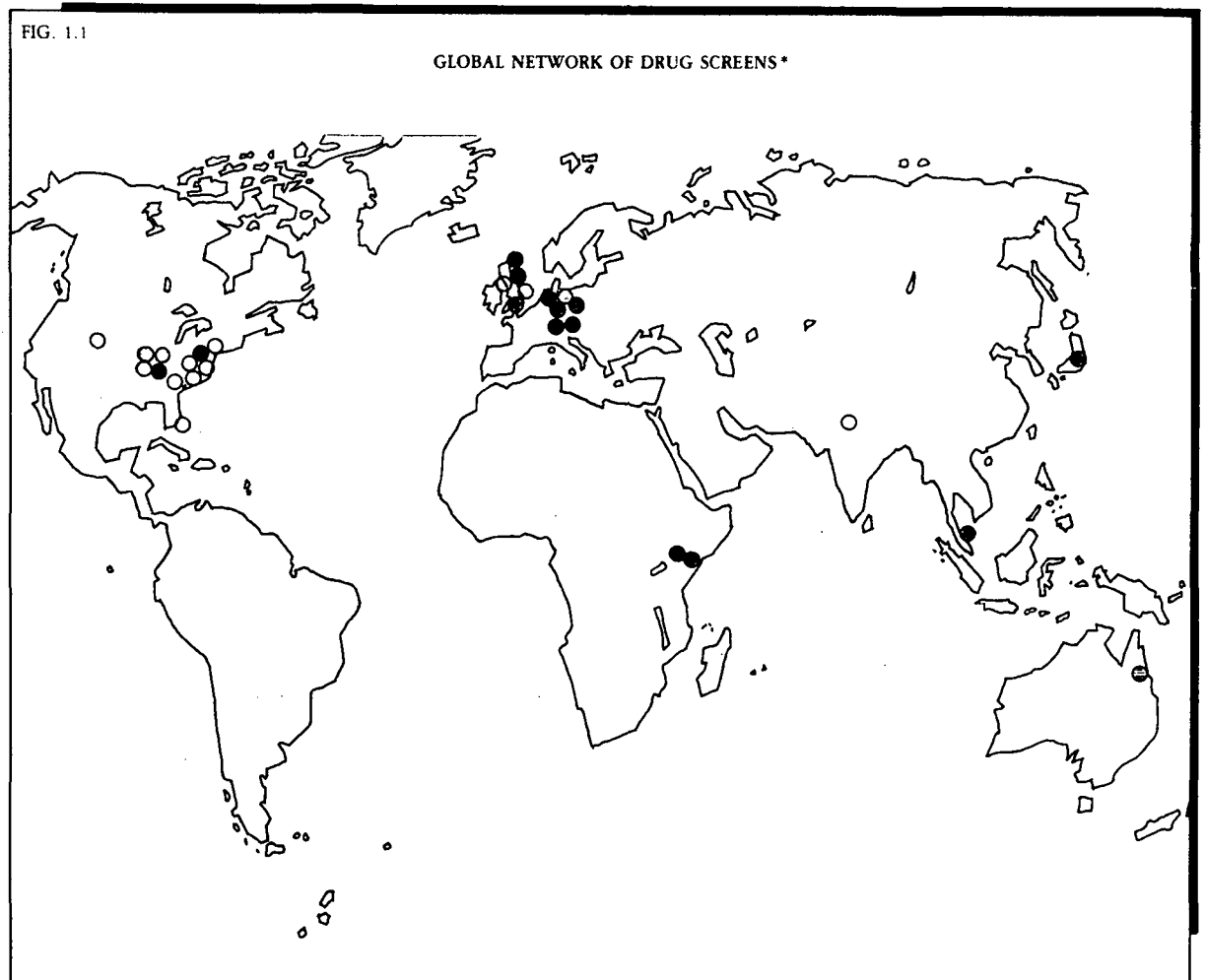
Tests ready for or in use

P. falciparum drug-sensitivity kits

The Programme has developed *in vitro* test kits, based on a method discovered outside the Programme, to determine the sensitivity of *P. falciparum* to drugs. "Macro" kits, requiring a relatively large quantity of blood (10 ml), have now been replaced by "micro" kits, which require only a few drops of blood (0.1 ml) obtainable by finger-prick. Kits are now available for testing *P. falciparum* sensitivity to chloroquine, amodiaquine, mefloquine, quinine and pyrimethamine, as well as to the sulfadoxine/pyrimethamine combination.

The trypanosomiases

A simple card agglutination test for the diagnosis of African trypanosomiases has been submitted for



GLOBAL NETWORK OF DRUG SCREENS*

Malaria:

- *In vitro* screen against cloned *P. falciparum* isolates (Washington, DC, USA)
- Long-acting blood schizontocidal activity (Miami, FA, USA)
- *In vitro* gametocidal activity against *P. falciparum* (New York, NY, USA)
- Exoerythrocytic activity against *P. berghei* *in vitro* (Rockville, MD, USA)
- ** Causal prophylactic and tissue schizontocidal activity *in vivo*, including *P. cynomolgi* screen (Lucknow, India)
- ** Causal prophylactic and blood schizontocidal activity *in vivo* (London, England)
- *In vitro* screen against cloned *P. falciparum* isolates (Chapel Hill, NC, USA)
- ** Blood schizontocidal activity *in vivo* (Washington, DC, USA)

Filariasis:

- * *In vivo* filaricidal screen using *D. immitis* in ferrets and dogs (Rahway, NJ, USA)
- *In vivo* screen using *Onchocerca* infection of cattle (Townsville, Queensland, Australia)
- *In vivo* screen using *B. pahangi* in jirds (London, England)
- *In vivo* screen using *B. pahangi*, *B. malayi*, *D. viteae* and *L. carinii* in multimammate rats (Giessen, Federal Republic of Germany)
- ** *In vivo* screen using *L. carinii* and *D. viteae* in rats and jirds (Frankfurt/Main, Federal Republic of Germany)
- ** *In vivo* screen using *B. pahangi* in jirds (Beckenham, England)
- *In vivo* screen using *B. malayi* or *B. pahangi* in *Presbytis* monkeys (Kuala Lumpur, Malaysia)

- *In vivo* screen using *L. carinii* and *B. pahangi* in jirds (Athens, GA, USA)
- ** *In vivo* screen using *L. carinii* in cotton rats (Basel, Switzerland)
- ** *In vivo* screen using *L. carinii*, *D. viteae* and *B. malayi* in jirds and mice (Basel, Switzerland)
- *In vivo* screen using *L. carinii* in cotton rats (Tokyo, Japan)

African trypanosomiases:

- *In vivo* trypanocidal activity in mice (Nairobi, Kenya)
- *In vivo* activity in vervet monkeys (Nairobi, Kenya)
- Activity against cerebral infection in mice (Glasgow, Scotland)
- ** *In vivo* activity in mice (Berlin [West])

Chagas' disease:

- *In vivo* primary screen (Athens, GA, USA)
- *In vitro* screen using compounds registered for use in man (Beckenham, England)

Leishmaniases:

- Primary screen against *L. donovani* in hamsters (Athens, GA, USA)
- *In vitro* screen against *Leishmania* promastigotes and amastigotes (Denver, CO, USA)

Leprosy:

- *In vivo* screen of new compounds (Atlanta, GA, USA)
- *In vitro* screen using "M. lufu" (Borstel, Federal Republic of Germany)

* Primary, secondary and tertiary screens refer to a progressive series of tests which use a variety of *in vitro* and *in vivo* models to select compounds effective enough to be tested in man.

** Not TDR-funded

further field evaluation and is now commercially available.

The miniature anion exchange column test (MAECT), developed outside the Programme, has been evaluated with TDR support for the diagnosis of African trypanosomiasis and is now available for operational use. Techniques for the serodiagnosis of Chagas' disease have been standardized, and sera from known infected and uninfected individuals are being provided by a TDR-supported central Reference Laboratory in Brazil to a network of 12 laboratories, of which 11 are in Latin America.

Tests at an advanced stage of development

Chagas' disease

A simple immunodiagnostic test has been developed for the rapid screening of stored blood for Chagas' infection and is now undergoing further evaluation before being made available for use.

Sporozoite infection in mosquitoes

The test devised at New York University by Fidel P. Zavala and associates for the identification of sporozoite species in mosquitoes, using monoclonal antibodies in a sensitive assay system, is now being developed into a field kit and should provide epidemiologically important information about malaria vectors in endemic areas, which could help malaria control personnel identify the precise roles of individual vectors in malaria transmission and target antivector measures more selectively.

Monoclonal antibodies in research on the leishmaniases and leprosy

Monoclonal antibodies are being widely used in research on all six diseases within TDR's mandate. They have been found particularly useful in the identification of *Leishmania* species and subspecies. Monoclonal antibodies recognizing epitopes present on *Mycobacterium leprae* but not on related organisms are now being exploited for possible leprosy-specific diagnostic tests.

DNA probes

DNA probes are being used to identify *Plasmodia*, *Leishmania*, trypanosomes (both *T. brucei* and *T. cruzi*) and other parasites, and suitable field methods are being developed.

Vector control

Biological control has greatly advanced during the past few years and some new agents are already being used in the field.

Vector control agents ready for or in use

Sporogenic bacteria

Discovered outside the Programme, *Bacillus thuringiensis* was developed in collaboration with industry and is now being used by WHO's Onchocerciasis Control Programme (OCP) to control *Simulium* vectors in the Volta River Basin area in West Africa. New formulations are also being tested for use in mosquito control.

Mechanical traps

Simple tsetse fly traps, originally designed in work conducted outside the Programme, have been further developed with Programme support and evaluated in different ecological settings. TDR has stimulated collaboration among scientists hitherto working separately; traps of greater efficacy have been designed and in some areas are now being used for sleeping sickness control.

Vector control agents at an advanced stage of development

Bacillus sphaericus

Three strains of *Bacillus sphaericus*—1593, 2297 and 2362—are currently being investigated. There is particular interest in the use of *B. sphaericus* for mosquito larvae control, as it is effective in polluted streams, and in some situations significant antilarval activity is maintained for several weeks after a single application.

Lagenidium giganteum

The development of another promising agent, *Lagenidium giganteum*, had previously been limited by the difficulty of cultivating the organism in the laboratory. This problem has been solved, and *L. giganteum* is being evaluated and developed into a usable tool.

Promising leads

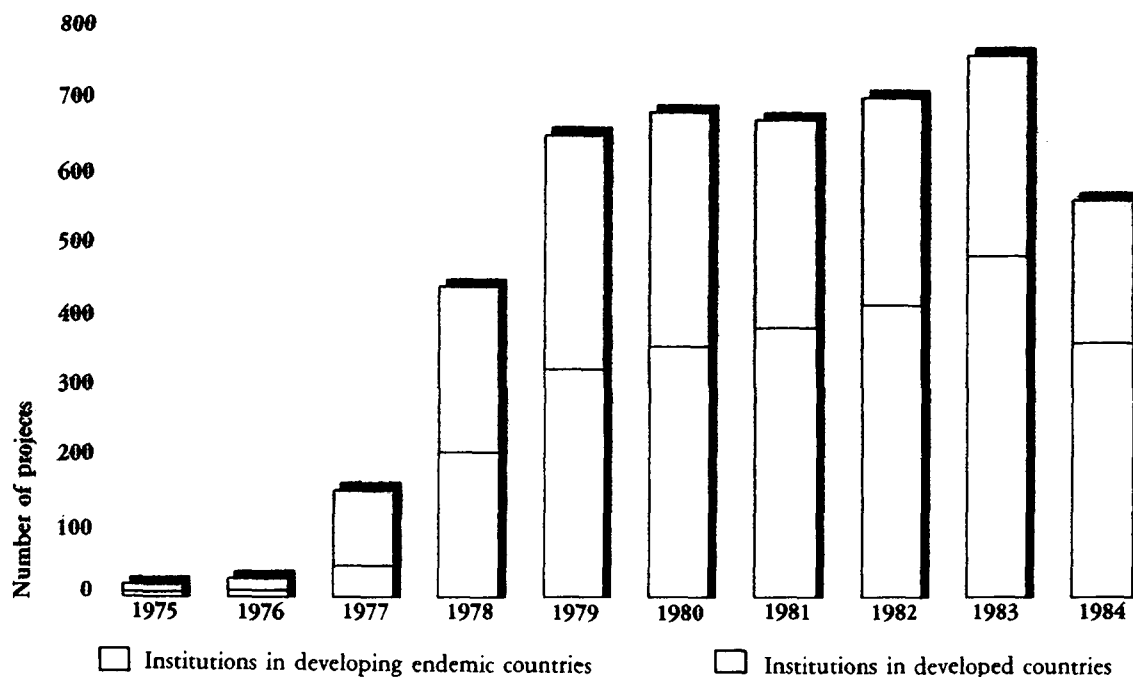
An asporogenic strain of *B. thuringiensis*, offering certain advantages over the original sporebearing strains, is being investigated, as are other promising agents, according to the WHO five-stage testing procedure.

Basic research

The advances so far described represent intermediate or final stages in research and development. The Programme also supports the more fundamental studies which provide the groundwork for future

FIG. 1.2

NUMBER OF ACTIVE PROJECTS SUPPORTED BY TDR FUNDING
TO INSTITUTIONS FROM 1975 TO 1984



advances. Some examples:

- The cloning of *P. falciparum* opens the way to a variety of genetic and biochemical studies, including research on drug resistance.
- A method has been discovered of cultivating *in vitro* the infective larvae of two *Brugia* filarial species and it is now being exploited to obtain filarial antigens for diagnostic studies.
- DNA analysis, using restriction enzymes and probes, is being used to identify parasites and study the relationships between parasites and disease.
- Monoclonal antibodies are being used to improve diagnostic tests, to identify parasite antigens and to serve as reagents for antigen preparation and assessment of immunity following passive transfer.
- Genetic engineering technology is being used to obtain antigens suitable for tests and vaccines.

These and other new concepts and techniques are moving research forward in ways unthinkable a few years ago, and are already having a profound impact on the development of new tools. Malaria vaccines, for example, could never have been developed to such an advanced stage were it not for these advances in biological sciences.

TDR's changing role in research and development

Initially, the role of TDR was to stimulate and support research on the six target diseases. From 1975 to the end of 1984, the Programme supported a total of 2290 projects, of which 1331 were in developing endemic countries (Fig. 1.2). One measure of the output of this research is the number of reports of TDR-supported work published in the scientific literature (Fig. 1.3), which had reached 3817 (plus a further 421 in press) by the end of 1984, compared with 2798 at the end of 1983 and 1800 at the end of 1982. Of these 3817, nearly half appeared in 1983-84, with a fairly even distribution between developing and developed countries.

In many cases, Programme support has been seminal, providing encouragement and often enabling scientists to obtain additional resources. Most of the scientists working on malaria vaccines, for example, received their first grants from WHO and TDR, and now obtain more extensive funding from other sources.

TDR is increasingly called upon to coordinate efforts now supported by a variety of funding agen-

cies. The Programme fulfils this new role in many ways: by creating a framework for future research through the organization of planning and review meetings and the publication of progress reports and state-of-the-art reviews; by conducting workshops for the standardization of reagents (e.g. monoclonal antibodies for research on malaria and leprosy); by setting up reference biological reagents and parasite strains (e.g. reference sera for serodiagnosis of *T. cruzi* infection and reference *Leishmania* strains); and by promoting exchange visits between scientists.

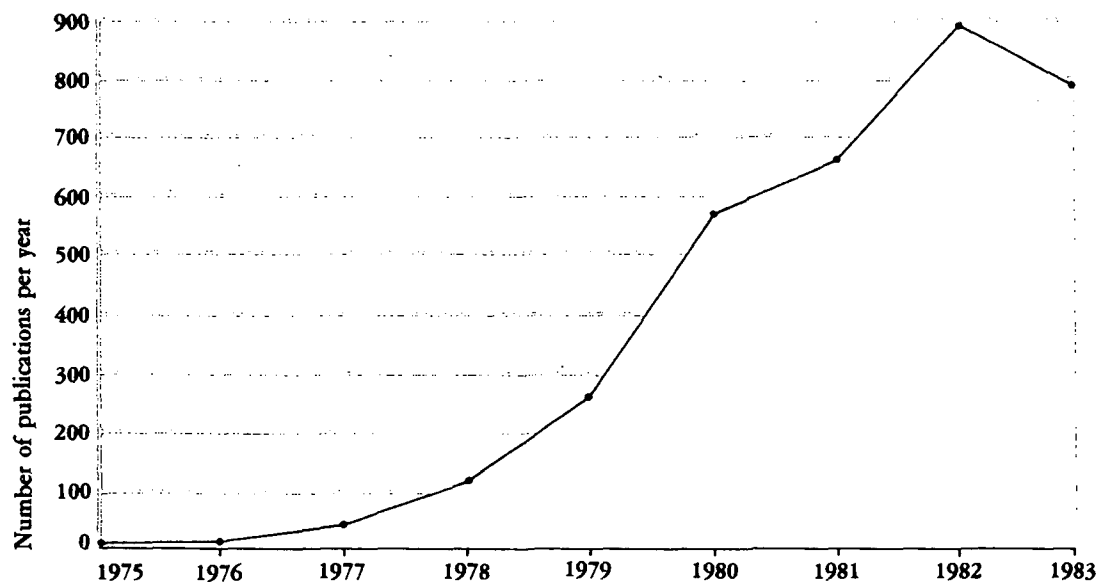
The Programme is also being called upon to collaborate with the pharmaceutical industry in the testing and further development of agents that were discovered, in some cases, with TDR support. The Programme's ability to organize the clinical and field evaluation of new products in endemic areas is being increasingly recognized (TDR is working with 20 companies on a variety of projects [Table 1.1]).

Research in the field

Effective disease control depends not only on drugs and vaccines but also on an accurate knowledge of the distribution of the diseases, the factors that predispose to their occurrence and the efficacy of specific control measures. This calls for research on the diseases as they occur in different populations and settings. As a result of TDR-supported studies, more is now known about the effectiveness of new anti-schistosomal strategies being implemented in several African countries, some of which are basing their control programmes on the findings of these studies. Similarly, new data derived from TDR-supported research on the distribution and extent of the leishmaniasis form the basis for control programmes in several countries affected by these diseases. Moreover, risk factors are now being identified for African trypanosomiasis and lymphatic filariasis.

FIG. 1.3

PUBLICATIONS (REPORTED TO TDR) FROM PROJECTS SUPPORTED BY TDR FUNDING TO INSTITUTIONS FROM 1975 TO 1983*



Year	1975	1976	1977	1978	1979	1980	1981	1982	1983	Cumulative Total 1975-1983
Institutions in developing endemic countries	2	1	8	39	62	190	245	396	408	1351
Institutions in other countries	1	5	30	78	188	371	411	498	379	1961
Cumulative Total 1975-1983	1351									
	= 3312									

* 1983 data incomplete

TABLE 1.1

TDR's collaboration with industry: Number of TDR projects contracted between 1975 and 1984

Country	No. of firms	No. of projects	TDR Component								TDR support (US\$)
			MAL	SCH	FIL	TRY	CHA	LEI	LEP	BIO	
Belgium	1	1	—	1	—	—	—	—	—	—	35 000
China	1	2	2	—	—	—	—	—	—	—	72 610
Germany, Federal Republic of	3	4	—	1	2	1	—	—	—	—	262 500
India	1	2	1	—	1	—	—	—	—	—	54 850
Switzerland	1	2	—	1	1	—	—	—	—	—	53 782
United Kingdom	1	4	—	—	2	—	1	—	1	—	476 652
United States of America	12	22	6	4	3	—	2	1	5	1	1 735 417
Total	20	37	9	7	9	1	3	1	6	1	2 690 811

MAL = Malaria
 SCH = Schistosomiasis
 FIL = Filariasis
 TRY = Trypanosomiasis

CHA = Chagas' disease
 LEI = Leishmaniasis
 LEP = Leprosy
 BIO = Biomedical sciences

Ultimately, to have some chance of success, disease control measures must be understood and accepted by the populations involved, as well as being affordable, in terms of money and effort, by these populations. TDR-supported research relating to malaria, filariasis and leprosy has shown how important a community's perception of disease is in determining whether people's behaviour will help or hinder disease control. Application of such findings to control programmes is now under study.

Methods of assessing the cost-effectiveness of control programmes are also the subject of several studies supported by the Programme, and ministries of health in a number of endemic countries are showing increasing interest in using the findings of these studies to improve their disease control services.

Strengthening research capabilities in developing countries

During 1983-84, a total of 25 institutions received grants, through TDR's Research Strengthening Group (RSG), in support of research activities, and 139 scientists and other research personnel received training grants. Institutions are also being strengthened through their participation in the research and development activities of the Programme, formal training courses have been supported by the Programme for Master of Science degrees in medical entomology, epidemiology and malacology, and short courses and workshops have been organized to promote the rapid transfer of technology to developing countries. Scientists and institutions in developing

countries have made major contributions to the development of tools for the treatment and control of the six target diseases, notably in activities best carried out in the endemic areas—epidemiological surveys, clinical trials, social and economic research. These activities have resulted in important scientific achievements: the discovery of *T. cruzi* schizodemes, test kits for malaria parasite sensitivity to drugs and the worldwide mapping of chloroquine sensitivity to drugs, to mention only a few. Strengthened institutions are providing training for scientists from other institutions in the same or different countries.

At the national level, institutions being strengthened by TDR are providing ministries of health with technical support for disease control activities, and data generated from field studies are being used to plan, modify and evaluate control programmes. Over the next two years, the RSG will focus on strengthening capabilities in field research and basic biomedical sciences.

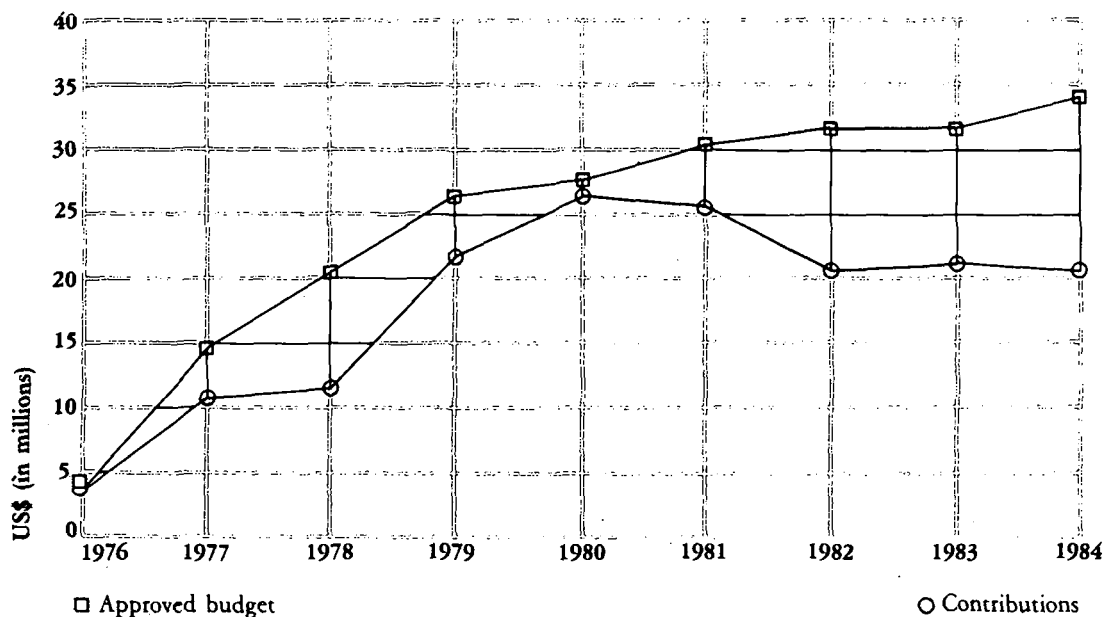
Maintaining the momentum

The mechanisms established by TDR to promote research and carry research findings through to the point where they become practical tools for disease control do seem to work. But if the initial investment in creating these mechanisms is to reap its full benefits, the effort must be sustained: promising scientific leads take time to develop into useful disease control tools—time and, of course, money.

Clouding TDR's horizon is the widening gap between the Programme's budget and the financial

FIG. 1.4

TDR'S APPROVED BUDGET AND "INCOME" FROM CONTRIBUTIONS



resources being made available to it through voluntary contributions from government agencies, philanthropic foundations and other sources (Fig. 1.4). Because of this gap, planned activities cannot be completed nor hoped-for results achieved as quickly as they might be, and the promise of some exciting leads cannot be fulfilled as urgently as it should be.

On the other hand, parasitic diseases are coming more and more to exert their own fascination as subjects of scientific scrutiny. More scientists than ever before are aware of the human problems related

to tropical diseases. And the interest and concern of research funding agencies and of industry have been aroused.

TDR's efforts are beginning to bear fruit and to justify the hope that lasting improvements can be made in the health of tropical peoples. It is now critical that adequate, sustained financial support be forthcoming so that these early results can be elaborated into a unique, powerful and realistic offensive against the diseases that have kept these peoples for so long under so heavy a burden of suffering.

2 Malaria

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Note on lists of publications

Publications and other documents relating to the subject of this chapter are listed, at the end of each section, under three headings: Publications acknowledging TDR support (list A), Publications from work outside the Programme (list B) and TDR and related WHO scientific reports which became available in 1983-84 (list C). Since most of the work described in the Report is too recent to have appeared in the literature, only a small proportion of publications listed relate directly to the text. List A comprises publications acknowledging TDR support that have not appeared in any previous Programme Report and list B, only those publications pertinent to specific points discussed in the text. The C list for the section of this chapter on Applied Field Research in Malaria includes an Inventory of Applied Field Research in Malaria, which is intended for scientists who do not have direct access to the complete literature on field research in malaria: the first edition was published in 1981 and an updated edition (C3, in this section), in 1984. All the documents in list C can be obtained by writing to: The Office of the Director, TDR, World Health Organization, 1211 Geneva 27, Switzerland; requests should carry the full document title and reference number.

2 Malaria

Introduction

The figures

Malaria is still the most important parasitic disease in the tropics. In 1982, the most recent year for which reliable figures are available, 365 million people—nearly one-twelfth of the world's population—were living in areas, mostly in Africa, where malaria is still highly endemic and where no specific antimalaria measures are being applied. A further 2217 million—46% of the world's population—were living in areas where malaria is still endemic but where control measures have reduced its level of endemicity to a certain degree.

This epidemiological picture (Fig. 2.1) has not changed significantly over the past decade and there is no reason to expect that it will in the near future, unless malaria is allowed to take root again in some parts of the world as a result of reductions or interruptions in control measures.

Excluding Africa south of the Sahara, 7.8 million malaria cases were *reported* to WHO in 1981 as opposed to 8.0 million in 1980 and 7.0 million in 1979. The provisional figures for 1982 were 6.5 million. These data must be considered with caution, since screening, diagnosis and notification efforts have declined over the past 15 years. Many countries have been forced to scale down malaria control measures, thereby reducing the availability of accurate information on the disease. The above figures may therefore not reflect a true reduction in global malaria incidence.

In areas other than tropical Africa the incidence of the disease is currently *estimated* to be around 20 million cases a year. In Africa south of the Sahara some 200 million people are believed to be chronically infected and of these, about one-third suffer acute manifestations of the disease in the course of a year. Accurate figures are not available for the number of deaths caused primarily by malaria, although in Africa alone it is estimated that the disease is responsible for the deaths of one million infants and young children each year.

Malaria incidence varies widely from one part of the world to another. In North Africa it has been drastically reduced and in Tunisia, eradicated altogether. In southern Asia, where a new peak was recorded only a few years ago, the incidence has fallen again, but fairly slowly. By contrast, in Central and South America there has been a steady, uninterrupted rise since 1974, whereas in tropical Africa—a mainly hyper- or holoendemic area—there has been little change, apart from an increasing involvement of urban and periurban areas. Europe, Australia and the United States have remained free of malaria transmission despite massive importation of cases from other countries.

The organisms

Plasmodium falciparum is the parasite responsible for most cases of malaria (80% worldwide) and for the most severe, often fatal, forms of the disease. It is deeply entrenched in tropical Africa. *P. vivax* is the commonest species in the Americas and in Asia. These two species account for most of the human suffering and economic loss due to malaria in the world today. In southern Asia, where renewed efforts at malaria control have shown recent successes, the incidence of *P. vivax* infection has fallen but that of *P. falciparum* has remained virtually unchanged. This is an ominous sign and apparently due to drug resistance and the impossibility of achieving radical cures in affected areas. Any relaxation of malaria control efforts in these areas could well cause an explosive upsurge in incidence.

Control

The repertoire of tools and methods available for malaria control has not increased significantly over the past three decades, and has even steadily declined in efficacy. In many areas, resistance of vector anopheline mosquitoes to insecticides has prompted a shift from chlorinated hydrocarbon insecticides, such as DDT and HCH, to organophosphorous compounds or the more expensive carbamides. Exophilic

(outdoor) vectors, such as the *Anopheles balabacensis* mosquitoes in South-East Asia or the *An. nuneztovari* group in South America, are not amenable to control by intradomestic insecticide spraying, and the inaccessibility and widespread nature of their breeding places make larval control practically impossible.

Resistance

Resistance of *P. falciparum* to drugs has probably become the most important threat to effective control of the disease. It has arisen largely through a combination of massive antimalarial drug deployment and a failure to combat transmission of the disease.

Box 2.1 The menace of malaria

- In temperate zone countries with originally unstable (predominantly epidemic) malaria—in the main, relatively affluent countries where economic growth outweighs population increases—the disease has been largely eradicated.
- In countries with originally stable hyper- or holoendemic malaria, the situation has not significantly changed:

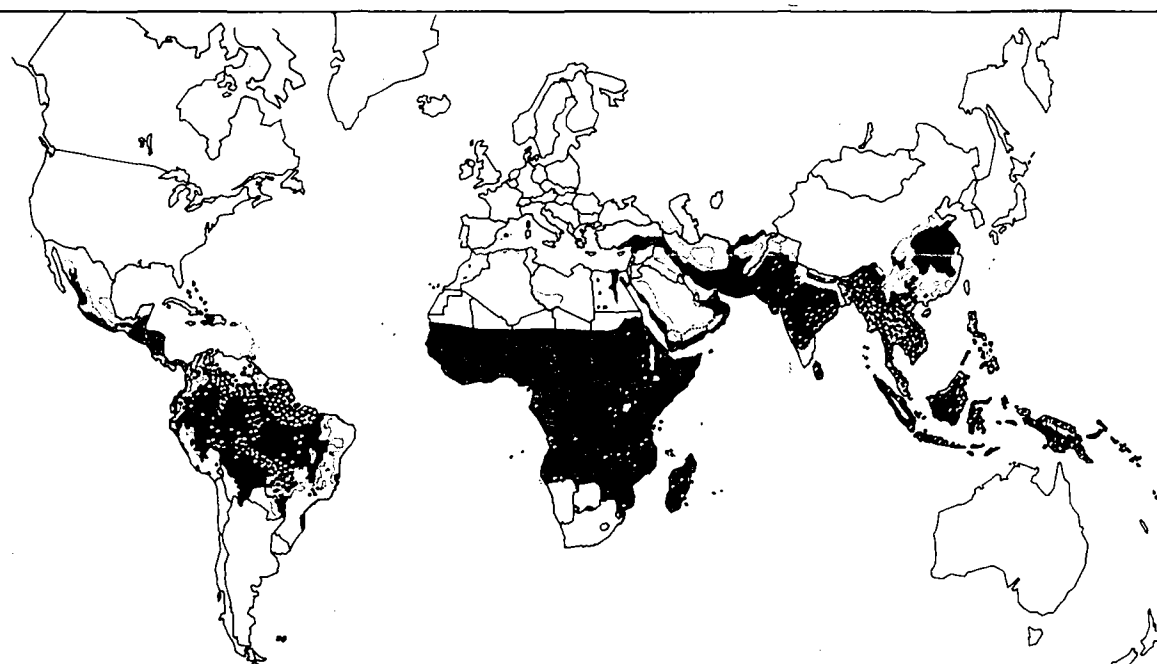
organized control efforts using presently available means offer little prospect of success and have therefore not even been attempted. Current measures—mainly treatment to reduce death and suffering from malaria—are increasingly threatened by the development and advance of drug resistance.

- In countries with originally moder-

ately stable meso- to hyperendemic malaria, control has yielded promising initial results and significantly curbed mortality and morbidity from the disease. However, a variety of technical problems and a shortage of resources have largely arrested further progress, and the disease has begun to strengthen its position.

FIG. 2.1

EPIDEMIOLOGICAL ASSESSMENT OF STATUS OF MALARIA AND CHLOROQUINE-RESISTANT *P. FALCIPARUM* *



- Areas in which malaria has disappeared, been eradicated or never existed
- ◻ Areas with limited risk
- Areas where significant malaria transmission occurs
- Areas of chloroquine-resistant *P. falciparum* reported by 1977
- Areas of chloroquine-resistant *P. falciparum* reported by 1984

* Map modified from WHO map 84092 (1984). Data on chloroquine-resistant *P. falciparum*: 1984 data modified from Wld. Hlth. Statist. Quart., 37: 161 (1984); 1977 data modified from W.H. Wernsdorfer. Die gelben Hefte, 19: 7 (1979).

Population movements have also played a part in the occurrence and spread of resistance.

Resistance to the new antimalarial mefloquine has been experimentally induced in rodent malaria models and in *P. falciparum* *in vitro*. Naturally occurring *P. falciparum* populations are known to vary considerably in their relative sensitivity to mefloquine. However, primary resistance to mefloquine (i.e. in patients not previously exposed to the drug) seems to be quite rare: to date, only two cases have been observed in two widely separated areas of the world. Current primary cure rates (up to 97% in some areas) for mefloquine treatment in *falciparum* malaria patients approximate those that were recorded for chloroquine shortly after its introduction as an antimalarial. Moreover, the majority of mefloquine treatment failures so far recorded have been associated with vomiting following administration of the drug and could therefore be attributed mainly to drug loss and inadequate treatment.

By the end of 1984 (Fig. 2.1), chloroquine-resistant *P. falciparum* was present in 15 countries in eastern Asia and Oceania, 10 in South America and 15 in Africa (mostly south of the equator, where it had reached the Atlantic coast in three countries). Resistance to the second-line sulfonamide/pyrimethamine combination is already widespread in the "hard-core" areas of South-East Asia and South America, and there have been recent reports of declining treatment efficacy in other areas, including East Africa. Although chloroquine and especially amodiaquine are still effective in most "semi-immune" individuals in tropical Africa, their ineffectiveness in an increasing number of non-immune patients poses a major threat to the lives of millions of infants and young children.

Apart from technical problems, malaria control has also suffered from inflationary increases in the costs of insecticides, drugs, equipment and fuel. In contrast, government budgetary expenditure on malaria

control has either not increased or, in many countries, has even been cut back. All in all, the balance favours the malaria parasite.

Current efforts at malaria control hold only limited promise. New approaches are clearly required, and this is the goal of the three Scientific Working Groups (SWGs) on Malaria, whose activities in 1983 and 1984 are described in the following pages.

- CHEMAL, the SWG on the Chemotherapy of Malaria, pursues the development of new antimalarial drugs. The recent registration of mefloquine and a mefloquine plus sulfadoxine/pyrimethamine combination was achieved largely on the strength of clinical trials conducted under CHEMAL auspices. Other drugs, most with entirely new mechanisms of action, are under development and innovative approaches to drug design are being pursued.

- IMMAL, the SWG on the Immunology of Malaria, has made considerable progress in the development of "causal" prophylactic (acting directly on the cause of the disease), clinically attenuating and transmission-blocking vaccines. Promising leads are expected to yield trial vaccines within the next few years and research on the mass production of vaccine antigens could lead to vaccines that are cheap enough for use even in the least developed countries.

- FIELDMAL, the SWG on Applied Field Research in Malaria, aims at the improvement of epidemiological and control technology and the validation and field application of new tools emanating from the work of CHEMAL and IMMAL. FIELDMAL spearheaded the establishment of a worldwide system for monitoring *P. falciparum* resistance to antimalarial drugs and is exploring new ways of achieving community participation in malaria control activities and of incorporating these activities within primary health care infrastructures.

Chemotherapy of malaria

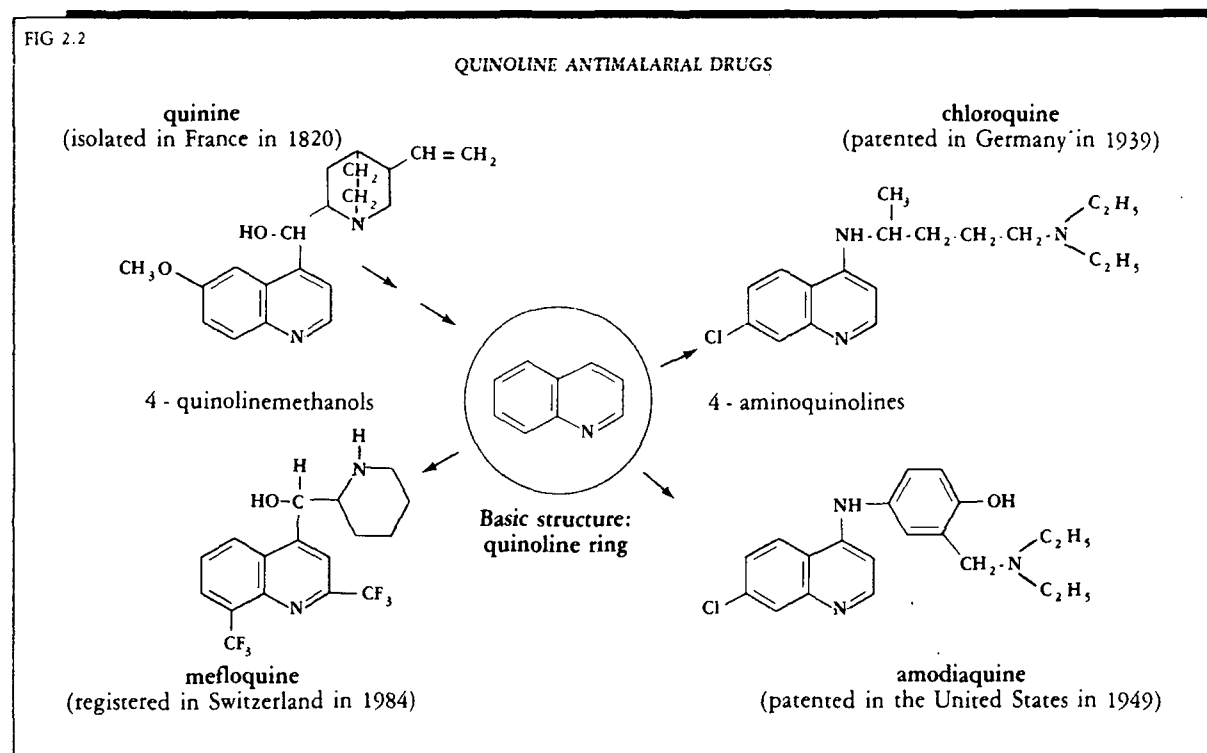
The context

□ Mefloquine, the first new antimalarial to be clinically tested in over 30 years, was made available to health authorities in several countries at the end of 1984. However, the chemotherapeutic armamentarium against malaria is still meagre. No suitable drugs exist for the long-term, mass treatment of communities exposed to epidemics of *Plasmodium falciparum* or for rapid, radical cure of *P. vivax* malaria, particularly in rural areas.

P. falciparum strains resistant to several antimalarial drugs—chloroquine, sulfadoxine/pyrimethamine combinations and quinine—are spreading rapidly throughout many parts of the world and threatening the lives of millions of people infected or at risk of infection with these multi-resistant organisms (see FIELDMAL section of this chapter, under “Global monitoring of drug resistance”).

New drugs are urgently needed that possess, respectively, blood schizontocidal, tissue schizontocidal and gametocytocidal activity. They must be inexpensive, safe, long-acting and effective in preventing relapses.

Mefloquine, a quinolinemethanol (Fig. 2.2), is a potent blood schizontocide active against multi-resistant *falciparum* malaria and continues to be developed by the Scientific Working Group (SWG) on the Chemotherapy of Malaria (CHEMAL) in collaboration with Hoffmann-La Roche and Company of Basel, Switzerland, and the Walter Reed Army Institute of Research (WRAIR), Washington, DC, USA. Studies carried out so far only permit its registration for prophylaxis and treatment in adults (males and nonpregnant females) and in children over two years of age. Trials of the “monosubstance”



(mefloquine alone) in pregnant women, in whom the effects of malaria can be devastating, will be completed as soon as possible.

Potential resistance to mefloquine is of great concern, for three main reasons: the drug is structurally similar to other currently used blood schizontocides, such as quinine, amodiaquine and chloroquine (Fig. 2.2); stable resistance has been induced in the laboratory; and isolated cases of mefloquine-resistant *falciparum* infection have been observed in the field. Everything must be done to prevent mefloquine resistance from becoming a major health problem. One approach is to ensure, as far as possible, that the drug will be used rationally: for example, in suitable combinations with other drugs—a strategy known to reduce the risk of resistance. One drug combination has already been developed, and WHO has issued recommendations for the use of mefloquine both alone and in combination with other drugs (B16).

Another approach is to develop a second generation of drugs, to be used if resistance becomes widespread. Such drugs should preferably have different structures and modes of action from mefloquine and the other drugs currently in use, lest crossresistance diminish their usefulness. CHEMAL is negotiating with pharmaceutical firms on at least five compounds undergoing preliminary clinical trials.

Qinghaosu (artemisinin), the active principle of the Chinese medicinal herb *Artemisia annua*, is a structurally novel antimalarial (Fig. 2.5). The parent compound and several derivatives have been widely studied by Chinese scientists and shown to have a rapid action in the treatment of chloroquine-resistant *falciparum* malaria. A CHEMAL meeting held in

Beijing in 1981 identified derivatives potentially suitable for the treatment of cerebral and other complicated forms of *falciparum* malaria. The assessment of this series of compounds is of extremely high priority.

Developing a drug from synthesis to clinical use can take many years (for mefloquine it took over 15). Priority is therefore already being given to the development of a third series of blood schizontocides. The steps in this process include: synthesis of "lead" compounds (e.g. simple trioxanes, structures derived from the more complex Qinghaosu molecule); evaluation of plant-derived compounds known to have antimalarial activity; studies on basic parasite biology and biochemistry to provide leads for the rational development of new compounds selectively toxic to the parasite (sparing the host). In view of the high financial risks involved, such long-term studies are not often carried out by industry.

The emergence and spread of resistance to widely used antimalarial drugs are regarded as inevitable. Susceptibility of the parasite to existing drugs must therefore be monitored and baseline sensitivity data obtained for new drugs. High priority is given to the development of tests for these purposes. Basic studies on the genetics of drug resistance are also in progress. It is hoped that these lines of research will lead to a more rational approach to the development of antimalarial drugs.

In circumstances where prophylactic measures are called for, a drug specially formulated to act for up to three months would ensure maximum patient compliance. Feasibility studies, using pyrimethamine as a model, are almost completed. Further development of long-acting formulations depends on the availability of drugs suitable for operational use. □

Box 2.2 Highlights of activities in 1983-84

- Mefloquine, the first new anti-malarial to be clinically tested in 30 years, has been registered in Switzerland for the prophylaxis and treatment of malaria in adult males and nonpregnant females and in children over two years of age. The triple drug combination comprising mefloquine, sulfadoxine and pyrimethamine has been registered in Switzerland and Thailand. Restricted use of the combination in adults and in children over two years of age began in Thailand in late 1984.
- Kits for the *in vitro* testing of

Plasmodium falciparum for susceptibility to sulfadoxine and pyrimethamine are being standardized in a multicentre study in Switzerland, Thailand and the United States, and field-tested in several malaria-endemic countries.

- Negotiations are under way with five pharmaceutical companies on the clinical development of new blood schizontocidal compounds belonging to a variety of chemical classes. Several simple trioxane ring compounds have also been shown to possess blood schizontocidal activity and several new

8-aminoquinolines, tissue schizontocidal activity. Primaquine itself has been the subject of intense research, essentially on pharmacokinetics, metabolism and mode of action, and significant findings are emerging.

- Exoerythrocytic stages of *P. vivax* have been successfully grown in culture, thus paving the way to development of *in vitro* screens for tissue schizontocidal activity of primaquine, its metabolites and other 8-aminoquinolines.

Report of activities in 1983-84

Blood schizontocides

Mefloquine

Clinical trials of mefloquine and mefloquine combinations for the treatment of falciparum malaria

By mid-1984, 17 clinical trials of mefloquine alone and of mefloquine in combination with sulfadoxine/pyrimethamine had been completed under the auspices of CHEMAL at clinical centres in Brazil, Thailand and Zambia. Findings from previous Phase I tolerance and bioavailability studies, and from Phase II and Phase III tolerance and efficacy studies in males, enabled Phase III studies to be extended to females and children. These trials and additional Phase III trials in males were completed in Thailand by mid-1984 (Table 2.1).

Mefloquine has been shown to be effective in a single dose for the treatment of adults and children. A dose of 750 or 1000 mg (base) caused early clearance of fever and of parasitaemia and radical cure in *falciparum* malaria patients, with no serious side-effects. Mefloquine was as well accepted as are standard antimalarial drugs, such as chloroquine and sulfadoxine/pyrimethamine, and more readily accepted than quinine plus tetracycline. Side-effects—nausea, vomiting, dizziness and abdominal pain—were generally dose-related, mild, of short duration and self-limiting. No biochemical or haematological abnormalities were reported (A42-43, A71, A88).

There are still gaps in clinical experience of mefloquine, notably with respect to its use in pregnant women. The need for a safe, effective drug is particularly pressing in this patient group, since formulations of pyrimethamine plus a sulfonamide are still regarded as contraindicated. An additional clinical trial centre was therefore established in Chantaburi,

Thailand, in 1983 and a comparative trial begun of mefloquine and quinine in pregnant women. This is the first trial of an antimalarial drug ever conducted in pregnant women. Studies on the pharmacokinetics and tolerance of mefloquine in malnourished patients and those with liver dysfunction (in whom drug bioavailability, metabolism and elimination may be impaired) were started at the Central Drug Research Institute in Lucknow, India, in 1984.

In an effort to delay the emergence of resistance to mefloquine, CHEMAL and Hoffmann-La Roche have undertaken studies on mefloquine combinations, including mefloquine plus sulfadoxine/pyrimethamine (Fig. 2.3), a combination now at an advanced stage of clinical development (Table 2.2). Mefloquine, sulfadoxine and pyrimethamine are pharmacologically well matched: given together, they have been found, in early animal studies, to considerably reduce the degree and delay the onset of resistance (A128, B12). Clinical studies using tablets containing 250 mg of mefloquine, 500 mg of sulfadoxine and 25 mg of pyrimethamine had been started by CHEMAL in late 1982, following pharmacokinetic and toxicity studies conducted by Hoffmann-La Roche. The need for these studies was highlighted by two confirmed reports in 1982 of resistance to mefloquine: one from Thailand (B1) was based on *in vitro* and *in vivo* results and supported by measurements of blood drug levels; the other, from the United Republic of Tanzania (B3), was based on *in vitro* observations. Reduced sensitivity to mefloquine has also been found in *falciparum* isolates from Gabon (B2) and the Philippines (B14), areas where mefloquine was either not available or had been used only in limited clinical trials.

Bioavailability of the triple combination appears satisfactory, the plasma half-lives of all components being indistinguishable from those observed after separate administration of mefloquine and sulfadox-

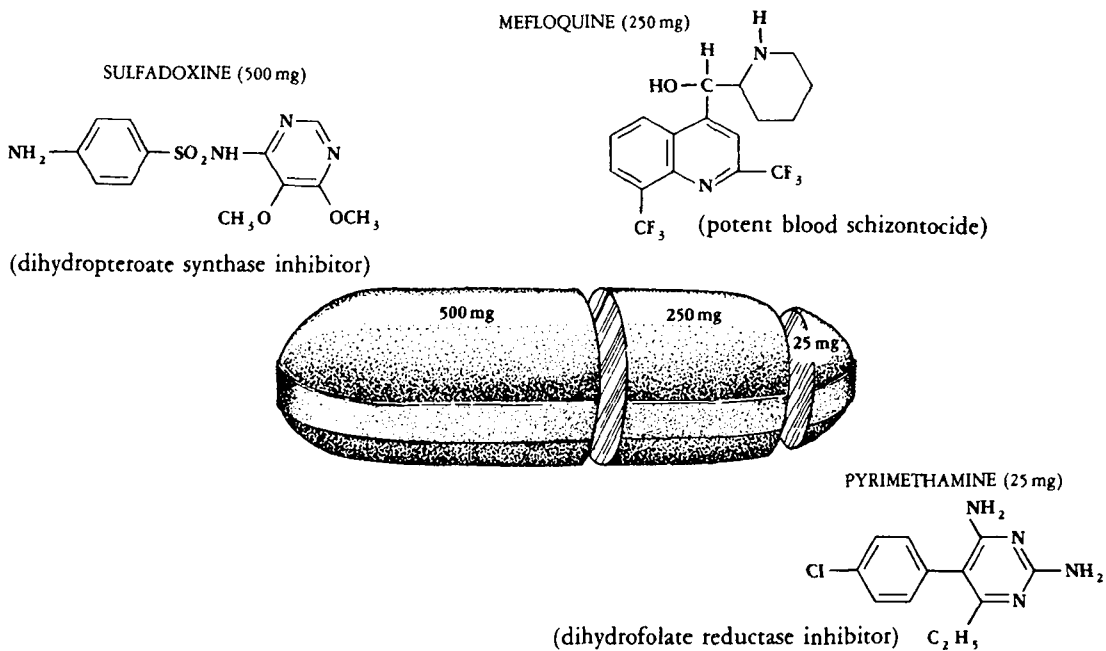
TABLE 2.1

Clinical trials of mefloquine (monosubstance) conducted by CHEMAL during 1982-84

Country	Phase	Type of trial	Type of case	No. of cases	Initiated	Completed
Thailand	III	Comparison of quinine and tetracycline	Males	101	1982	1983
Thailand	III	Noncomparative	Females	47	1982	1984
Thailand	III	Comparison with quinine	Pregnant women	150	1983	Ongoing
Thailand	III	Dose-finding study	Children (>2 yr)	73	1982	1984
Zambia	I	Multi-dose pharmacokinetic study (steady-state concentrations)	Males	6	1984	1984

FIG 2.3

MEFLOQUINE + SULFADOXINE/PYRIMETHAMINE: THE TRIPLE COMBINATION



ine/pyrimethamine. Similar observations have been made in Caucasian and African subjects, and tolerance has been good in all trials. Some gastrointestinal side-effects have been observed, but these have been mild and short-lived, and have required no specific treatment. No biochemical or haematological changes have been reported.

Dose-finding studies using one, two or three tablets of the triple combination for the treatment of *falciparum* infection were begun by CHEMAL in Brazil, Thailand and Zambia. In the Zambian study, there were no recrudescences in any patient group during the 63-day follow-up. This very high radical cure rate, achieved with a single tablet, may be due to the absence in the area of resistance to sulfadoxine/pyrimethamine. In Brazil and Thailand, where such resistance is common, recrudescences occurred after the administration of only one tablet. In Brazil, radical cure was obtained with two or three tablets. The study in Thailand has been completed and the results are being analysed.

On the basis of these trials and others conducted by Hoffmann-La Roche, a field trial on the use of the triple combination for the curative treatment of *falciparum* infections in an area of multiple resistance was conducted by FIELDMAL in Thailand (see FIELDMAL section, under "Field trials of drugs").

This was followed by a large-scale field trial conducted by Thai authorities in collaboration with WHO. These trials are still under way, but preliminary results show cure rates similar to those observed clinically. Tolerance of the combination has been excellent.

There is evidence that in most cases the administration of a single dose of the gametocytocidal drug primaquine together with an effective blood schizonticide prevents or eliminates gametocytaemia, which should reduce the transmission and spread of resistant *Plasmodium falciparum* (B16). In a CHEMAL study conducted at Rush-Presbyterian St. Luke's Medical Center in Chicago, IL, USA, the administration of primaquine and mefloquine did not influence the toxicity or pharmacokinetics of either compound. Primaquine given in a single dose (45 mg base), with either mefloquine or the triple combination, was well tolerated. There was evidence of an increased gametocytocidal effect when primaquine was administered to gametocytaemic patients on the same day as mefloquine rather than three days later. Further studies are in progress to determine optimal timing of drug administration and whether primaquine is effective in patients who become gametocytaemic following mefloquine treatment.

TABLE 2.2

Clinical trials of mefloquine + sulfadoxine/pyrimethamine (S/P) combination supported by CHEMAL during 1983-84

Country	Phase	Type of trial	Type of case	No. of cases	Initiated	Completed
Brazil	I	Comparison with S/P	Males	20	1982	1983
Brazil	II/III	Dose-finding study	Males	150	1983	1984
Brazil	II/III	Combination with primaquine	Males	120	1984	Beginning
Zambia	II/III	Dose-finding study	Males	150	1983	1983
Zambia	III	Dose-finding study	Children (>2 yr)	150	1984	Ongoing
Zambia	II/III	Comparison of final pharmaceutical formulation with chloroquine	Males	40	1984	1984
Zambia	III	Comparison with S/P	Males	100	1984	Ongoing
Thailand	III	Dose-finding study	Males	150	1983	1984
Thailand	III	Combination with primaquine	Males	124	1984	Ongoing
Thailand	III	Dose-finding study	Children (>2 yr)	150	1984	Ongoing

Clinical trials of mefloquine and mefloquine combinations used suppressively

Although population-wide drug suppression is no longer an acceptable malaria control measure, the suppressive or prophylactic use of drugs may be considered for special groups and circumstances (B16). Clinical studies on suppression using mefloquine and the triple combination are being carried out chiefly by Hoffmann-La Roche. In Zambia, a pharmacokinetic study of mefloquine, given in weekly doses to achieve steady-state drug levels, was completed by CHEMAL in 1984 and the results are being analysed.

Registration and deployment of mefloquine and mefloquine combinations

In February 1984, mefloquine (monosubstance) was registered in Switzerland, under the trade name Lariam^R, for prophylaxis and treatment in adults and in children over two years of age. Authorization of its use in pregnant women and in children under two years of age was not applied for, since adequate clinical data were not available at the time.

The triple combination—mefloquine plus sulfadoxine/pyrimethamine—has been registered, under the name Fansimef^R, in Switzerland and Thailand,

Box 2.3 Recommendations of a WHO scientific group on the chemotherapy of malaria

"The Group, recognizing the urgent need to protect mefloquine and ensure its optimal deployment, strongly RECOMMENDS:

(a) that governments should legislate for strict control of the importation, distribution, and utilization of mefloquine alone or in drug combinations;

(b) that the use of mefloquine by communities in endemic areas should

be restricted to the treatment of acute malaria attacks that are likely to be due to multiple drug-resistant *P. falciparum* ...;

(c) that, when available, drug combinations known to delay the development of drug resistance (as might be the case with the mefloquine/sulfadoxine/pyrimethamine combination currently under development) should be used for prophylaxis or treatment,

when necessary, instead of mefloquine; (d) that mefloquine should *not* be distributed for use as a single prophylactic drug by residents in endemic areas" (B16).

These recommendations will be presented to the Executive Board of the World Health Organization in January 1985.

for restricted use in adults and in children over two years of age. Future registration of the combination will be restricted to countries where chloroquine-resistant *P. falciparum* strains are present. The marketing and distribution of the two drugs have been agreed upon by WHO and the manufacturer, taking into consideration the need to maintain the usefulness of the drug in endemic countries and the recommendations formulated at a WHO meeting in September 1983 (See Box 2.3).

New blood schizontocidal compounds

Discussions are in progress with five pharmaceutical companies on the further clinical development of new blood schizontocidal compounds. Several compounds of a different chemical class from drugs in current use and from mefloquine are undergoing early, limited clinical trials in man. One, the phenanthrenemethanol halofantrine (Fig. 2.4), was originally thought not to be effective against mefloquine-resistant *Plasmodium*. However, since recent studies have shown that crossresistance involving this drug is not absolute, clinical trials are now being considered.

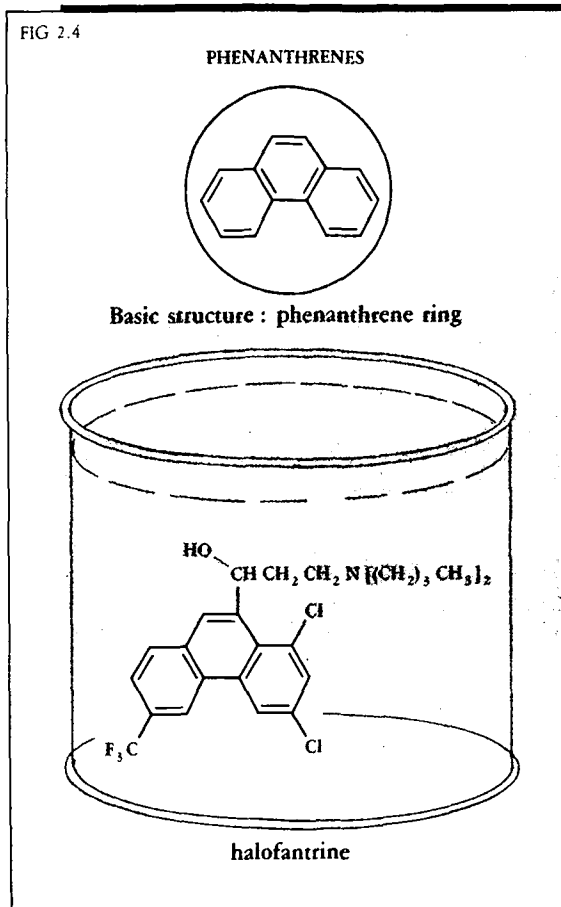
Plant-derived substances

Qinghaosu (artemisinin)

The therapeutic effectiveness of Qinghaosu and its derivatives (sesquiterpene lactones) and their possible development in accordance with international standards have continued to be the subject of intense study. These naturally occurring peroxide compounds have been extensively studied in China since the 1970s. Their novel chemical structures (Fig. 2.5) (B4) and known effectiveness against chloroquine-resistant malaria (B5-6) justify the keen interest they are arousing.

Research on the hemisuccinyl derivative, artesunate, and the methyl ether derivative, artemether—focusing on production methods, the standardization of formulations and the development of methods for pharmacokinetic and metabolic studies—is being carried out in collaboration with Chinese scientists, working both in China and in institutions in the United States and Europe.

Following the development of sensitive methods to measure artesunic acid and its decomposition product, dihydroartemisinin, the formulation and stability of artesunate are being studied in China and the United States. As a solid, the free acid form of the compound has been found to be more stable than the sodium salt, which was previously used for the production of artesunate, but in aqueous solution it

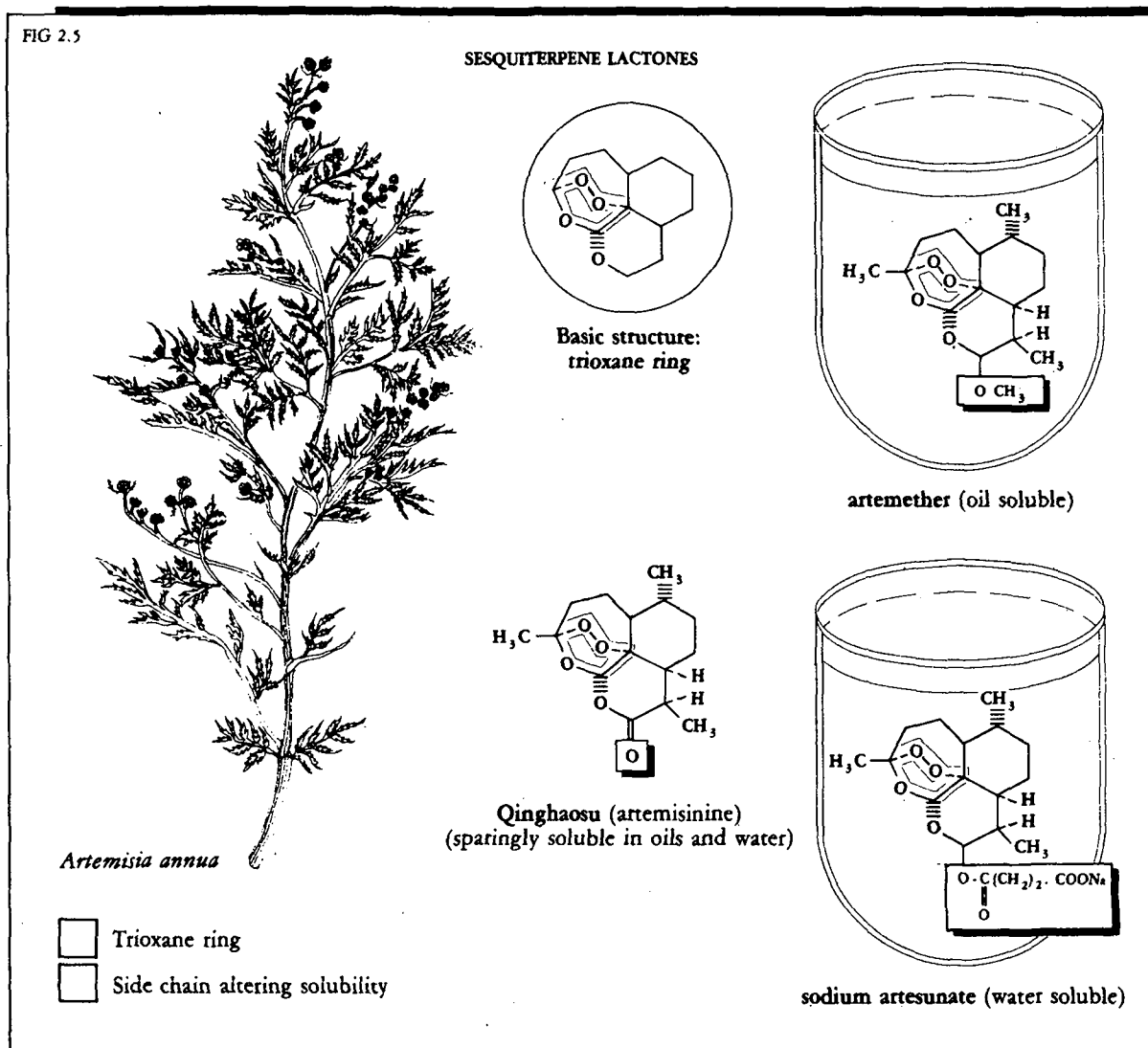


is unstable and should therefore be freshly prepared and used immediately. A stable water-soluble form is certainly required.

Current methods of measuring these compounds in biological fluids are far from ideal (A148, A184) and new methods are being developed (A104). Pharmacokinetic studies with radioactively labelled Qinghaosu or its derivatives show that they have a relatively short plasma half-life, which varies markedly between different animal species (A97). ¹⁴C-labelled artemether, with the label in the methyl ether side chain, has already been produced. Although useful, this compound is not ideal, and other forms of the drug, labelled with ³H and ¹⁴C, are urgently required. A workshop on the development of methods that could be used for the detection of Qinghaosu and its derivatives in body fluids was held in Beijing in October 1983 under the auspices of CHEMAL and the Ministry of Public Health of China.

Preliminary studies indicate that Qinghaosu compounds have a different mode of action from those of other antimalarials (B5). They appear first to affect the parasite plasma membrane structure, then

FIG 2.5



the nucleus, but not, apparently, as a result of lipid peroxidation. There is, in fact, evidence that the primary mode of action may be inhibition of parasite protein synthesis (A67-68, A99). The drugs are concentrated in malaria-infected red cells by a mechanism which appears to be different from that of other antimalarial drugs, including chloroquine. These studies, which were conducted by Chinese scientists at the London School of Hygiene and Tropical Medicine in the United Kingdom, also indicate that integrity of the four rings of the Qinghaosu molecule is required for full antimalarial activity.

Studies of combinations of artemisinin with other antimalarial drugs have shown artemisinin and mefloquine to be synergistic and to markedly enhance suppression of erythrocytic infection with a mefloquine-resistant *P. falciparum* isolate. Potentia-

tion was also observed with artemisinin and primaquine against the *P. berghei* N strain and the primaquine-resistant P strain. The effects of combinations of artemisinin and chloroquine were additive against the N strain, whereas antagonism was observed between artemisinin and antifolate drugs, such as pyrimethamine, cycloguanil and sulfa drugs.

Studies on the Qinghaosu series of drugs are continuing, in collaboration with Chinese scientists. CHEMAL now considers the development of these compounds to be of such high priority that active collaboration with other groups is also being sought.

Quinine and related compounds

The increased need for quinine in control operations has led to a re-examination of this and related compounds to identify more active, less toxic drugs

and regimens offering greater patient compliance.

Recent clinical (B15) and *in vitro* studies indicate that quinidine may be more effective against *P. falciparum* than its stereo-isomer quinine. Although crossresistance to the two compounds was observed *in vitro*, quinine-resistant lines were more susceptible to quinidine than to quinine. Similarly, the *in vitro* responses of *P. falciparum* to quinine and mefloquine in these studies also appeared to be related, but the phenanthrenemethanol halofantrine retained full activity against quinine-resistant isolates. If its reported cardiotoxicity does not prove a problem, quinidine may be an alternative to quinine where the latter is not available. In India, a comparative study of quinine and quinidine in monkeys failed to reveal marked differences in cardiovascular effects, as judged by ECG recordings.

New quinine and quinidine analogues synthesized by the pharmaceutical industry show not only the expected blood schizontocidal effect but also surprisingly high gametocytocidal activity against *P. falciparum in vitro* and *P. berghei in vivo*, but the gametocyte stage at which they act has not been determined.

Other plant-derived substances

Other sesquiterpene lactones, quassinoids from Simaroubaceae and coleones and rolyeanones from the Labiatae, are currently being screened for activity against *P. falciparum in vitro* and some have been shown to be active against the parasite.

Lead-directed synthesis

As the antimalarial activity of the Qinghaosu series of compounds appears to depend upon the 1,2,4-trioxane ring structure of the basic molecule (see Fig. 2.5), a number of simpler compounds containing this ring have been synthesized (A80-85) and 20 are now being evaluated for efficacy against *P. falciparum*. Some do appear to have blood schizontocidal activity.

Screens for blood schizontocidal activity

So many lead compounds are being submitted for evaluation that two new centres (one at the University of North Carolina, USA, another at WRAIR) were set up to screen drugs against clones of *P. falciparum* of known drug sensitivity. About 100 compounds are being screened annually, and initial results will be reviewed early in 1985.

Tissue schizontocides

Primaquine

Primaquine, an 8-aminoquinoline (Fig. 2.6), the only tissue schizontocide currently available for the radical treatment of *P. vivax* and *P. ovale* infections, also shows gametocytocidal and some blood schizontocidal activity. Its usefulness is offset by innate toxicity and the need for treatment to be continued over several days. Moreover, the mechanisms of its antimalarial action and of its toxicity are unknown. It is not clear, for example, whether its therapeutic effect is due to the parent drug or to its metabolites. Studies now in progress on the pharmacokinetics, metabolism and mode of action of the drug have already identified new 8-aminoquinolines with antimalarial potential.

As a gametocytocidal compound, primaquine used in association with fully effective blood schizontocidal therapy may also have a role in combating malaria transmission, particularly that related to the spread of drug-resistant parasites. Studies on the gametocytocidal effects of this compound and its metabolites and on the identification of new gametocytocidal drugs are therefore being given priority.

Most current research on primaquine is probably being conducted under CHEMAL. New assay methods have been used to measure primaquine and its metabolites in body fluids of animals and man (A9, A122, A133, A171). High-pressure liquid chromatography (HPLC), in particular, has been shown to provide a sensitive, specific and relatively inexpensive assay system.

Two metabolic pathways have now been discovered, one affecting the aromatic A ring, the other, the 8-N-aminobutyl side chain (Fig. 2.6). The first pathway leads to the formation of 5-hydroxyprimaquine and 5-hydroxydemethylprimaquine (A133, A153) (both metabolites are active *in vivo* and cause the formation of methaemoglobin). The second pathway, originally observed in bacteria, affects the 8-N-aminobutyl side chain and results in the formation of N-acetylprimaquine and a desaminocarboxylic acid. N-acetylprimaquine has not been found in significant concentrations in human plasma or urine, and its pharmacological activity is unknown. However, the desaminocarboxylic acid, the major metabolite of primaquine in human (A26, A114), rat, hamster and monkey (A9) plasma, has not, with one exception, shown antimalarial activity in screens, and it is uncertain if it contributes to the activity of primaquine.

Primaquine is now known to be reasonably well absorbed from the gastrointestinal tract and not to

undergo extensive initial degradation in the gut and liver in man, as it did in earlier animal studies. Several pharmacokinetic parameters relating to the use of primaquine in man show a wide range of individual variability. The drug's apparent volume of distribution suggests some localization in tissues but not to the same extent as chloroquine. Its elimination half-life after a single dose is six to eight hours in man (A26, A114), and dose-related variations in half-life are currently under study. Primaquine and its carboxylic acid metabolite appear in urine and there is evidence to suggest enterohepatic recycling. The ultimate fate of the carboxylic acid metabolite is unknown, but after a single dose of the parent compound plasma levels are about ten times higher than those of primaquine.

The major toxic effects of primaquine are methaemoglobinaemia, abdominal pain and haemolysis in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. The cause of pain is unknown, but it is sometimes too severe to allow repeated drug administration.

The role of primaquine metabolites in causing *in vivo* biochemical side-effects is largely unknown. *In vitro*, many metabolites (but not the carboxylic acid metabolite) have shown a greater propensity to cause methaemoglobin formation than has primaquine itself. One structural requirement for methaemoglobinaemia appears to be the presence of a 5-hydroxy group, with or without a side-chain (A8) (Fig. 2.6). *In vitro* studies also suggest that primaquine metabolites may produce methaemoglobin at concentrations several orders of magnitude less than the parent substance, which may be one reason why it has hitherto proved difficult to identify a toxic metabolite *in vivo*, since even the most sensitive of current assay methods would clearly be unable to detect it in such low concentrations.

Studies in man have shown that the methaemoglobinaemia produced by a 14-day course of primaquine slowly disappears over the subsequent seven to ten days. This is seemingly in contrast with what is known about primaquine kinetics in man, where the half-life after a single dose is six to eight hours. Further research is needed on the mechanisms of toxicity and the kinetics, including individual variability, of primaquine metabolites after multiple dosing. Any metabolites responsible for the formation of methaemoglobin may either impair normal methaemoglobin reductase activity or persist for several days, exerting an oxidant effect.

The methaemoglobinaemia and haemolysis associated with primaquine could possibly both be explained by metabolic conversion of the compound to a quinone and quinonimine. A method of testing

this hypothesis has been developed outside the Programme (B9) and studies have been started under CHEMAL.

In addition, *in vitro* screens have been set up to test primaquine and its known metabolites for tissue schizontocidal and gametocytocidal activity (A11, A108-109, B11). Preliminary results have shown that primaquine, demethylprimaquine, 5-hydroxyprimaquine and 5-hydroxydemethylprimaquine have gametocytocidal activity *in vitro* against *P. falciparum*, whereas only the parent drug has shown *in vivo* activity (in a rodent model). *In vitro* tissue schizontocidal activity has been detected with primaquine and its carboxy metabolite but *in vivo* tissue schizontocidal activity has only been seen with the parent drug, not the metabolite. Truly "causal" prophylactic activity (protecting directly against sporozoite invasion) has been observed, in a rodent model only, with the parent drug and 5-hydroxyprimaquine. Surprisingly, causal prophylactic and gametocytocidal activity has also been observed with certain new 8-aminoquinolines lacking the 8-N side chain. These compounds (synthesized with support from the Programme) and the metabolites identified to date (Fig. 2.6) are now being produced in the larger amounts required for further pharmacokinetic, toxicological and efficacy studies.

In view of the information now available on primaquine, the development of repository (i.e. extended-release) formulations is now considered worthwhile.

Other tissue schizontocides

Recent work, especially at WRAIR, has uncovered a large number of very active causal prophylactic compounds belonging to a number of distinct chemical classes, as well as several drugs with radical curative activity (B7). These leads are being explored with WRAIR. In addition, there is considerable interest in the naphthoquinone series of compounds, which show both blood and tissue schizontocidal activity.

Drug resistance

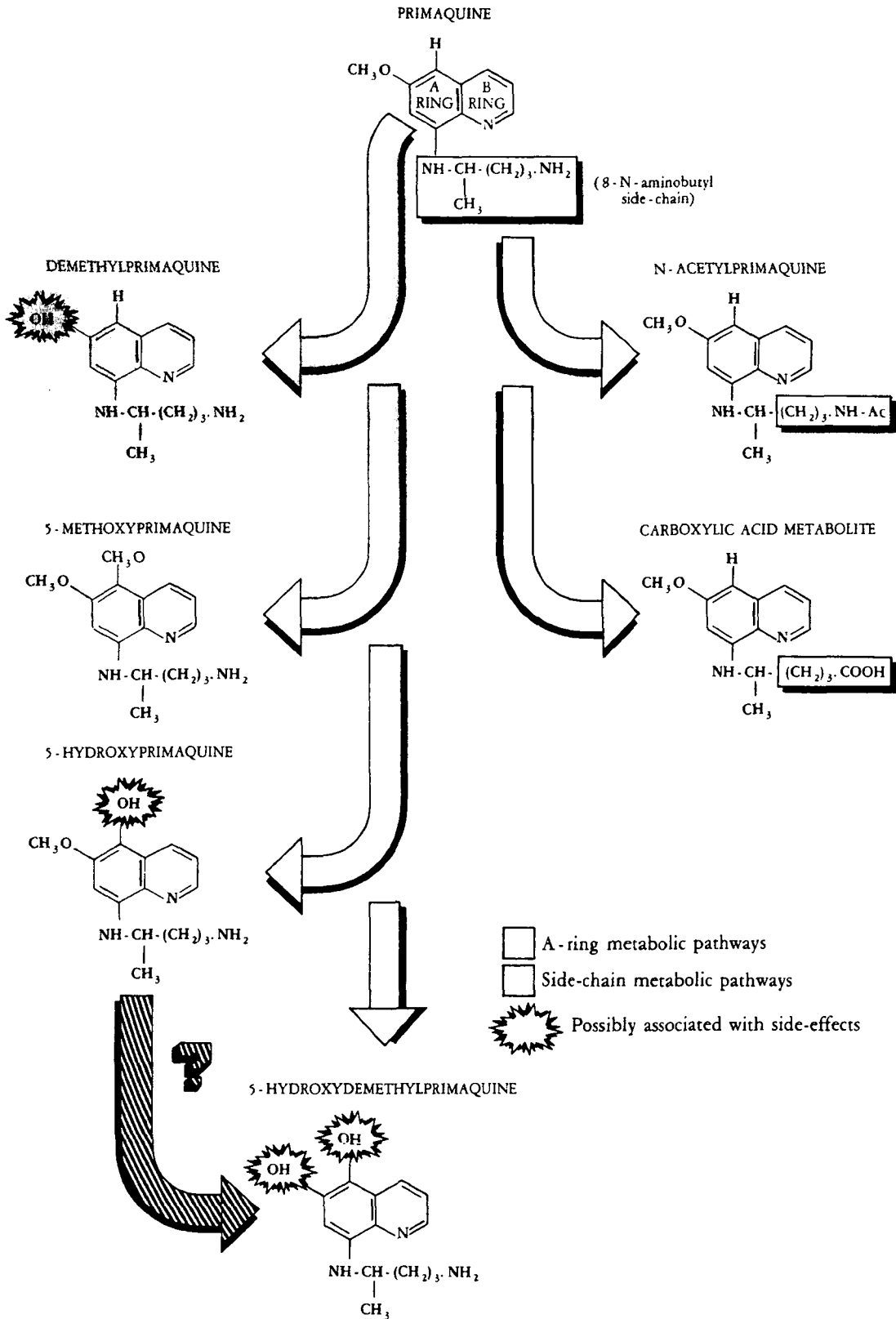
Development of microtests for drug susceptibility

A number of advances have been made in methodology for evaluating *P. falciparum* drug sensitivity:

- In addition to the currently available "micro" kits for testing the susceptibility of *P. falciparum* to chloroquine and mefloquine, kits for amodiaquine, quinine and quinidine have now been developed.
- Standardization of a test for the sulfadoxine/pyrimethamine combination is the object of a multi-

FIG 2.6

PUTATIVE METABOLISM OF PRIMAQUINE



centre study, now in an advanced stage, being conducted in Switzerland, Thailand and the United States. Preliminary field assessment has been completed in Brazil and Haiti and is continuing in Thailand. It has been found that the inhibitory effect of these drugs, used either singly or in combination, can be assessed with parasites incubated in a modified version of RPMI 1640 medium containing reduced levels of *p*-aminobenzoic acid (PABA) and folic acid. The production of medium for use in the field is still a problem.

- Test kits for some blood schizontocides currently under industrial development are now being assessed in preparation for baseline studies (prior to clinical trials) on *P. falciparum* drug sensitivity.
- A prototype portable incubator and modified candle jars appropriate for parasite cultivation in the field have been built and are now being assessed.
- A visual test for determining the sensitivity of *P. falciparum* to 4-aminoquinolines, developed by Rieckmann in 1982 (B13), was assessed in the field during 1983 by WHO's Malaria Action Programme (MAP) in collaboration with the South-East Asia Regional Office (SEARO) of WHO and with CHEMAL. A comparison of this test with standard, relatively simple *in vitro* methods suggests that its adaptation to field use would require major technical modifications which may not be warranted.

Development of drug combinations

In addition to the important findings on drug combinations referred to under "Mefloquine" above, preliminary studies at the London School of Hygiene and Tropical Medicine suggest that the effects of combining mefloquine or halofantrine with antibiotics, such as tetracycline, spiramycin, clindamycin and minocycline, are purely additive.

Combining chloroquine with the macrolide antibiotic erythromycin has an enhancing effect against chloroquine-resistant *P. berghei*, as was confirmed in two CHEMAL-supported animal studies, although subsequent clinical studies have failed to demonstrate the operational effectiveness of the combination in man (A129).

Drugs: modes of action and mechanisms of resistance

Appropriate systems are required to study modes of drug action and mechanisms of drug resistance. Early research on *P. berghei* and *P. falciparum* suggested that chloroquine resistance is related to the formation and sequestration of ferriprotoporphyryn IX (A58-60). Mössbauer effect spectroscopy of in-

fect cells (A177) and research on the interactions of haemin and chloroquine with phospholipid monolayers (A13) failed, however, to support this hypothesis in connection with *P. falciparum*.

Evidence has accumulated that cloned parasites are needed for these studies. A simple and efficient cloning method has in fact been developed, by which a single red cell can be isolated, examined under the microscope and transferred rapidly and efficiently to culture. Thanks to this method, *P. falciparum* clones with stable resistance to mefloquine have been established for the first time. The changes observed in these clones were similar to those produced by gene amplification in other systems (e.g. resistance to dihydrofolate inhibitors of *Leishmania*).

These and other recent advances in the cultivation of all stages of the parasite should make it possible in the future to conduct genetic studies on mechanisms of drug resistance. Meanwhile, strain characterization studies suggest that *P. falciparum* constitutes a single worldwide population of interbreeding organisms, with individual isolates containing genetically distinct parasite clones. These findings were confirmed by studies at Chulalongkorn University in Thailand, which showed for the first time that *P. falciparum* isolates resistant to chloroquine, amodiaquine, sulfadoxine/pyrimethamine and pyrimethamine alone contained clones ranging in drug susceptibility from sensitive to highly resistant (A157, A159). Chloroquine-sensitive parasites were also found to grow *in vitro* more slowly than chloroquine-resistant parasites, suggesting that the latter have a biological advantage, which may contribute to the spread of chloroquine resistance in the field. In similar studies with an isolate from an RI mefloquine treatment failure in Thailand, cloning of isolates obtained on admission (before mefloquine treatment) produced a heterogeneous parasite population in which mefloquine-sensitive organisms predominated. In the course of unsuccessful mefloquine treatment, selective drug pressure produced a homogeneous mefloquine-resistant parasite population. Mefloquine-resistant parasites, unlike chloroquine-resistant clones, appear not to have a biological advantage over mefloquine-sensitive clones but even to grow more slowly. In Thailand, the potential for mefloquine resistance already seems to exist in the indigenous *P. falciparum* gene pool and adds urgency to the search for antimalarial drugs of novel structures and modes of action.

Parasitology and experimental chemotherapy

Several institutes have continued to study parasite cultivation, biochemistry and host-cell invasion with

the aim of developing the required methodology for applied chemotherapeutic studies and identifying leads for the rational development of new drugs.

Following the development of methods for cultivating the exoerythrocytic (EE) stages of rodent malaria parasites (see TDR's Sixth Programme Report, p. 25), two research groups in Paris, France—one at the Museum of Natural History, the other at the Hôpital Salpêtrière—have successfully grown *P. vivax* EE stages in normal human hepatocytes (A108-109). Very recently, a United States group funded by CHEMAL has grown *P. vivax* EE stages in a transformed hepatoma cell line.

These techniques are now being used in CHEMAL-supported research to assess the *in vitro* schizontocidal activity of primaquine and its metabolites. In spite of the discovery (made in 1982 during work conducted under CHEMAL auspices) of a latent hypnozoite *P. vivax* stage, there is no *a priori* reason to believe that these *in vitro* methods will be suitable for the identification of anti-relapse drugs. Some, albeit tentative, evidence does exist, though, that hypnozoite stages might be present in the *P. vivax* cultures. The question remains to be explored.

Unfortunately, attempts to maintain the erythrocytic stages of *P. vivax* and *P. malariae* in continuous culture *in vitro*—a major priority of CHEMAL—have continued to be unsuccessful, the parasites only surviving for a limited number of generations. The use of continuous cultures of *P. falciparum* blood stages has, however, led to major advances in the understanding of the biochemistry of the parasite. CHEMAL-supported studies have concentrated on the modes of action of existing drugs or on known differences in metabolism between host and parasite.

Studies on the development of a microtest for sulfa-pyrimethamine combinations have shown that both folic acid and PABA interfere with the activity of sulfadoxine against the susceptible Liberian (De Paul) *P. falciparum* isolate, suggesting that the parasite is capable, through a hitherto unrecognized metabolic pathway, of utilizing red-cell folates in the form of polyglutamated 5-methyltetrahydrofolate (A115). It has now been shown that ³H-folic acid or ³H-PABA is incorporated into the parasite, possibly as pterylpolyglutamate. This metabolic pathway and its relation to purine and pyrimidine metabolism are now under further study.

The potential of purine and pyrimidine metabolism as targets of chemotherapy has been recognized for many years. A number of enzymes isolated from, on the one hand, human red cells and those of other animal species and, on the other, from malaria parasites show marked differences between host and parasite. These enzymes are: orotate phosphoribosyl-

transferase (EC 2.4.2.10); orotidine-5'-phosphate decarboxylase (EC 4.1.1.23); adenosine deaminase (EC 3.5.4.4); hypoxanthine-guaninephosphoribosyltransferase (EC 2.4.2.8); and purine nucleoside phosphorylase (EC 2.4.2.1). The first two were isolated and characterized from *P. falciparum* (A135); the others were studied in *P. lophurae* (A139). Evidence has also been obtained that adenosine deaminase from *P. falciparum* differs from that in the red cell.

It has been suggested that pyrimidine metabolism may be linked to oxygen utilization through a cytochrome chain including cytochrome oxidase and ubiquinones and that this pathway may be the target of naphthoquinone activity (B10). Evidence for this hypothesis is still lacking, but the presence of a functional mitochondrion and of mitochondrial protein synthesis in *P. falciparum* is suggested by studies conducted at Michigan State University in the United States.

Marked host-parasite differences have also been observed in phospholipid biosynthesis, which is related to membrane structure (A167). Parasite-specific enzymes of the Kennedy pathway for phosphatidylcholine synthesis have been identified in malaria parasites, and their locations within the infected cell are being studied. *P. falciparum* phospholipids have also been shown to have a different fatty acid composition from those of the host.

Structural changes have been observed in the membranes of *P. falciparum*-infected red cells. New techniques have been developed for the study of anion (A24), amino acid (A65), and purine and chloroquine transport, and have led to the identification of a "permeability pathway" involving pore-like structures which develop on the red-cell membrane as the parasite grows within the cell (A66, A93).

Much light has recently been thrown on the mechanism of red-cell invasion by the parasite, and possible receptors on red cells and merozoites have been identified (in studies conducted both within and outside the Programme). In addition, histidine-rich parasite-specific proteins have been implicated both in this process and in the formation of the knob-like projections found on *P. falciparum*-infected cells (see IMMAL section, under *Erythrocyte surface*). CHEMAL-supported studies conducted at the National Institutes of Health in the United States, in collaboration with WRAIR, have now shown that several L-histidine analogues have significant activity *in vitro* against isolates of knobproducing *P. falciparum*. Substitution on the imidazole ring of the analogues appears to be critical for antimalarial activity. Since several known histidine analogues are toxic to mammals, it remains to be seen whether these observations will lead to the develop-

ment of new compounds with selective toxicity to the parasite.

Diagnostic tests

Current methods of diagnosing malaria involve examination of stained blood films under the microscope, a reasonably sensitive and specific procedure but one that is time-consuming and expensive and requires trained personnel. Serodiagnostic techniques are being developed by IMMAL (see IMMAL section, under "Immunodiagnosis of malaria"), whereas CHEMAL is exploring the diagnostic potential of DNA probes, which have already been developed

for *P. falciparum* and *P. vivax* in a CHEMAL-supported study conducted at Harvard Medical School in Boston, MA, USA. Similar studies with *P. falciparum* have been conducted by others outside the Programme (B8). A major advantage of DNA probes is the possibility of rapid testing of many blood samples, a particularly useful asset in large population surveys. DNA probes appear to have adequate specificity, although their current sensitivity is less than that of methods employing well-trained microscopists. Studies aimed at increasing the sensitivity of DNA-probe methods and at developing simple, non-radioisotopic methods are being conducted with CHEMAL support; field tests are planned for 1985.

Box 2.4 The future

The following areas are expected to be of high priority during 1985-86:

- completion of the Phase III clinical trial of mefloquine in pregnant women;
- completion of Phase III clinical trials of the mefloquine plus sulfadoxine/pyrimethamine combination in children over two years of age in Thailand and Zambia;
- completion of Phase III trials of mefloquine and mefloquine plus sulfadoxine/pyrimethamine, each with primaquine, in Brazil and Thailand;
- start of Phase I and II trials of other blood schizontocidal drugs currently under development;
- synthesis of primaquine metabolites and 8-aminoquinolines in large amounts for extended efficacy and toxicity testing;
- preclinical studies on selected Qinghaosu derivatives;
- further studies on the development of appropriate drug combinations;
- further development of tests for sensitivity of *P. falciparum* to selected antimalarial drugs;
- further development of simple qualitative and quantitative tests for drugs in blood and urine;
- studies on the mechanisms of drug resistance;
- development of *in vitro* cultivation systems for erythrocytic stages of *P. vivax*;
- biochemical studies in selected areas for the identification of parasite-specific drug targets.

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Immunology of malaria

The context

□ The development of vaccines and the improvement of immunodiagnostic methods have remained the two major research priorities of the Scientific Working Group (SWG) on the Immunology of Malaria (IMMAL) since it was set up in 1976.

Malaria vaccine research is now being vigorously pursued in a large number of laboratories and there has been a virtual explosion of scientific publications on the subject. Pessimism about the feasibility of developing effective vaccines against malaria has been largely replaced by a mood of cautious optimism, based, first, on recent advances in applying molecular biology to malaria research and, second, on the expectation that new vaccine production technology will be applicable to malaria.

The complex structure and life-cycle of the malaria parasite (Fig. 2.7) and the nature of its interaction with its human host preclude any rapid solution to the vaccine problem. The protozoan parasite of the genus *Plasmodium* undergoes a multi-stage development cycle, part of which takes place in the vertebrate host and part in the mosquito vector. Changes in both morphology and antigen expression occur during the cycle. The parasite confronts the host with a large number of antigenic components, each developmental form of the parasite (Fig. 2.7) containing distinct, stage-specific antigens. Only a small proportion of the many antigens present, however, are likely to stimulate protective immune responses. The rest are either irrelevant to protection or may induce undesirable host responses. Vaccination using whole parasites is therefore not feasible. Nor is it practical, since malaria parasites cannot be obtained in sufficient quantities or in sufficiently pure form. The strategy for malaria vaccine development is therefore based on the identification and characterization of those parasite antigens which specifically stimulate protective immune responses. The next phases involve cloning of the genes coding for the protective antigens or antigenic structures (epitopes), their expression in bacteria, analysis of their nucleotide sequences, deduction of the amino-acid sequences of the encoded molecules and pro-

duction of the molecules by genetic engineering methods or chemical synthesis.

Of the hundred or so species of *Plasmodia* that exist in nature, four are generally pathogenic to man: *P. falciparum* (the most dangerous), *P. vivax*, *P. ovale* and *P. malariae*. Much of the malaria parasite's life-cycle involving the vertebrate host takes place within the liver or blood cells, where the organisms are relatively well protected from immune attack. Interest is therefore focused on the extracellular forms—sporozoites and merozoites—which come into direct contact with the immune system, and also on the sexual stages, which develop within mosquitoes and could be possible targets of vaccines capable of blocking transmission of the disease.

Most current research on malaria vaccine centres on the analysis and production of antigens of these various parasite forms. Stage-specific antigens are of special interest, since protective immunity is apparently stage-specific. Thus, a sporozoite-based vaccine would not protect against malaria induced by transfused blood containing asexual-stage parasites, nor a gamete-based vaccine against infection by sporozoites and the development of disease due to the asexual blood stages.

At the end of the last biennium (1981-82), malaria vaccine research was entering a particularly productive phase following the identification of several potentially protective antigens. The protective sporozoite surface antigens of *P. berghei* and *P. knowlesi* had been identified and the gene coding for that of *P. knowlesi* had been cloned. Several asexual blood-stage antigens which may be involved in protection had been studied in various species of *Plasmodium*: one pure *P. yoelii* and one *P. knowlesi* antigen had been found to induce protection in vaccination experiments. Antigens reacting preferentially with immune sera or with monoclonal antibodies (MABs) that block merozoite invasion *in vitro* had been characterized, although the gamete antigens responsible for transmission-blocking immunity had not been identified.

It is now reasonable to expect that experimental

malaria vaccines will in the near future be brought to the stage of initial testing for efficacy, tolerance and toxicity. The Special Programme collaborates closely with agencies in the United States actively involved in this work, including the United States Agency for International Development (USAID), the Walter Reed Army Institute of Research (WRAIR) and the National Institutes of Health (NIH). The Programme's goal is to develop a vaccine which could be effectively incorporated into public health measures for malaria control.

The remarkable recent progress made in the iden-

tification and production of potentially protective antigens contrasts strikingly with the still fragmentary understanding of the mechanisms and possible indicators of protective immunity in man. At this stage of their development, the potential effectiveness of malaria vaccines for disease control remains to be demonstrated. Their effectiveness in protecting individuals and in stemming transmission of the disease will have to be carefully evaluated under the conditions of infection, nutrition, genetics and transmission obtaining in tropical countries. □

Box 2.5 Highlights of activities in 1983-84

The most striking advance in malaria vaccine research—the main focus of the Scientific Working Group (SWG) on the Immunology of Malaria (IMMAL) and one that has seen a burst of activity in the last two years—has been the cloning of the gene coding for the major protective surface protein of sporozoites of *Plasmodium falciparum*, the parasite species responsible for the most severe form of malaria. As a result of this achievement, reported in the summer of 1984 independently and almost simultaneously by IMMAL-supported scientists at New York University and by researchers at the National Institutes of Health (USA), the first malaria vaccine should soon be ready for initial toxicity, tolerance and efficacy testing.

Over the past two years other

noteworthy advances have taken place in research, much of which was supported by IMMAL. These include:

- the identification, in several species of *Plasmodium*, including *P. falciparum*, of potentially protective antigens on the surface of asexual blood-stage forms of the parasite and on the surface of infected erythrocytes;
- the development of a simple, highly sensitive, species-specific assay—the Zavala test—to detect sporozoites in infected mosquitoes: this is a striking example of the application of monoclonal antibody techniques to immunodiagnosis, the result being a powerful new epidemiological tool;
- the identification of a stage-specific antigen of *P. falciparum* exoerythrocytic (liver) stages: the antigen has yet to be characterized and evaluated for

immunoprotective potential, but with so little known of the antigenicity of these parasite forms or of the liver's role in protective immunity, this finding represents an important step towards a more complete "mapping" of the immune response in malaria;

- the analysis and amino-acid sequencing of *P. falciparum* "S-antigens", which are released in large quantities at the time of schizont rupture; S-antigens differ from other plasmodial antigens in being heat-stable, water-soluble and antigenically diverse; their biological function, still obscure, is stimulating intense interest;
- the identification of the *P. falciparum* gamete antigens which are the targets of transmission-blocking immunity.

Report of activities in 1983-84

Sporozoites

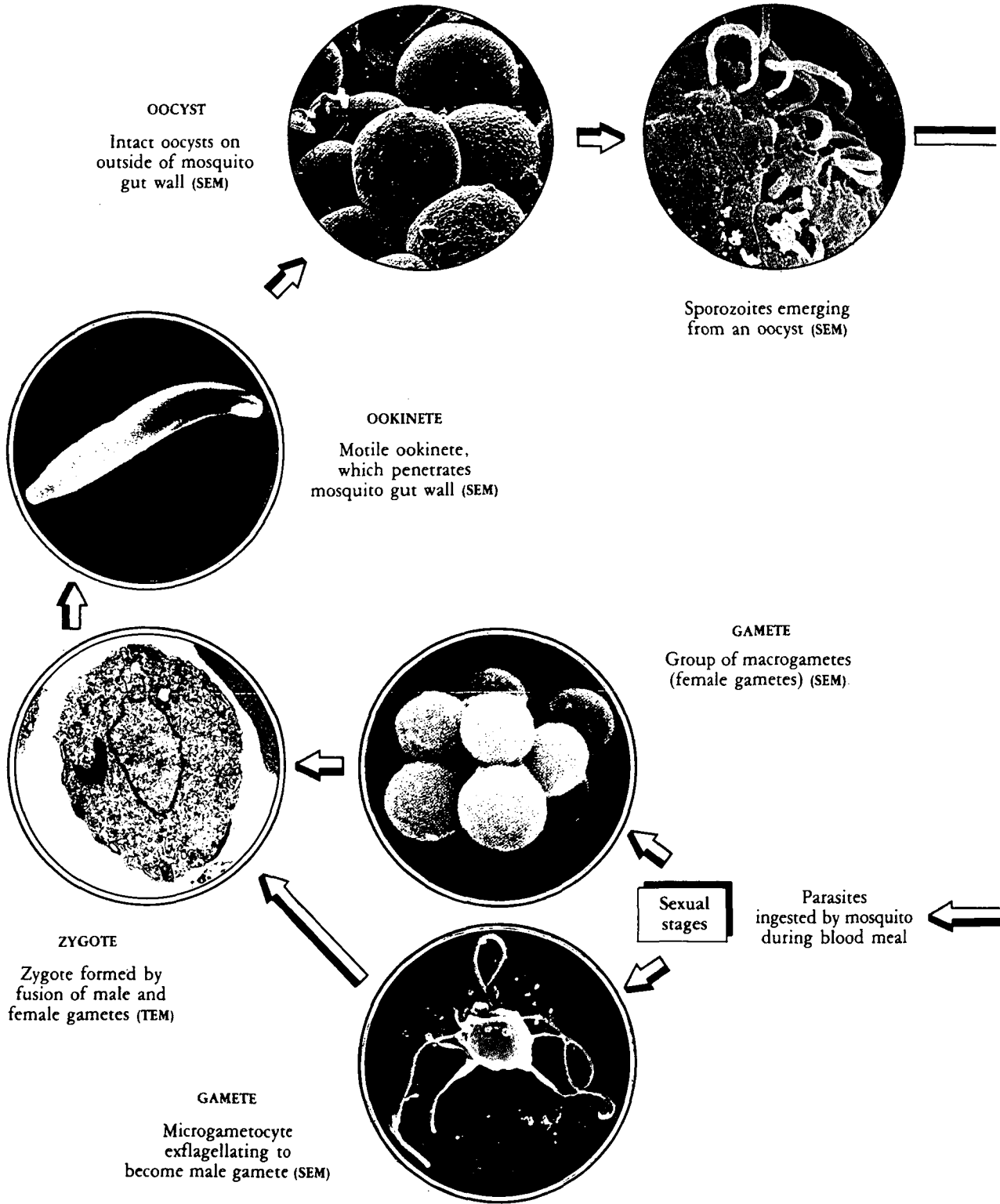
The protective antigen of the sporozoite—its major surface membrane protein—has been characterized by scientists at New York University (NYU) for several species of *Plasmodium*. These "circumsporozoite" (CS) proteins, which can be visualized (Fig. 2.8) using an appropriate monoclonal antibody, are immunodominant molecules differing among the different species but sharing a number of common features (A103). Following the identification of the *P. berghei* and *P. knowlesi* CS molecules, those of *P. falciparum*, *P. vivax* and *P.*

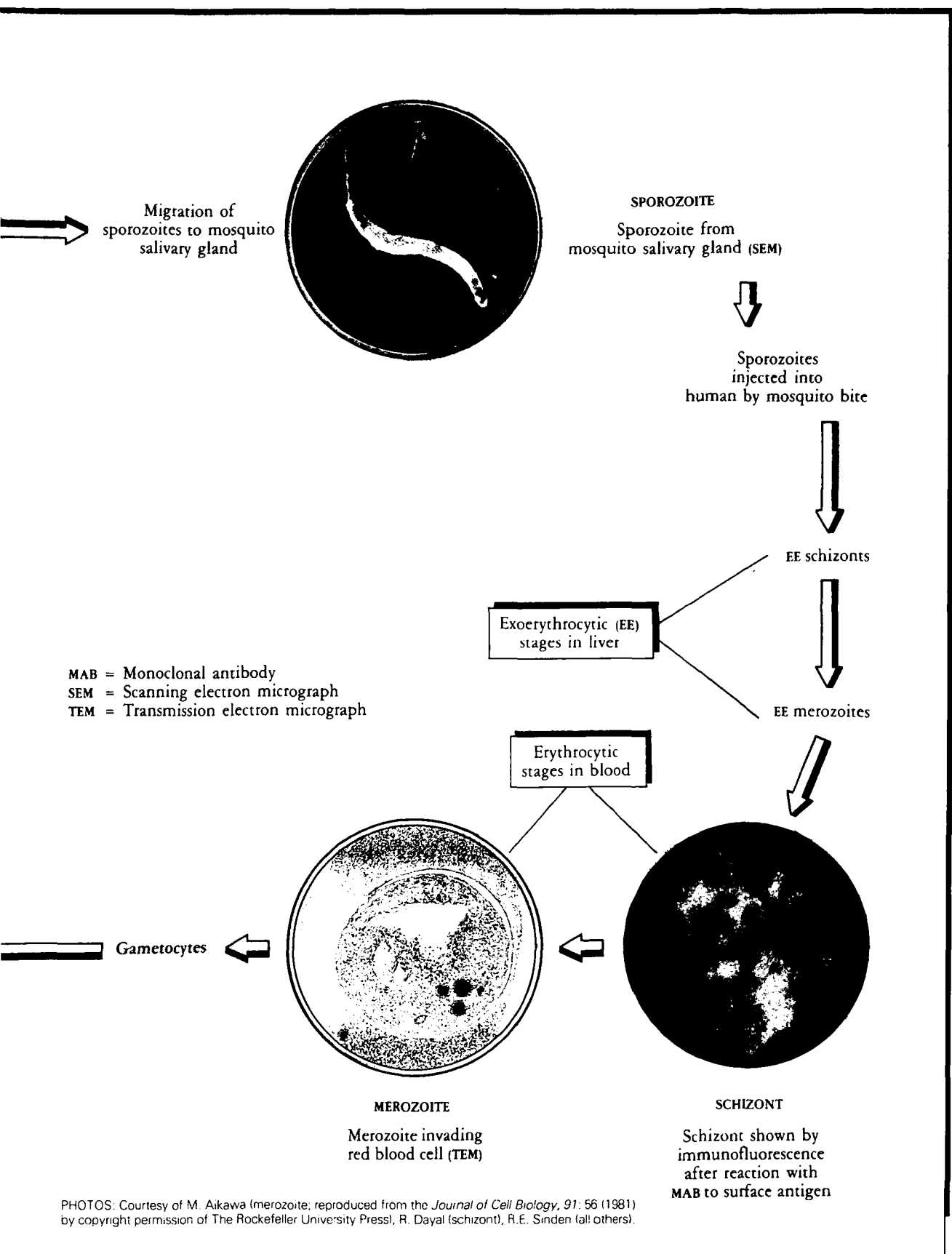
cynomolgi have now been characterized and designated, respectively, Pf58, Pv45 and Pc48, to indicate species and relative molecular mass (Mr) $\times 10^{-3}$.

The CS molecule may play a major functional role in sporozoite invasion of hepatocytes. Fab fragments of MABs recognizing CS protein have been shown to inhibit sporozoite attachment and entry into hepatocytes, suggesting that the antibody-reactive epitope is identical, or in close proximity, to the part of the CS molecule involved in the parasite-target cell interaction. This finding has been used to develop a functional *in vitro* assay which measures

FIG. 2.7

LIFE-CYCLE OF THE MALARIA PARASITE. MAJOR VACCINE TARGETS ARE HIGHLIGHTED IN GREEN: SPOOROZOITES, MEROZOITES, GAMETES, ZYGOTES AND OOKINETES





PHOTOS: Courtesy of M. Aikawa (merozoite; reproduced from the *Journal of Cell Biology*, 91: 56 (1981) by copyright permission of The Rockefeller University Press), R. Dayal (schizont), R.E. Sinden (all others).

FIG. 2.8

SPOROZOITE SHOWING CIRCUMSPOROZOITE (CS) PROTEIN REACTION



the capacity of antibodies to *P. falciparum* and *P. vivax* sporozoites to inhibit sporozoite entry into cultured human hepatoma cells (A63).

Greater understanding of the properties of CS proteins has accrued from the cloning and nucleotide sequencing of the corresponding genes. The first such gene to be cloned was that of *P. knowlesi* (see TDR's Sixth Programme Report, p. 28). Sequencing analysis has subsequently revealed that the immunodominant epitope on the CS molecule contains a sequence of 12 amino acids tandemly repeated 12 times (A89). The sequence was chemically synthesized and the synthetic molecule was recognized by monoclonal antibody to the native CS protein (A54). The gene has also been inserted into vaccinia virus, and cells infected with the recombinant virus have produced the sporozoite antigen (A110). Moreover, rabbits immunized with the virus produced antibodies that reacted with the sporozoite surface.

P. falciparum CS protein

In a recent major advance, the gene coding for the *P. falciparum* CS protein has been cloned indepen-

ently and almost simultaneously by scientists at NYU and, outside the Programme, at the National Institutes of Health (NIH) in Bethesda, MD, USA. The group at NYU used standard methods to construct a cDNA library from sporozoite-extracted mRNA (A47). At NIH, in a novel approach, mung bean nuclease was used to excise directly from the genome the gene coding for CS protein (B1). In this case, the sporozoite gene was obtained from asexual blood-stage parasites. Sequence analysis carried out by both research groups showed that the immunodominant epitope contains repeated sequences of short amino-acid chains (two chains of four amino acids each). The repeat sequences were synthesized and found to share common antigenicity with native CS protein. It will now be of critical importance to determine whether the synthetic peptides can induce protective immunity.

P. cynomolgi CS protein

The gene coding for the *P. cynomolgi* CS protein has also been cloned (A46). This species is unusual among those studied to date in that the CS molecules of different strains of the parasite are immunologically distinct. The immunoreactive epitope was found to contain ten repeats of a short amino-acid sequence, which differed between parasite strains.

Repetitive sequences within CS molecules are likely to play a major biological role, but it is not yet certain that this part of the molecule is responsible for the induction of protective immunity. The possibility is being explored that other regions, outside the repeating sequences, may stimulate protection. This is important, in view of a recent observation made outside the Programme that *P. falciparum* asexual blood stages have an antigen which crossreacts with the sporozoite surface and reacts with human immune serum (B7).

Liver-stage parasites

The liver or exoerythrocytic (EE) stages of *Plasmodium* are not yet the subject of vaccine research. Little is known of the antigenicity of EE parasites or of the role of the liver in protective immunity to malaria. Recently developed methods for the *in vitro* cultivation of EE parasites do, however, open the way to studies of their antigenic composition. An important recent observation, made in a study begun outside and now continuing within the Programme, is the presence of stage-specific antigen in EE stages of *P. falciparum* (B3). The antigen has not yet been characterized, and it is not clear whether EE stage-specific antigens can induce protective immune responses.

Asexual blood-stage parasites

Of the many antigens belonging to this extremely complex group, some occur in intimate contact with host cell components and some undergo processing to yield lower Mr products during the cycle. Identification and analysis of potentially protective asexual-stage antigens are therefore difficult. Comparison of results from different laboratories is complicated by the common practice of designating antigens according to their apparent molecular weights, which may vary between strains of the same parasite species and between individual assays. In view of this problem, IMMAL recently undertook a systematic comparison of *P. falciparum* asexual-stage antigens. Antibodies being used by various research groups to identify antigens central to their different research interests were compared, by means of the immunofluorescent antibody (IFA) test and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), at a workshop held in March 1984 in conjunction with the sixth IMMAL SWG meeting (C1). The relationships between a number of antigens were clarified at the meeting, which was so successful that it was decided to set up a reference centre where comparison of malaria antigens could continue.

Different criteria have been used to identify a variety of potentially protective antigens on asexual blood-stage parasites: presence on the surface of schizonts and merozoites, and exposure to the immune system; reactivity with monoclonal antibodies that inhibit parasite growth *in vitro*; preferential reactivity with sera of immune adults from endemic areas; recognition by IgG from sera of immune animals and induction of protective immunity on passive transfer; and critical importance in parasite function, such as invasion of erythrocytes. A great deal of information has been obtained on these various antigens during the past two years.

Schizont antigens

A high molecular-weight schizont antigen identified in several species of *Plasmodium* is of particular interest for vaccine studies: in rodent malaria models, it has been shown that monoclonal antibodies to the surface proteins of the different species confer protection following passive transfer (A10, A79) and, in a study outside the Programme, purified protein from one parasite species induced a substantial degree of protective immunity in vaccinated mice (B6). Lower molecular-weight fragments of the antigen are produced at the time of schizont rupture and remain on the merozoite surface membrane after their

release. Analogous molecules found in different parasite species share a number of common features: they are in the 190 000-230 000 Mr range, with slight differences both between species and between isolates of the same species; the antigens are synthesized only by mature intraerythrocytic parasites and can be demonstrated on the surfaces of trophozoites and schizonts; in most, but not all, species studied, they are glycoproteins, as indicated by ³H-glycosamine incorporation; and, finally, the intact molecule is lost when the schizont ruptures (A39, B5, B9). Crossreactivity has been shown between analogous proteins in several species of *Plasmodium* and there is evidence for antigenic diversity of the molecule within species (A80). In *P. falciparum*, this molecule (195 000 Mr) contains strain-shared and strain-specific epitopes.

Serum from squirrel monkeys immunized against *P. falciparum* has been used to identify another schizont surface antigen that may be protective (A48). Purified IgG from immunized animals was used in passive transfer experiments. The reactivity of sera conferring protection was compared with that of nonprotective sera. The recognized antigens were purified and used in vaccination experiments (A43). A 96 000 Mr antigen which induced a substantial degree of protection was shown to be synthesized by trophozoites and early schizonts but not by mature schizonts.

Merozoite antigens

Merozoite surface antigens are important because they come into direct contact with the immune system and include components involved in recognition and penetration of erythrocytes. There is now good evidence that the receptor for merozoite attachment is glycophorin, the major sialoglycoprotein of erythrocyte membranes. Attachment may be a ligand-like interaction involving carbohydrates on the glycoproteins of the erythrocyte surface (B11). Merozoite proteins of 155 000 and 130 000 Mr have recently been identified that specifically bind to glycophorin and appear to be transferred from the merozoite to the erythrocyte surface during the invasion process (A94). In a study outside the Programme, a 155 000 Mr molecule has been identified on the surface of newly-invaded erythrocytes, and antibodies to this molecule block merozoite invasion *in vitro* (B14). Merozoite surface antigens involved in protection have also been identified in *Plasmodium knowlesi*.

S-antigens

The S-antigens (so named for their "stability" or

resistance to heating at 100 °C) of *P. falciparum* are the subject of a great deal of current research, although their biological function remains obscure. They differ from other malaria antigens in having the three characteristics of heat stability, water solubility and antigenic diversity, and are released into the plasma or culture medium in large amounts at the time of schizont rupture (B17). One MAB to an S-antigen blocks merozoite invasion *in vitro*, but there is no other evidence of a protective role. The S-antigens are now known to include polypeptides in the 120 000 - 250 000 Mr range and to contain different repeated amino-acid sequences. The genes coding for two S-antigens have recently been cloned from cDNA libraries, and clones expressing malaria antigens have been recognized by immune sera from Papua New Guinea (A32). The repeating sequence of one S-antigen contains eleven amino acids and that of the other S-antigen, eight amino acids. Variation in S-antigen size may be due to variation in the size and number of repeating sequences.

Erythrocyte surface

The surface of the infected erythrocyte becomes modified during *P. falciparum* infection. Cells containing late trophozoites and schizonts develop surface knobs—sites of attachment of infected erythrocytes to capillary endothelial cells (Fig. 2.9). As a result, erythrocytes containing mature parasites are sequestered, disappear from the peripheral circulation and thus evade destruction in the spleen. This is probably an important mechanism in cerebral malaria, in which cerebral vessels are blocked by parasitized erythrocytes. Immune serum can prevent or reverse this cytoadherence (A120). Indeed, it has been shown that the surface knobs contain antigens of parasite origin and could therefore be possible vaccine targets.

Knob production is associated with the synthesis of a histidine-rich protein (HRP) similar in amino-acid content to the *P. lophurae* HRP, which induces protective immunity to *P. lophurae* in ducks (A71). *P. falciparum* HRP is now known to be localized to the knobs in close association with electron-dense material immediately beneath them. Recently, HRP-related genes and transcription products have been identified in *P. falciparum* and highly repetitive DNA sequences found in the coding region of the HRP gene (A130).

The surface of *P. knowlesi* schizont-infected erythrocytes bears a variant antigen—a 190 000 - 210 000 Mr molecule of parasite origin—recently identified in a study conducted outside the Programme (B8). Antigenic variation of erythrocyte surface antigens

PHOTO: Courtesy of M. Aikawa, reproduced with permission of the Journal of Parasitology (A3).

FIG. 2.9
PARASITIZED ERYTHROCYTE SHOWING SURFACE KNOBS



has also been demonstrated in *P. falciparum* (B13). Serum from immunized squirrel monkeys reacts with new antigenic determinants on the surfaces of erythrocytes containing mature asexual-stage parasites, as revealed by IFA applied to fresh unfixed erythrocytes (A81). Strain-specific determinants have been recognized in this way, their expression being modified by the presence or absence of the spleen. With cloned parasites, differences have been found between the surface antigens in primary infections and those in secondary and recrudescence peaks. The *P. falciparum* variant antigen has not been identified and its significance for protective immunity is not clear. With the same surface IFA technique, human immune sera have been shown to react with the membranes of erythrocytes infected with mature asexual parasites.

Host factors

Several recent studies have shown that host factors, as yet not understood, may modulate the expression of parasite antigens during malaria infection. In the study cited above, erythrocyte surface antigen was detectable on *P. falciparum*-infected erythrocytes from intact but not splenectomized squirrel monkeys. The variant surface antigen of *P. knowlesi* is also only expressed on the surfaces of cells from intact monkeys. In toque monkeys infected with *P. fragile* (associated with a *P. falciparum*-type malaria), sequestration of infected erythrocytes was shown to be a spleen-dependent phenomenon. Moreover, sequestration of *P. falciparum*-infected cells in

squirrel monkeys is reduced in splenectomized animals. These findings have important implications for vaccine experiments in nonhuman primates.

Sexual-stage parasites

Sexual-stage parasites do not produce illness in the vertebrate host and until very recently were not thought to induce an immune response during malaria infection. A study now in progress in Sri Lanka suggests that antigametocyte antibodies occur frequently in patients with *P. vivax* malaria, particularly after two or more infections. Antibodies which block the development of parasites within the mosquito have been produced in animal models following immunization of the vertebrate host with gametes. When ingested as part of a blood meal, these antibodies act on the sexual-stage parasites after their release from erythrocytes in the mosquito midgut and prevent their further development. Since sporozoite production is thereby prevented, transmission of the parasite does not occur: hence the term "transmission-blocking immunity".

A vaccine based on antigens of sexual-stage parasites could greatly reduce the frequency of malaria in endemic areas by reducing the level of parasite transmission. Since the vaccinated individual would not be protected against sporozoite infection or the subsequent development of disease due to the asexual blood stages, a transmission-blocking vaccine would probably have to be used as part of a multi valent vaccine incorporating protective antigens of other developmental forms of the parasite.

Progress in research on transmission-blocking vaccines was reviewed at the sixth IMMAL SWG meeting (C1).

Gamete antigens

MABs have been used in several recent investigations to study gamete antigens. In one, conducted outside the Programme, *P. gallinaceum* gametes were used to raise transmission-blocking MABs, which reacted with three gamete surface molecules, agglutinated male gametes and prevented fertilization (B12). Other MABs subsequently identified another zygote surface molecule and prevented the development of ookinetes. In *P. yoelii*, transmission-blocking MABs identified a 70 000 Mr gamete antigen, which was purified and used to immunize mice. Blood from these mice showed markedly reduced infectivity for mosquitoes during challenge infection. Evidence for both humoral and cellular immune

mechanisms in transmission-blocking immunity has been found in the *P. yoelii* model.

With the development of a method for stimulating gametocyte production in *in vitro* cultures of *P. falciparum*, it has been possible to obtain sufficient numbers of gametocytes for transmission-blocking studies in this species. The target antigens have now been identified in a study at NIH, begun outside and continuing within the Programme (B15). Transmission-blocking MABs reacted with gamete surface molecules of 260 000 Mr, 60 000 Mr and 55 000 Mr that closely resemble the *P. gallinaceum* antigens identified earlier. They are synthesized by gametocytes, and the lower Mr molecules do not appear to be derived from the larger Mr molecule.

Transmission-blocking immunity is also being studied in *P. vivax*. Because of the lack of a method for continuous *in vitro* cultivation of *P. vivax*, gametocytes must be collected from infected blood of gametocyte carriers. Gametocytes obtained from the blood of *P. vivax* malaria patients in Sri Lanka were used to produce a suspension of pure female gametes, which were used, in turn, to immunize rabbits. The resulting antiserum reacted in the IFA test with *P. vivax* macrogametes and abolished the infectivity to mosquitoes of blood from gametocyte carriers. Studies are in progress to identify the antigens involved.

Primates for malaria vaccine research

Now that a number of candidate antigens have been identified and some of the corresponding genes cloned, it can be expected that initial efficacy and toxicity testing of trial vaccines will soon begin. Nonhuman primates will be needed.

Both the owl monkey, *Aotus trivirgatus*, and the squirrel monkey, *Saimiri sciureus*, support the growth of *P. falciparum*, although neither provides an ideal experimental model for human infection and both are in short supply (the scarcity of *Aotus* is particularly critical). Monkeys should, therefore, only be used in fully warranted experiments that can be expected to yield unequivocal results.

IMMAL has drawn up guidelines (C1) for the use of nonhuman primates in malaria vaccine research and established criteria for: the selection and definition of antigens that would merit testing in monkeys; the data to be obtained in laboratory rodent species prior to primate experiments; the examinations to be carried out on the monkeys before, during and after vaccination and challenge; and the samples to be deposited in reference centres where the immunization experiments will be monitored.

Malaria vaccine production

Recent results show that immunogenic epitopes of plasmodial antigens can be produced in several ways, including genetic engineering of bacteria, use of live recombinant virus and chemical polypeptide synthesis. It remains to be determined which method will prove most suitable. The repetitive amino-acid

sequences that characterize a number of plasmodial antigens would appear to lend themselves to production by chemical synthesis, particularly as some of the repeats are remarkably short. Short chains of amino acids will require carrier molecules and probably adjuvants for the induction of immunity. Live recombinant virus has the advantage of providing a continuous source of vaccine antigen in the host. The

Box 2.6 Strategy for the development of malaria vaccines

- TARGETED RESEARCH TO DEVELOP VACCINES AGAINST THREE PARASITE STAGES:
- SPOROZOITES
- ASEXUAL BLOOD STAGES
- SEXUAL STAGES

STATUS OF VACCINE RESEARCH AND DEVELOPMENT

● SEQUENCE OF RESEARCH ACTIVITIES:

SEXUAL STAGES

ASEXUAL BLOOD STAGES

SPOROZOITES

- Choice of parasite stage
- Analysis of antigens
- Identification, purification and characterization of protective antigens
- Cloning of genes coding for protective antigens
- Expression of genes in *E. coli*
- Analysis of gene nucleotide sequence
- Deduction from the gene's nucleotide sequence of the antigen's amino-acid sequence
- Production of the antigen by chemical synthesis or genetic engineering
- *In vitro* testing of the antigen
- Testing of the antigen in animals for:
 - Immunogenicity and toxicity
 - Protective immunity

● SEQUENCE OF DEVELOPMENT ACTIVITIES:

- Efficacy, tolerance and toxicity trials
- Large-scale production of antigens
- Development of formulations: choice of adjuvant and carrier molecules
- Pilot-scale production: Phase I, Phase II trials
- Field trials in endemic areas: Phase III trials in human volunteers
- Registration and commercial production
- Field trials of efficacy, epidemiological impact and tolerance of licensed vaccines in large populations: Phase IV trials

Malaria vaccine research will soon progress from the stage of fundamental laboratory investigation to the developmental stages, involving pilot-scale production and initial efficacy, tolerance and toxicity testing. Although several candidate antigens have been identified on which future malaria vaccines might be based, there is still a long way to go before any malaria vaccine will be ready for extensive field trials. Among the problems to be solved are the production and formulation of malaria vaccines and the selection of suitable adjuvants and carrier molecules. It is therefore impossible to predict when a malaria vaccine will become commercially available, but the present rate of progress and the commitment of industry to develop malaria vaccines justify optimism.

use of vaccinia for this purpose is now being explored for a number of potential new vaccines, including a malaria vaccine. A recombinant vaccinia virus would offer a number of advantages: low cost, ease of administration, high stability, and the possibility of combining several vaccine antigens and incorporating them into a single vaccine strain given as a single inoculum. This promising approach is now being pursued, but more work is required on the attenuation and production, and the control, safety and efficacy testing of vaccinia-based vaccines. Other potential microbial vectors will also be evaluated.

Modern vaccine production technology should be applicable to future malaria vaccines, and it is encouraging that several commercial concerns are now committed to the development of malaria vaccines. IMMAL held an informal consultation with interested companies in October 1983, which was attended by representatives from the United States Agency for International Development (USAID) and from 13 industrial companies (C3). Several companies expressed their intention to collaborate with WHO/TDR in future malaria vaccine development and a number of meetings have already been held with several of these companies.

Immunodiagnosis of malaria

The second main objective of the IMMAL programme is the development of new and the improvement of existing immunodiagnostic methods for malaria—methods which could provide valuable epidemiological tools for a variety of purposes, including the field evaluation of future malaria vaccines.

Vaccine trials will probably involve examination of patients for the presence of circulating parasites and for the assessment of immune status, two parameters that are the focus of current studies.

The detection of malaria parasites in thick blood films is rapid and accurate when parasitaemia is relatively high, but slow and less reliable when parasitaemia is low and when large numbers of slides have to be examined. Large-scale studies would be greatly facilitated by a simple, highly sensitive serological test to detect parasites in peripheral blood. Both radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) methods have been devised for the detection of *P. falciparum* parasites in blood and useful levels of sensitivity have been achieved. These are solid-phase assays, based on competition or inhibition of specific antibody binding to parasite antigens in the presence of infected erythrocytes. However, greater precision and standardization will be required before such tests are

ready for widespread field application. Defined reagents, another prerequisite, are only now becoming available. As more synthetic plasmodial peptides are produced, it can be expected that they will replace the use of whole parasites as sources of antigens for immunodiagnostic tests. A striking example of how a MAB can be used to provide a powerful new epidemiological tool is illustrated by the Zavala test—a simple solid-phase sandwich assay, in which a MAB to CS protein is used to detect sporozoites in infected mosquitoes (A137). Detection is highly sensitive, quantitative and species-specific.

Monoclonal antibody registry

The registry for monoclonal antibodies to malaria antigens, established two years ago in Geneva, continues to collect MABs to asexual blood-stage antigens. These are being evaluated as potential immunodiagnostic reagents for use in existing and in new test systems.

Another recently introduced approach to malaria diagnosis is DNA hybridization, using cloned *P. falciparum* repetitive DNA as a probe to detect corresponding DNA sequences in infected blood (B4). Results published so far suggest that sensitivity is lower than that of RIA or ELISA.

As yet there is no test for protective immunity to malaria. Several methods are available for measuring serum antibodies to blood-stage parasites—IFA, indirect haemagglutination (IHA), RIA, ELISA—but the antibody titre does not provide an index of protection. Several functional assays have been devised in which antibody reacts with antigens that appear to be important for parasite growth and development. Merozoite invasion inhibition is one such assay, in which merozoite reinvasion is quantitated in *P. falciparum in vitro* cultures (A13, A33). Many immune human and *Aotus* sera have been shown to be inhibitory: some, however, have an enhancing effect on parasite growth, while some inhibit certain *P. falciparum* strains but not others. In one study in the Sudan, it was found that "immune" adult sera retarded parasite growth and induced the intra-erythrocytic production of "crisis forms"; the serum factor responsible for this effect has not yet been identified (B10). Another functional assay uses an *in vitro* model of the mature *P. falciparum* parasite sequestration that occurs in natural infections. The binding of trophozoite-containing erythrocytes *in vitro* to endothelial cells or amelanotic melanoma cells was shown to be inhibited or reversed by immune *Aotus* sera (A120). This phenomenon is now being studied, using human sera from individuals with different levels of exposure to malaria. Tests for

cell-mediated immunity in malaria are also being developed, but this work is still at an early stage.

Immune mechanisms and immunopathology

The rational development of malaria vaccines requires an understanding of the mechanisms underlying both protective immunity and the immunopathological complications of malaria. The ideal vaccine should specifically stimulate protective responses and not others which may have undesirable effects. IMMAL is therefore supporting studies in this area.

Humoral immunity in malaria has received a great deal of attention recently, with much interest focused on MABs that identify protective plasmodial antigens. However, cell-mediated immunity (CMI), although to date a relatively neglected research area, is also important in malaria and was reviewed at the seventh meeting of the IMMAL SWG (22-23 October 1984) (C2).

Cell-mediated immunity

There is good evidence from experimental animal models that CMI responses (Fig. 2.10) are involved in protective immunity to malaria, particularly to the asexual blood stages. Experimental evidence indicates that T-cells are required for the development of effective immunity and that immunity can be induced in the absence of antibody. Macrophages are activated during malaria infection and secrete products capable of killing malaria parasites within red cells (A87). T-cell control of macrophage activation and secretory function is exerted through a mechanism involving a series of lymphokines. Research on the T-cell receptor involved in antigen recognition, however, is still at an early stage.

Evidence from several studies suggests that CMI may correlate more closely with immune status than do antibody levels. Thus, in vaccinated mice, protection is improved by the administration of saponin or *Bordetella pertussis*, both of which increase CMI without affecting antibody titres. Recent studies on lymphocyte responses from malaria patients have produced some interesting findings. In one study, lymphocytes taken from patients a year after a single malaria infection proliferated *in vitro* in response to antigens in *P. falciparum* culture supernatants. Lymphocytes from acutely ill patients with *P. falciparum* malaria proliferated in response to parasite antigens for only three to four days after adding antigen to the cells *in vitro*. Thereafter, interleukin-2 secretion ceased. In immune subjects, proliferative responses were maintained for longer and high levels of

γ -interferon (IFN- γ) were secreted. It was concluded that IFN- γ secretion by T-cells in response to malaria antigen may prove to be a useful index of immune status.

Antimalaria factors

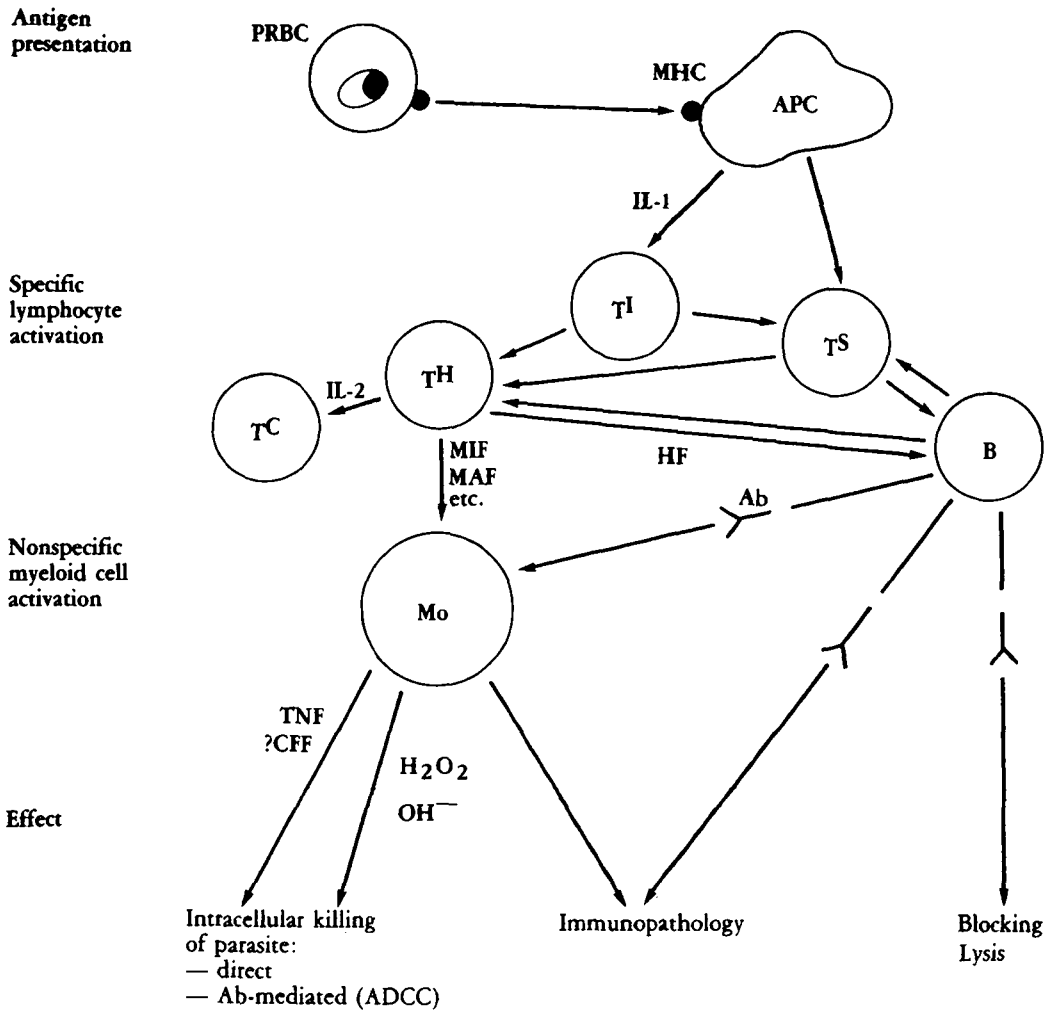
Activated macrophages secrete a variety of substances, of which at least two groups may be capable of killing malaria parasites: reactive oxygen intermediates (ROI) (A27) and tumour necrosis factor(s) (TNF) (B16). It has been shown that intra-erythrocytic malaria parasites, particularly those in mature or aged red cells, are highly susceptible to oxygen stress (A27, A86, B2). The susceptibility of older red cells may be due to their reduced antioxidant content, which may explain why crisis forms are seen only with parasites in mature cells. Serum from mice treated first with BCG and then with endotoxin kills parasites *in vitro* and reduces parasitaemia *in vivo*. The responsible factors have been termed TNF because such sera cause necrosis of a number of transplantable mouse tumours. TNF probably includes several molecules, of which none has been characterized. Detectable levels of TNF have been found in serum from malaria-infected mice given doses of endotoxin too low to affect normal mice, suggesting that TNF production is increased during malaria. It is not yet clear, however, which type(s) of cells may be capable of producing TNF.

Serum from healthy adults from southern Sudan has been shown to kill *P. falciparum* *in vitro*, followed by the appearance of crisis forms (B10). The responsible factor was not found in a purified IgG fraction from this serum, and the apparently immune adults had comparatively low antimalaria antibody titres. The crisis form factor has not yet been identified, and it remains to be determined whether its high prevalence in southern Sudanese is unique to this population.

The fact that vaccination against blood-stage parasites requires the use of adjuvants that induce CMI suggests that the protective immune response includes a cellular component. Successfully vaccinated mice have been shown to develop a number of CMI-associated changes, including increased inflow of lymphocytes to the liver and spleen, increased production of ROI and increased macrophage killing of tumour cells. Parasites thus appear to be killed by activated macrophages at sites where the two come into close contact, such as the sinusoids of the liver and spleen. T-cells may attract and activate the macrophages and contact between macrophages and infected red cells could be promoted by antibody. Experiments in squirrel monkeys also indicate that

FIG. 2.10

CELL-MEDIATED IMMUNITY IN MALARIA:
THE MAIN CELLS AND MEDIATORS INVOLVED IN REGULATION OF THE IMMUNE RESPONSE



- | | |
|---|---|
| Ab = Antibody | MAF = Macrophage activation factor (= γ -interferon) |
| ADCC = Antibody-dependent cell-mediated cytotoxicity | MHC = Major histocompatibility complex |
| APC = Antigen-presenting cell | MIF = Migration inhibition factor |
| B = B-cell | Mo = Macrophage |
| CFF = Crisis-forming factor | PRBC = Parasitized red blood cell |
| HF = Helper factors | T ^C = Cytotoxic T-cell |
| IL-1 = Interleukin-1 (= lymphocyte activation factor) | T ^I = Inducer T-cell |
| IL-2 = Interleukin-2 (= T-cell growth factor) | T ^H = Helper T-cell |
| LT = Lymphotoxin | TNF = Tumour necrosis factor(s) |
| | T ^S = Suppressor T-cell |

Box 2.7 The future

IMMAL Workplan for 1985-87

<i>Area of study</i>	<i>Specific topics</i>	<i>Specific activities</i>
Antigen analysis	Sporozoite Asexual blood forms Gametes Exoerythrocytic stages	— Identification, isolation and characterization of immunogenic antigens — Typing of parasite isolates and clones — Identification of variant antigens
Antigen production	Comparison of different methods	— Recombinant DNA — Polypeptide synthesis — Recombinant live virus
Vaccination experiments	Preliminary testing of candidate vaccines	— Efficacy testing } monkeys — Toxicity testing } — Efficacy testing } human — Toxicity testing } volunteers
Immune response	Analysis of cellular immune responses Effector mechanisms	— Analysis of T-cell subsets in response to malaria — Role of histocompatibility antigens — Identification of immunosuppressive factors — Analysis of specific macrophage activation — Nonspecific macrophage activation by endotoxin-like substances — Role of mediators released by activated macrophages
Immunopathology	Tropical splenomegaly syndrome (TSS) Cerebral malaria	— Identification of genetic factors affecting susceptibility — Analysis of cellular control mechanisms in TSS — Analysis of immunological factors involved in aetiology (animal models) — Comparison of immunological parameters in serum and CSF from affected children
Immunodiagnosis and seroepidemiology	Registry for monoclonal antibodies	— Standardization of existing tests — Development of new tests
Comparison of antigens	Reference centre for malaria antigens	— Comparison of asexual blood-stage antigens of <i>P. falciparum</i> from different laboratories

protection induced by vaccination against *P. falciparum* may be largely cell-mediated (A48). Protective immunity does not seem to correlate with the ability of serum from vaccinated animals to confer immunity on passive transfer.

CMI, in addition to its protective role, may be responsible for certain immunopathological complications of malaria. The immunosuppression associated with malaria appears to be macrophage and T-cell-mediated, although the mechanisms involved

are complex and not fully understood. Glomerulonephritis and anaemia are probably antibody-mediated in malaria. However, CMI may play a role in cerebral malaria, as studies in rodents suggest. In certain strains of rats and mice, *P. berghei* can cause early death from cerebral malaria that can be prevented by T-cell depletion (A51). Unexpectedly, the transfer of antigen-specific *helper* T-cells prevents cerebral complications without affecting parasitaemia

levels (A50). It is possible that cerebral malaria may be related to inadequate levels of T-helper activity.

Recommendations for further studies on CMI in malaria were drawn up during the seventh IMMAL SWG meeting (C2). Further investigation of cellular responses in both man and owl monkeys, as well as of the response to immunization with individual antigens, is clearly needed.

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Immunology of malaria

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Applied field research in malaria

The context

□ The aims of the Scientific Working Group (SWG) on Applied Field Research in Malaria (FIELDMAL) are to promote the best use of existing tools for the control of malaria and, on the strength of reliable epidemiological data, to evaluate new tools and methods for malaria control and field research.

Over the past two years FIELDMAL has been con-

cerned mainly with: the growing severity and increasing spread of resistance of *Plasmodium falciparum* to chloroquine; the complex pattern of "family relationships" among the different *Anopheles* species and the roles of different members of these families in malaria transmission; the integration of malaria control into local primary health care systems. □

Box 2.8 Highlights of activities in 1983-84

- A global system for monitoring the spread of *Plasmodium falciparum* resistance to drugs has been set up and integrated into the routine epidemiological activities of national health services in several countries in South-East Asia, Africa, and Central and South America.

- Kits to test the sensitivity of *P. falciparum* to 4-aminoquinoline drugs and

to the new antimalarial mefloquine are being produced in the Philippines, initially under a research agreement with the Special Programme.

- Ways of gearing antimalarial treatment to the specific needs and circumstances of individual communities are being developed from research findings in Guatemala, Kenya, Thailand and the United Republic of Tanzania.

- Chromosomal and biochemical analyses are emerging as promising tools for studying the different functional "families" or species complexes of mosquitoes and their roles in disease transmission, and could provide the basis for more rational malaria control strategies.

Report of activities in 1983-84

Plasmodium falciparum resistance to chloroquine, first reported almost simultaneously in Colombia and Thailand in the early 1960s, has since spread to many other areas of the Americas and Asia, and more recently to Africa, and is a major obstacle to the control of malaria.

Global monitoring of drug resistance

A global resistance monitoring programme, begun as a project of the Scientific Working Group (SWG) on Applied Field Research in Malaria (FIELDMAL) in 1981, was incorporated into WHO's Malaria Action Programme at the end of 1983. The monitor-

ing programme has three main objectives: to develop standard methods of recording, storing, processing and analysing drug sensitivity data; to set up a databank; to report periodically on drug sensitivity throughout the world. The system provides a synopsis of the geographical distribution and levels of susceptibility/resistance (Fig. 2.1), and calls attention to significant differences in responses to individual drugs between different geographical areas and at different times (Fig. 2.11). Although of fairly recent introduction, the system will very likely become the main source of data on worldwide antimalarial drug sensitivity and provide a basis for the planning of countermeasures to drug resistance (Fig. 2.12).

Resistance can be detected by *in vivo* observation of patients' responses to therapy and by *in vitro* testing. In the absence of resistance, parasitaemia diminishes rapidly following therapy. Some patients show a recrudescence of parasitaemia following initial resolution (RI). Others, with more resistant parasites, show a significant reduction but no resolution of parasitaemia (RII). At the highest level of resistance (RIII), therapy has little or no effect on parasitaemia (B12). *In vivo* drug resistance depends not only on factors related directly to the parasite but also on several host-dependent variables, including drug metabolism and pharmacokinetics, and on the immune status of the individual. In patients with some degree of immunity to malaria ("semi-immunes"), chemotherapy may effectively control parasitaemia, despite a certain degree of parasite drug resistance. *In vitro* resistance is assessed by measuring the effects of different concentrations of antimalarial drugs on parasites in culture.

Test kits

Test kits readily applicable to field conditions in the tropics have been developed, largely through the efforts of CHEMAL, to detect *P. falciparum* resistance to chloroquine, amodiaquine, quinine and mefloquine.

As part of its global resistance monitoring system, FIELDMAL has promoted, through appropriate funding and workshops, the production of standard test kits, the training of personnel to gather *in vivo* and *in vitro* data on resistance, and the development of field projects for the assessment and monitoring of resistance.

Production of standard WHO kits for testing *P. falciparum* sensitivity to 4-aminoquinolines and to mefloquine has continued at the National Malaria Service of the Ministry of Health of the Philippines, initially under a research agreement with the Special Programme and later, towards the end of 1983, as a financially autonomous project administered in collaboration with WHO's Western Pacific Regional Office. Quality control is provided by the University of North Carolina, in the United States, and financially supported by CHEMAL.

During the reporting period, production of "macro" kits, which require 10 ml of blood, was switched to that of "micro" kits, which require only 0.1 ml of blood: 337 basic and 1467 replenishment macro kits were produced between 1979 and 1982, and 100 basic and 200 replenishment micro kits during 1983 and 1984. Macro kits are at present distributed free of charge and micro kits sold at cost for US\$ 390 per basic ("A") kit (for testing 48

samples against two drugs) and US\$ 130 per replenishment ("B") kit (for 24 samples against two drugs).

Field work

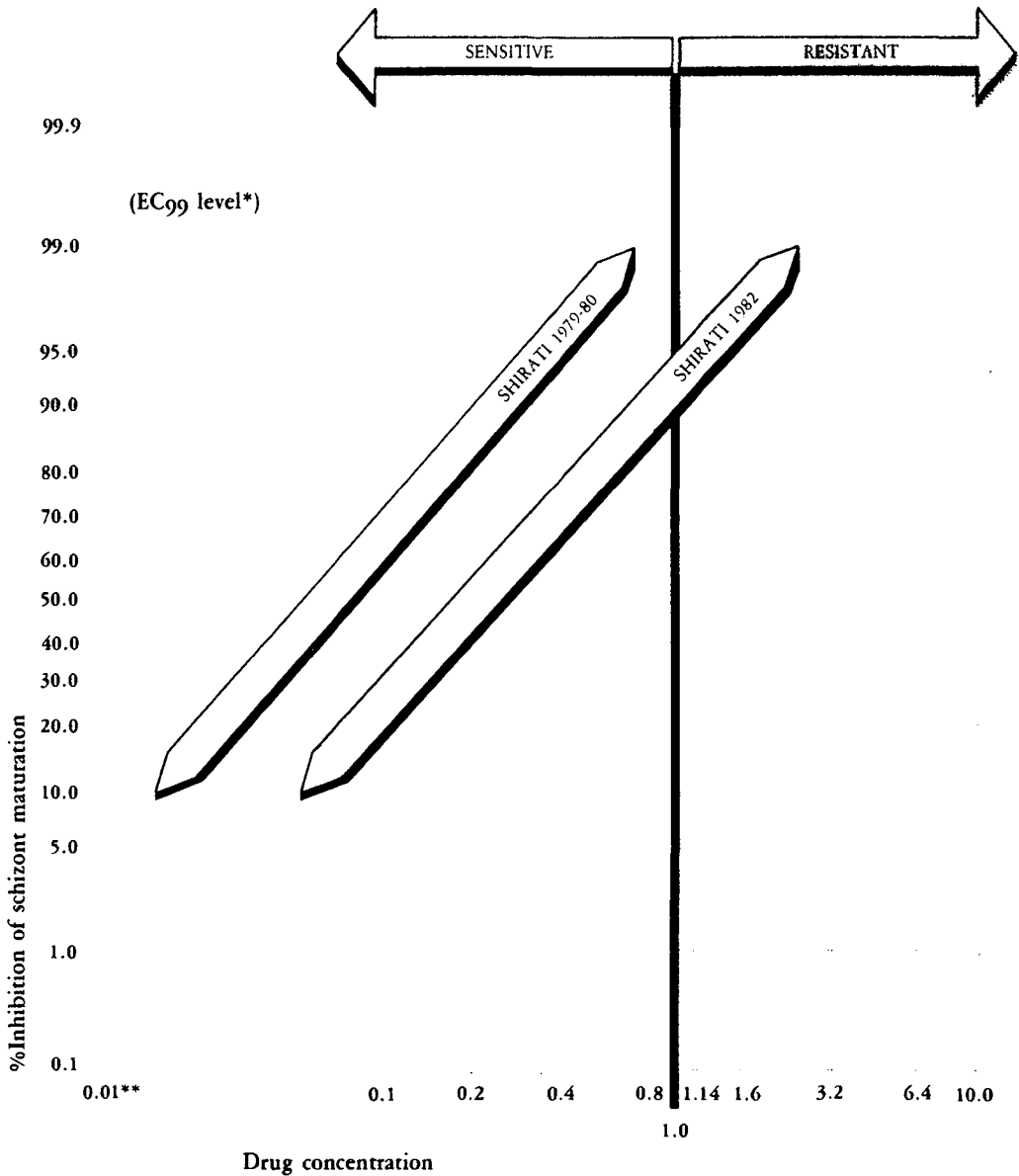
Research conducted outside the Programme in semi-immune populations of Kenya, the Sudan, and the United Republic of Tanzania, including Zanzibar, has confirmed the spread of *P. falciparum* resistance to 4-aminoquinolines in Africa (B7, B9). In South-East Asia, chloroquine resistance has reached, in India, the states of Bihar, Gujarat and Rajasthan, and the Andaman and Nicobar Islands. In the Americas, confirmed cases of resistance have for the first time been reported from Bolivia and Peru (B6). *P. falciparum* resistance to the second-line sulfa-pyrimethamine drugs had been observed several years ago in Democratic Kampuchea and Thailand and to some extent in other South-East Asian countries. There is now evidence that it is becoming increasingly common in these areas: in Thailand, particularly the border area between Thailand and Democratic Kampuchea, about 40% of patients have shown resistance to these drug combinations (B8). Treatment failures with sulfa-pyrimethamine combinations had also been reported over the last few years in South America, but are now also appearing in East Africa, notably Kenya (B5) and the United Republic of Tanzania (B2-3, B10).

Field work conducted with TDR support was initially concentrated in and around areas where drug resistance first appeared: in South-East Asia, the Western Pacific and the Americas. This work has become, or is rapidly becoming, part of the routine epidemiological activities of several national health services in these regions. Technical Officers have been appointed by WHO's South-East Asia and Western Pacific Regional Offices to coordinate these activities.

- In the South-East Asia Region, regional regular and extra-budgetary funds (from the Swedish International Development Authority) are being used to support field activities.
- In Africa, where resistance first appeared in the late 1970s and has since spread with increasing rapidity throughout East Africa and, in the past year or so, also appears to be spreading westwards (see Fig. 2.12 and Box 2.9), several studies have been undertaken over the past four years (1980-84).
- In some areas of the Eastern Mediterranean Region resistance has also made its appearance, a development highlighted at a FIELDMAL workshop held in Amman, Jordan, in the spring of 1984.

FIG. 2.11

RESPONSE OF *P. FALCIPARUM* TO CHLOROQUINE IN SHIRATI,
UNITED REPUBLIC OF TANZANIA, IN 1979-80 AND IN 1982



* EC₉₉ = Effective drug concentration required for 99% inhibition of schizont maturation

** All values x 10⁻⁶ mol chloroquine/l blood

The parasite's response to chloroquine has shifted in two and a half years from sensitive to resistant, in an area of intense prophylactic drug "pressure". In vitro tests were carried out on random blood samples from about 250 children in the area of Shirati, United Republic of Tanzania. Sensitivity or resistance is determined by the effective concentration of drug required for 99% inhibition of schizont maturation (EC₉₉). An EC₉₉ less than 1 x 10⁻⁶ mol/l denotes sensitivity, an EC₉₉ over 1 x 10⁻⁶ mol/l, resistance. Over the two-and-a-half-year period, the EC₉₉ shifted, in Shirati, from about 0.7 to over 2 x 10⁻⁶ mol/l.

Box 2.9 Resistance of *Plasmodium falciparum* to drugs: New findings from major FIELDMAL projects (in two WHO Regions)

Western Pacific Region

In vivo and *in vitro* resistance to chloroquine has been reported in six provinces of the Lao People's Democratic Republic; in various Malaysian states, including Sabah; in Malaita Island and the central provinces of the Solomon Islands; throughout Papua New Guinea (except in the highlands, where the situation regarding resistance is not clear) and in the southern districts of Vanuatu (A4) (the number of cases of *P. falciparum* malaria has increased in the north, suggesting growing resistance there too). Treatment failures with sulfadoxine/pyrimethamine have also been reported in the Lao People's Democratic Republic.

Some variability in *P. falciparum* sensitivity to mefloquine has been observed *in vitro*, but *in vivo* studies undertaken in three different regions do not suggest that this finding has any therapeutic significance.

On the strength of increasingly frequent reports of chloroquine resistance, the sulfadoxine/pyrimethamine combination has been adopted for general use in Sabah and for recrudescence infection (in which case it is used together with quinine) in the Solomon Islands. Clinical evidence from this

area suggests an increase in the numbers of patients responding to chloroquine alone, possibly because of reduced drug pressure or increased immunity — a finding which, if confirmed, merits further study.

African Region

Dominating the situation in this area over the past two years have been the rapid spread and the growing severity of parasite resistance (Fig. 2.12).

Resistance to chloroquine, initially at low levels, has been observed in four districts of the United Republic of Tanzania. There have been recent reports, however, of RII and RIII cases in the area.

Resistance of *P. falciparum* to amodiaquine has been observed *in vivo*, although at levels much lower than that to chloroquine. Resistance to sulfadoxine/pyrimethamine has also occasionally been observed *in vivo*. Again, as in the Western Pacific Region, tests of sensitivity to mefloquine have shown some variability, but the findings do not suggest reduced *in vivo* sensitivity. Full clinical and parasitological response to mefloquine, however, has been observed in

extensive clinical trials conducted in Zambia.

Chloroquine given in a dose of 10 mg/kg failed to achieve cure in children, a finding which therefore also rules out its prophylactic use. An alternative drug, proguanil, has been evaluated for prophylaxis and the results have been encouraging, although too few patients have been assessed to warrant definitive conclusions.

In Kenya (A32), 60 to 70% of parasite isolates from patients living in coastal areas have been found to be resistant to chloroquine *in vitro*, although *in vivo* resistance in the same patients was not so marked. In western Kenya, sporadic RI and RII cases have been reported, and here again amodiaquine has proved much more effective than chloroquine. An experimental *in vitro* test of sensitivity to sulfadoxine/pyrimethamine showed the prevalence rate of resistance to this drug combination to be about 15% in this area—a finding consistent with *in vivo* observations.

In Uganda, *P. falciparum* has been shown still to be sensitive to chloroquine, but not in Burundi, where resistance is well established, at least in an area that was subjected to high drug pressure in the past.

Field trials of drugs

FIELDMAL is concerned with the field evaluation of drugs of clinically proven efficacy and safety and with the improvement of strategies for the use of these drugs.

The increasing menace of *P. falciparum* resistance to chloroquine calls for a number of changes in choice of drugs and drug regimens: 1) an increase in recommended chloroquine dosage from a single dose of 10 mg/kg to a fully curative total dose of 25 mg/kg given over three days; 2) use of an alternative 4-aminoquinoline, such as amodiaquine, which has proved effective in some regions for some time after the appearance of chloroquine resistance; 3) use of combinations of sulfonamides or sulfones with pyri-

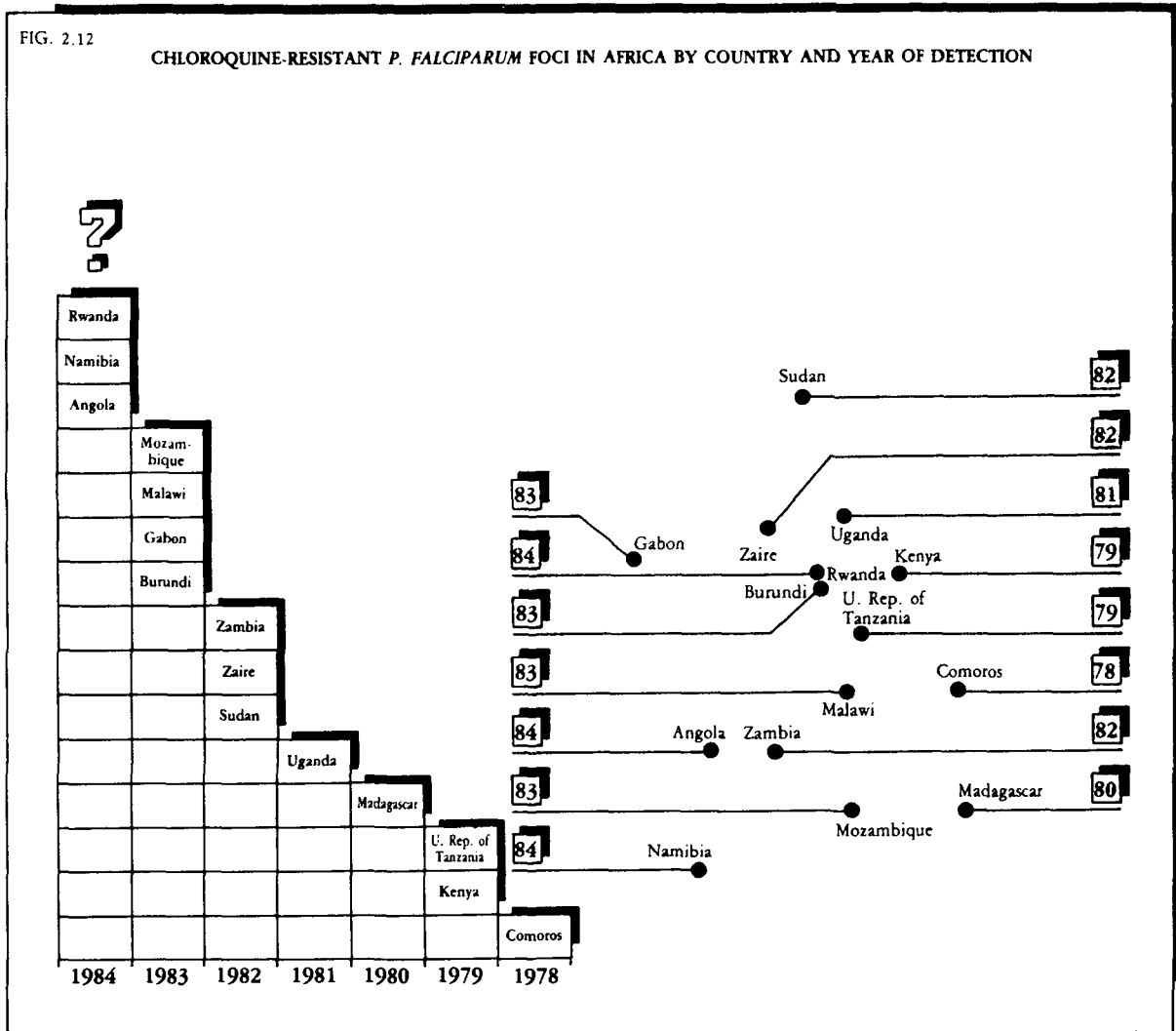
methamine, a strategy whose effectiveness against *P. falciparum* has been found to vary from place to place; 4) a longer treatment schedule (usually up to seven days), using quinine and tetracycline, in cases where standard regimens have failed; 5) use of the new antimalarial mefloquine, alone and in combination with other drugs, within the limitations outlined under CHEMAL (Box 2.3).

These new approaches are being evaluated under the global monitoring programme described above and are all based on the use of existing drugs or drug combinations. If and when new drugs and new drug combinations currently being tested under CHEMAL become available for field testing, they will come within the scope of FIELDMAL.

• In Thailand, double-blind, randomized, com-

FIG. 2.12

CHLOROQUINE-RESISTANT *P. FALCIPARUM* FOCI IN AFRICA BY COUNTRY AND YEAR OF DETECTION



parative trials in adults have been carried out on mefloquine given in single doses of 750 mg or 1000 mg, in association with primaquine (as a gametocytocide, which may therefore prevent transmission arising from sexual forms of parasites that escape the effects of mefloquine) given either on the same day as mefloquine (day 0) or three days afterwards. All regimens tested were well tolerated and showed almost identical efficacy in lowering fever and clearing the blood of parasites (100% clearance of asexual forms by day 4 with both dose regimens). Gametocyte levels declined more rapidly in the patients receiving primaquine on day 0 than in those given the drug on day 3. In both groups gametocytes had disappeared by day 7, suggesting that there is no appreciable advantage in giving primaquine on day 3 rather than on day 0. Trials are also under way on primaquine and a triple association of mefloquine, sulfadoxine and pyrimethamine.

- In Sri Lanka, comparative trials are being conducted on chloroquine used either alone or in conjunction with a 5- or 14-day regimen of primaquine in *P. vivax* infections, which are often relapsing, require prolonged radical therapy and are therefore poorly accessible to field follow-up. Preliminary findings show that chloroquine plus the 5-day primaquine regimen is associated with one-half as many relapses as chloroquine alone. Although data on the 14-day regimen are still being analysed, it is likely that the 5-day regimen will prove to be a poor substitute for the 14-day regimen.

Epidemiological surveillance and drug distribution: trials of different methods

Antimalaria programmes depend on prompt detection of cases and institution of treatment, neither of which is always easy to achieve. Different

approaches to this problem are being investigated.

- In Guatemala, the national malaria services have set up a network of village volunteers and two systems are being tested: in one, volunteers administer treatment over three days to all individuals with suspected or self-diagnosed malaria; in the other, a single dose of antimalarial medication is given, followed by blood film examination and administration of a three-day regimen in confirmed cases only. The two systems are being compared with the traditional, more costly, national surveillance scheme. With the new approaches there has been a marked increase in the number of patients receiving treatment and a marked reduction (by about half) in the delay between blood sampling and institution of a full treatment regimen. It was also observed during the study that malaria cases tended to cluster in a limited number of houses, that chloroquine was widely believed to be contraindicated in pregnancy and that itching (previously unknown outside Africa as a side-effect of chloroquine) was a frequent reason for interrupting treatment. The encouraging preliminary results of this trial, which are still being evaluated, prompted the organization in Guatemala of a regional workshop to study the possibility of transferring some facets of the surveillance methodology to other countries, particularly in Central America.

- Another approach is being tried out in South Mara in the United Republic of Tanzania, where traditional birth attendants are asked to distribute antimalarial drugs and keep records under the supervision of village midwives. The project has encountered serious logistic and administrative difficulties that preclude objective evaluation of the results, but the traditional birth attendants did respond enthusiastically to requests for cooperation, mainly because of government recognition of their work.

- The Saradidi community near Kisumu in Kenya has established a comprehensive community health system that is based on local clinics and volunteer village health workers, and is partly financed by the community. Primary health care services include malaria prophylaxis for pregnant women and treatment of all malaria patients. This experiment in community participation has elicited a very enthusiastic response from the local population. The findings are being analysed and prepared for publication.

- In a different context, four new systems of epidemiological surveillance are being assessed in Thailand in relation to local conditions and the epidemiological situation. Village volunteers are present in all areas, but up to now slide examinations and radical treatment have been conducted in centralized malaria

clinics, with the result that treatment delays and poor patient compliance are common. Alternative schemes are being studied: in one area, microscopists visit villages at regular intervals and slides are examined on the spot; in another, a mobile malaria clinic provides weekly services to each village; in another, with low endemicity and easy accessibility, the supervisor's weekly visits are to become monthly; and in yet another, highly malarious, area, volunteers are responsible for administering radical treatment and will have weekly consultations with supervisors.

Chemoprophylaxis

Chemoprophylaxis has long been advocated as a means of reducing infant and child mortality in hyper- and holoendemic areas. This approach is now seen to have more disadvantages than advantages, in particular the risk of inducing drug resistance. In Burkina Faso, chemoprophylaxis in children was compared with the treatment of children actually suffering from a malaria attack. Despite a much lower compliance rate, chemoprophylaxis was associated with a threefold greater drug consumption than chemotherapy, as well as with a reduction in antimalaria antibody titres and some reduction in malaria prevalence. Neither regimen, however, had a demonstrable effect on mortality rates (A3).

These findings in children do not invalidate the use of chemoprophylaxis in expectant mothers, a group especially vulnerable to the severe effects of malaria. In the Saradidi (Kenya) research project described above, chemoprophylaxis was confined to expectant mothers. It was originally decided to give chemoprophylaxis also to children under five years of age, but when *P. falciparum* resistance made its appearance in the area, they were given only curative treatment as needed. Preliminary results from the project suggest that mortality rates among young children were little affected by malaria chemotherapy and that what effect there was, resulted more from other community health activities and mass vaccination campaigns (especially against measles) than from the chemotherapy. Other data from this study are being analysed.

Drugs may also be used under special conditions in attempts to reduce or interrupt malaria transmission. In Thailand, population movements and the presence of sylvatic mosquitoes contribute to the maintenance of malaria transmission in some communities. In three villages in a hypoendemic area, studies were conducted on the mass administration of primaquine, using 30 mg doses at 10-day intervals for three to four months. Prevalence of *P. falciparum* infection declined almost to nil very rapidly,

and that of *P. vivax* somewhat less rapidly. The role of drugs in bringing about these changes is, however, uncertain, due to the unusual weather conditions prevailing at the time of the project. Primaquine, which can produce untoward side-effects in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency if given continuously over several days, caused no adverse reactions in the G6PD-deficient individuals participating in this project, in which the drug was given intermittently. Further data from the project are being analysed.

Vectors

Mosquito vectorial status

Effective vector control requires an understanding of vector behaviour and other biological characteristics. This, in turn, requires identification of the mosquito species or subspecies responsible for transmission of the disease.

Studies on mosquito species are in progress in two areas of Sri Lanka, one an endemic dry area, the other an intermediate wet area. Epidemics of malaria have recently occurred in both areas. Mosquitoes of 15 indigenous *Anopheles* species collected in the field and studied in the laboratory all proved susceptible to infection with *P. vivax*, the prevailing *Plasmodium* species in the area. In the field, all 15 have some degree of contact with man. The known malaria vector, *An. culicifacies*, however, is the most anthropophilic. This species, like *An. subpictus*, is also highly endophilic (indoor). *An. culicifacies*, *An. subpictus*, *An. jamesi*, *An. karwari* and *An. vagus* were all found to carry sporozoites under natural conditions. These observations greatly extend the known spectrum of malaria vectors in Sri Lanka.

Research being conducted in the Amazon Basin in Brazil makes simultaneous use of three techniques for the detection of infected mosquitoes: the traditional dissection method for detection of sporozoites and oocysts, the more recently developed Zavala test—an immunoradiometric assay (IRMA) devised by Fidel P. Zavala and associates at New York University, (see IMMAL section, under "Immunodiagnosis of malaria")—and an enzyme-linked immunosorbent assay (ELISA). The IRMA and ELISA both use monoclonal antibodies to detect sporozoites in infected mosquitoes and to differentiate between parasite species. *P. vivax* and *P. falciparum* sporozoites have been detected in *An. darlingi*, and only *P. vivax* in *An. albitarsis*, *An. triannulatus*, *An. nuneztovari*, *An. oswaldi* and *An. evansi*. These new immunochemical tests are clearly suitable for field identification of infected malaria vectors and of the sporozoite species they are carrying.

Vector behaviour and insecticides

Insecticides are extremely effective weapons in the fight against malaria. They do, however, have serious limitations. Their wide use for health and agricultural purposes resulted in an upsurge of vector resistance: the vector not only became less susceptible to, but also avoided contact with, insecticides—two genetically determined traits "selected" by insecticide pressure. Under these circumstances, the choice of an insecticide and methods of applying it must take into account not only a vector's susceptibility but also its behaviour.

In Indonesia, fenitrothion was used in two ways against DDT-resistant *An. aconitus*: spraying of interior walls up to 3 m or in a single swath 10 to 85 cm high (most mosquitoes rest between these levels). The more complete coverage caused a more rapid decline in mosquito density and parasite counts, but selective coverage also proved quite effective and was only a third of the cost. Moreover, with selective spraying, cholinesterase levels remained higher, denoting less toxicity, in spraymen.

Studies in Thailand have shown that DDT diverts *An. minimus* from man to animals (A22). This effect is obtained with less frequent applications of the insecticide than are needed to actually kill mosquitoes and thus makes for a considerable saving in costs.

Vector species complexes

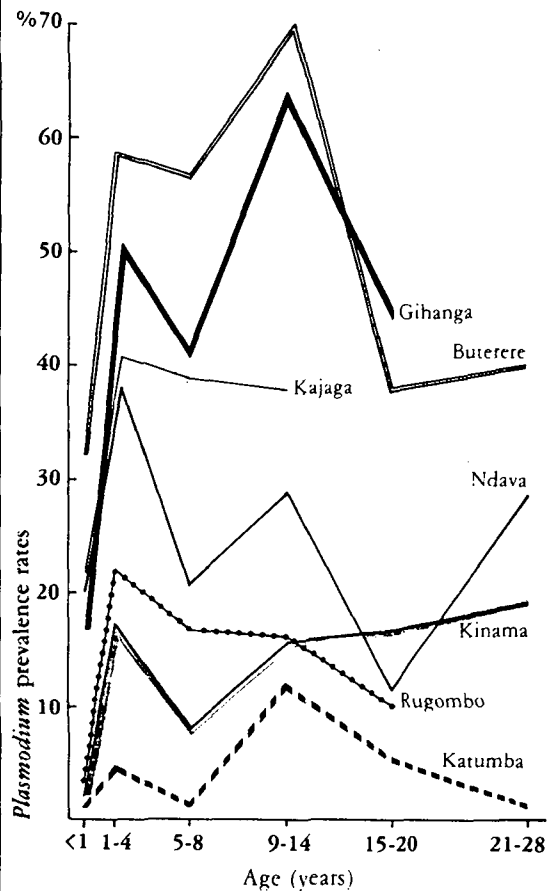
Chromosomal analysis and biochemical methods have in recent years made it possible to distinguish between more species of *Anopheles* and intraspecific variants than was hitherto possible using morphological characteristics. Ecological and behavioural differences observed in such variants are often of epidemiological importance and may have implications for malaria control.

In Mali, *An. gambiae sensu stricto* was found to comprise three variants of mosquitoes: the Bamako type, found in the humid areas in the south; the Mopti type, found in areas subject to flooding along the river Niger and coexisting with the Bamako type; and the Savanna type, found in drier areas.

In an irrigated area close to Bobo-Dioulasso in Burkina Faso, *An. gambiae sensu lato* exhibits four times the man-biting rate than in a surrounding dry area, but much lower sporozoite rates, with a resulting lower level of malaria endemicity. One reason for this diversity is that *An. gambiae s.l.* is made up of three different populations with distinct intraspecific variations in polytene chromosomes: the Mopti type, with a short life expectancy, predom-

FIG. 2.13

AGE-SPECIFIC PLASMODIUM PREVALENCE RATES
IN DIFFERENT VILLAGES
ON THE RUZIZI PLAIN (BURUNDI)



Malaria prevalence varies widely and consistently between villages, being highest in those close to rice fields. Control activities may therefore be based primarily on water management and other environmental control measures.

inating in the irrigated area; the Savanna and *An. arabiensis* types, with longer life expectancies, predominating in dry areas.

In the United Republic of Tanzania, *An. gambiae* s.s. predominates in humid areas and *An. arabiensis* in dry areas, and where the two coexist both species carry chromosomal inversion polymorphisms which apparently make them adaptable to extreme conditions. Differentiation between the two is more reliable by cytotoxic analysis than electrophoretic methods. Initial observations on infection rates suggest that *An. gambiae* may play a leading role in the transmission both of malaria and of bancroftian

filariasis. *Anopheles* mosquitoes show chromosome inversion polymorphisms reflecting specific adaptive traits in relation to the environmental conditions from which they originated. For *An. gambiae* and *An. arabiensis*, in particular, geographical variations in inversion frequencies have been found to relate closely to climatic and ecological conditions. Chromosomal arrangements found in mosquitoes during the rainy season resembled those found in mosquitoes from humid areas, whereas chromosomal arrangements found during the dry season resembled those associated with dry areas (B1).

In Thailand, *An. dirus* s.l., the forest malaria vector, was shown to comprise at least four species (A2, A45): A, B, C and D, with A common in central and northern Thailand; C found only in Kanchanaburi, to the north-west of Bangkok; B confined to southern Thailand; and D occurring sympatrically with the others in the central and eastern parts of the country. Moreover, heterochromatin analysis revealed intraspecific variations within species A. Similarly, *An. maculatus* consists of four sibling species: A, B, C and D, with A and B common in central, eastern and western Thailand, and C in Kanchanaburi and in Chiang Mai in the north. All four species are found together at Pakchong, 220 km north-east of Bangkok. Moreover, B is polymorphic for paracentric inversions, one of which, form E, probably includes those mosquito populations of the Thai-Malaysian peninsula thought to be malaria vectors. Crossing experiments confirmed that *An. nivipes* is distinct from *An. philippinensis*.

Another important vector in Thailand is *An. minimus* s.l. Cytotoxic analysis and electrophoretic studies revealed the presence at Kanchanaburi of five species and three types of this vector complex, of which type M bites mainly man (outdoors) and type P, mainly buffaloes. The latter is more susceptible to DDT, probably because it has been less exposed to this insecticide.

Mark-release-recapture experiments carried out in one area of Indonesia revealed heterogeneity in the man-versus-cattle feeding habits of *An. aconitus*. Different types could not be distinguished on the basis of morphological characters, and they will now be subjected to cytotoxic analysis.

In Nepal, *An. annularis* was shown to consist of two species, A and B: A is ubiquitous but B is only found in places where malaria transmission continues.

Studies conducted outside the Programme have shown that *An. culicifacies* constitutes a complex of three sibling species: species A, B (B4) and C (B11). Species A and B are found in northern India, B and C in western India, A and B (together) or B and C

(together) in southern India, and all three in central India. Mosquito densities varied between the three species, the pattern of variability differing over the different seasons. Species B has been reported in Nepal and Sri Lanka, and both A and B have been found in Pakistan.

In view of the complexity of this research, an *ad hoc* consultation was organized in Bangkok at the end of 1984 at which entomologists and taxonomists, examining the role that vector species research might play in epidemiology and disease control, identified priority research topics for the different tropical regions: in Africa, research on species complexes is well advanced and future concerns should, the meeting concluded, be focused on translating research results into operational strategies; in other WHO Regions, basic research is still needed. The meeting also highlighted the need for trained personnel for field and research work, reference centres to which material from the field could be sent and increased collaboration among all countries involved.

Studies on the epidemiology and control of malaria

The development of new tools for malaria control, including vaccines, calls for more information on the

disease in a variety of different epidemiological situations. New and more sensitive epidemiological evaluation methods also need to be validated for field use.

- In Burundi, an attempt is being made to analyse the situation in an irrigated area where a number of insecticides have been used agriculturally and where over the past 16 years chloroquine and pyrimethamine have been used for chemoprophylaxis. Malaria is unstable in this area: its degree of endemicity varies from village to village, being highest (50-60% prevalence rate) near the rice fields and hypo- to mesoendemic in drier areas. In the dry season, transmission is higher, due to longer mosquito survival. *P. falciparum* is the predominant species. Age-specific parasite prevalence rates show two peaks, one at 2-4 years, the other at 9-14 years (Fig. 2.13). Antibody titres in the immunofluorescent antibody (IFA) test rise steadily from lower to higher age-groups, reaching a plateau in the 15-20 year age-group, when parasite prevalence becomes stable (A7). Since the serological data indicate continuity of host-parasite contact, the relatively low parasite prevalence rate in the 5- to 8-year age-group may possibly be associated with the chemosuppressive use of antimalarial drugs. Several drugs and drug combinations are being tried in an attempt to overcome

Box 2.10 Malaria vector control

Insecticides

Resistance of *Anopheles* to insecticides or of people to spraying calls for alternative insecticidal approaches. Both problems are experienced in Turkey and are the main reasons for the persistence of malaria transmission in certain areas of the country. Pirimiphos methyl was effective for up to three months when applied on walls but had little effect in ultra-low volume (ULV) space spraying. The former method can now be evaluated in a larger trial in order to determine its effectiveness, cost and acceptability to the population.

Biological control

In semi-arid areas of northern Somalia, it was shown that malaria can be controlled by the introduction of a species of larvivorous fish (*Oreo-*

chromis spilurus spilurus) into mosquito breeding sites (C1). Other species of fish are now being tested in the different ecological conditions found in the south. A preliminary survey, conducted under less arid conditions, revealed two local species of *Nothobranchius*, an annual fish (eggs resist drought between two rainy seasons): *N. microlepis* and *N. cyaneus*. Both can be reared in the laboratory, are effectively larvivorous and are likely candidates for further trials, for which three areas were selected: one for the seeding of adult fish, another for the seeding of fish eggs and a third as a control area. Baseline data are being collected in these areas.

Spraying devices

The established method of spraying residual insecticides is laborious, requiring preparation of houses and

large quantities of insecticides. It also causes particles to rebound from the walls as the substances are applied under pressure. An electrostatic spraying device already used in agriculture is being adapted to vector control and overcomes many of these difficulties. In the field, insecticides applied with this device had a considerable residual effect and produced a high mosquito mortality rate. A prototype sprayer is being prepared for village trials.

Conventional spraying methods require the use of water-dispersable powders, which rapidly erode the sprayer's nozzle tips and cause overdosing. Standard methods for testing the abrasiveness of wettable powders are therefore being elaborated and criteria for assessing the acceptability of abrasiveness in commercial formulations are being developed.

Box 2.11 The future

FIELDMAL programme status and workplan 1985-86

Priority areas	Objectives/needs
The malaria parasite	<p>Global assessment and monitoring of <i>Plasmodium falciparum</i> sensitivity to antimalarial drugs</p> <p>An understanding of the mechanism(s) involved in the development and spread of <i>P. falciparum</i> resistance</p> <p>Studies on the mechanism(s) involved in the spread and the possible containment of resistance, e.g.:</p> <ul style="list-style-type: none">— retrospective and prospective surveys— the effect of withdrawing chloroquine from an area with resistance— the use of primaquine to retard resistance <p>Definition of important biological characteristics of <i>P. vivax</i> and radical treatment of <i>P. vivax</i> infection</p> <p>Studies on <i>P. vivax</i> infection relapse patterns and anti-relapse treatment</p>
The malaria vector	<p>Studies on vector bionomics</p> <p>Methods to overcome insecticide resistance</p>
Use of antimalarial drugs	<p>Trials of new drugs and treatment regimens</p> <p>More studies on treatment regimens in relation to immunity and drug resistance</p> <p>More trials on mefloquine and other new drugs</p>
Epidemiology and control of malaria	<p>An understanding of the epidemiology of malaria in areas not responding well to control measures</p> <p>Epidemiological studies to improve malaria control</p> <p>Trials of integrated control measures</p> <p>Evaluation of methods of malaria prophylaxis and treatment</p> <p>Evaluation of the effect of chemoprophylaxis in pregnancy and of treatment in children and other age-groups on mortality and morbidity in highly endemic areas where drugs are the main weapons for malaria control</p> <p>Baseline data for future vaccine trials</p> <p>Epidemiological field studies and refinement of basic evaluation methods</p> <p>Evaluation of different patterns of delivery of antimalaria services, with special emphasis on primary health care and community participation</p> <p>Trials of new insecticides and methods of application</p> <p>More trials on control of exophilic vectors and ways of overcoming non-acceptance of residual spraying</p> <p>New insecticide application apparatus</p> <p>Trials of biological control methods: larvivorous fish and bacterial agents</p>
Promotion of applied field research in malaria	<p>Promotion and creation of field research facilities</p> <p>Promotion of national malaria field research units, programmes and networks of collaborating institutions</p> <p>Training of personnel in applied field research</p> <p>Organization of workshops and training courses</p> <p>New techniques for field research and for the evaluation of malaria control methods</p> <p>Validation and transfer of new technologies developed by basic research (i.e. field trials, production and training)</p>

P. falciparum chloroquine resistance. Future control will, in all likelihood, rely mainly on environmental management (such as water drainage and clearing of vegetation to facilitate water flow), on treatment of malaria patients and, to a lesser extent, on residual spraying for mosquito control.

- In Burkina Faso, villages near the irrigated rice fields are the least malarious, a situation different from that in Burundi. This can probably be explained by the different vectors involved, i.e. *An. arabiensis* in Burundi and *An. gambiae sensu stricto* (Mopti type) in Burkina Faso.

- In Shandong Province in China, where *An. sinensis* transmits *P. vivax*, epidemiological and entomological studies are being intensified to provide baseline data for a trial on selective insecticide coverage and improved treatment of malaria cases, with the ultimate aim of eliminating transmission.

- Serological studies using the IFA test are being carried out in Thailand to supplement and improve case detection, assess the low levels of residual ende-

micity and increase the reliability of an early warning system for epidemics. Methods have been standardized and practical ways found to collect and transport serum samples from the field to the laboratory.

- In Papua New Guinea, baseline studies have commenced on antibody levels and their correlation with malariometric indices and with responses to chemotherapy, on assays of potential value in assessing protective immunity and on the collection of data for vaccine trials. Preliminary results show a good correlation between ELISA serological indices, slide positivity and splenometric indices in children, but not in adults. Two assays pertinent to protective immunity were studied: serum inhibition of intracellular schizont growth and inhibition of merozoite invasion of red cells. Sera from children were found to be more active in inhibiting schizont growth, whereas those from adults showed more activity in the merozoite test.

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3 Schistosomiasis

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Note on lists of publications

Publications and other documents relating to the subject of this chapter are listed, at the end of the chapter, under three headings: Publications acknowledging TDR support (list A), Publications from work outside the Programme (list B) and TDR scientific reports which became available in 1983-84 (list C). Since most of the work described in the Report is too recent to have appeared in the literature, only a small proportion of publications listed relate directly to the text. List A comprises publications acknowledging TDR support that have not appeared in any previous Programme Report and list B, publications pertinent to specific points discussed in the text. The B list for this chapter includes reviews relating to the work of the Scientific Working Group on Schistosomiasis, and also the bimonthly publication Schisto Update, which is produced by the Edna McConnell Clark Foundation in the United States and lists current publications on schistosomiasis. All the documents in list C can be obtained by writing to: The Office of the Director, TDR, World Health Organization, 1211 Geneva 27, Switzerland; requests should carry the full document title and reference number.

3 Schistosomiasis

The context

The disease

□ Contact with water can be dangerous in tropical countries. Streams, rivers, ponds and lakes, natural or man-made, are the sites of transmission of schistosomiasis—a disease that is still spreading, in association with water and agricultural development projects, in the 74 countries where it is endemic and where over 600 million people are exposed to the risk of infection and an estimated 200 million more are actually infected.

There are four types of schistosome worms infective to man: three, *Schistosoma mansoni*, *S. japonicum* and *S. intercalatum*, cause intestinal schistosomiasis and the fourth, *S. haematobium*, urinary schistosomiasis. WHO's strategy for all four types of infection aims at reduction of morbidity rather than eradication of disease transmission, since: man himself causes and spreads schistosomiasis; children are the most frequently and heavily infected; in children there is a direct association between intensity of infection, as measured by urinary or faecal egg counts, and severity of disease; current approaches to control of the snail intermediate hosts of schistosomes are expensive, require skilled field personnel not usually available locally and demand long-term, repeated interventions.

Control

In contrast to the other diseases within TDR's mandate, a realistic, demonstrably effective control strategy (recently endorsed by a WHO Expert Committee) has been established for schistosomiasis. It is based on: quantitative epidemiological evaluation, chemotherapy, supplemental mollusciciding, follow-up of patients at predetermined intervals, community education and integration of control operations into health care delivery systems. The success of this strategy depends on the resolve of endemic countries to adapt it to local needs and to mobilize the political and administrative resources required for its implementation.

Research

The Programme's Scientific Working Group (SWG) on Schistosomiasis supports research focused on the identification and development of new scientific tools that should make for more efficient, less expensive and more easily achieved disease control.

Outside the Programme, research on several aspects of schistosomiasis is supported by a number of agencies. The Edna McConnell Clark Foundation has played a major role in globally orienting schistosomiasis research, for which it has provided nearly 16 million US dollars over the past ten years. TDR has collaborated closely with the Foundation since 1977. The Foundation's Tropical Disease Research Programme is now initiating support in other areas, and current funding for schistosomiasis is expected to be reduced.

Other agencies supporting schistosomiasis research include: the United States National Institute of Allergy and Infectious Diseases (NIAID), which includes schistosomiasis in its domestic Tropical Medicine Program; the Great Neglected Diseases of Mankind Program of the Rockefeller Foundation; the European Economic Community, which established a programme on tropical disease research in 1983 and funds 58 projects, five of which relate to schistosomiasis.

In planning its activities, TDR takes into account research needs not covered by work conducted outside the Programme. More specifically, it recognizes a need for applied field research on new drugs, diagnostic tests, snail control agents, drug delivery methods, disease control in agricultural development projects, disease control operations and their applicability in different epidemiological conditions, and the distribution of potential intermediate snail hosts in the environment and its relation to human infection.

There is also special interest in the immunology of schistosomiasis, including schistosome antigens, immunological aspects of the schistosome surface

membrane, the development of immunodiagnostic tests suitable for control programmes, and the potential for vaccination.

Other research topics currently within the focus of the Programme include: identification of schistosome strains and species, development of new drugs

based on parasite biochemistry, the schistosome surface membrane, parasite neurophysiology, mechanisms of drug resistance, pharmacokinetics and drug metabolism, snail metabolism and schistosome embryogenesis. □

Box 3.1 Highlights of activities in 1983-84

- A new syringe urine filtration technique has been tested in Zanzibar and found effective, and is now being evaluated in Cameroon, the Congo, Malawi, Mozambique and the Niger.
- Biochemical studies on schistosome purine and pyrimidine metabolism are providing promising pointers to new antischistosomal drugs.
- Monoclonal antibodies are being used successfully to identify schistosome antigens that might form the basis of protective vaccines.
- Applied field research projects have been conducted in Burundi and Zanzibar with a view to assessing control strategies in different locations and have demonstrated the feasibility of large-scale treatment programmes using available diagnostic techniques and antischistosomal drugs.

Report of activities in 1983-84

Applied field research

Much field research is conducted in collaboration with ministries of health but is hampered by a lack of well-trained research workers. Moreover, national health plans often give low priority to schistosomiasis control and make no provision for field research on the disease.

Two TDR-supported projects, however, were used as a basis for national control activities. In Burundi (A78), a large-scale field programme, conducted under Ministry of Health auspices for the purpose of testing disease control strategies in different localities, has demonstrated the feasibility of using quantitative parasitological diagnostic techniques and of treating intestinal (*S. mansoni*) schistosomiasis with praziquantel and oxamniquine. This programme is now the basis for a national control programme which extends throughout the Ruzizi Valley.

In Zanzibar, the effectiveness of a primary health care approach (Fig. 3.1) in reducing the overall prevalence of urinary schistosomiasis and that of heavy infection was confirmed (Tables 3.1 and 3.2). Nearly all children in the study area with egg counts greater than 50 per 10 ml of urine had blood in their urine, detectable by chemical reagent strips. If reagent strips were available at low cost, they could be used on a large scale to identify heavily infected children.

Epidemiology and snail control

Three different approaches have been studied for the control of schistosomiasis due to agricultural irrigation.

- In the Blue Nile Health Project in the Sudan, which is partly supported by WHO, it was confirmed that molluscicides can be effective when restricted to small well-defined areas where human activity in water is high. Molluscicides had previously been used over large areas, and this new approach, which offers a less expensive alternative, is now being evaluated on a larger scale.

- A large rice irrigation project in the Niger used to be an important focus of urinary schistosomiasis. "Selective population chemotherapy" (treatment of all infected members of a given population) with praziquantel was undertaken by the Meningitis and Schistosomiasis Research Centre (Centre de Recherches sur les Méningites et les Schistosomiasis) in Niamey; the infection prevalence rate fell in one year by about 60% and the average egg count of those not cured, by 95%.

- A comparative evaluation is being made, within an ongoing national control programme in Morocco, of techniques for the diagnosis of *S. haematobium* infection to be used by itinerant health workers in remote areas. By means of a plastic syringe, urine is passed through filters of polycarbonate

(Nuclepore[®]), nylon (Nytrel[®]) or filter paper, which are then transported by bicycle to central laboratories—an approach that should facilitate schistosomiasis control in areas where access by motor vehicles is difficult or impossible.

Chemical molluscicides have been used for more than 60 years to control the intermediate snail hosts of schistosomes and in some endemic areas they have been applied repeatedly for more than 20 years. Resistance to molluscicides has not yet been documented. In a laboratory study, *Biomphalaria glabrata*, the major intermediate host of *S. mansoni* in the Western Hemisphere, was repeatedly exposed to molluscicides: the snails became tolerant to higher doses but not resistant. Furthermore, this species can avoid molluscicides by burrowing in mud

up to a depth of one metre. It has also been found that not all *Biomphalaria glabrata* snails in a given snail population are susceptible to *S. mansoni* infection. Snails resistant to infection have been shown to have a characteristic enzyme marker, which could serve to determine whether snails remaining after initial mollusciciding are susceptible to infection.

Chemical molluscicides are generally effective, but only one agent is currently recommended by WHO, and it is too costly to be used in most endemic countries. New molluscicides are needed, as are ways of improving the use of existing molluscicides. Research on plants with molluscicidal properties was reviewed at a Scientific Working Group meeting in 1983 (C2), which concluded that, although at first sight apparently inexpensive, effective and immediately

FIG. 3.1

SCHISTOSOMIASIS FIELD PROJECT IN ZANZIBAR



PHOTO. Courtesy WHO/K. Mott

Children taking part in a TDR-supported field project in Zanzibar, which involved a survey of the total study population and treatment of infected individuals. Drug therapy administered through a primary health care approach led within two years to a reduction of over 50% in the overall prevalence of urinary schistosomiasis.

TABLE 3.1

Prevalence of *S. haematobium* infection in total Kinyasini (Zanzibar) population before and after metrifonate treatment

Prevalence rate (%):	Age-group (years)						Total N = 2679*
	0-4 N = 365	5-9 N = 671	10-14 N = 529	15-24 N = 382	25-44 N = 403	45 + N = 329	
Initial	19.2	62.0	69.9	51.8	36.2	36.8	49.3
At 24 months	9.6	31.4	36.9	19.0	6.0	10.5	23.2
% reduction	50.0	49.4	47.2	63.3	83.4	71.5	52.9

* Numbers of individuals are those at the first survey. About 35% fewer were seen at the 24-month follow-up.

TABLE 3.2

Prevalence of heavy *S. haematobium* infection (>50 eggs/10 ml urine) in total Kinyasini (Zanzibar) population before and after metrifonate treatment

Prevalence rate (%):	Age-group (years)						Total N = 517*
	0-4 N = 15	5-9 N = 203	10-14 N = 175	15-24 N = 67	25-44 N = 30	45 + N = 27	
Initial	4.1	30.3	33.1	17.5	7.4	8.2	19.3
At 24 months	3.4	12.8	11.9	3.5	0.9	1.1	7.3
% reduction	17.1	57.8	64.0	80.0	87.8	86.6	62.2

* Numbers of individuals are those at the first survey. About 35% fewer were seen at the 24-month follow-up.

applicable, these plants should be studied further—as thoroughly as chemical molluscicides—before being recommended for widespread use. The first research priorities should be to define the chemical structure and structure-activity relationships of the active compounds and their modes of action and toxic potential.

Plants with promising molluscicidal activity include *Phytolacca dodecandra*, *Ambrosia maritima*, *Anacardium occidentale* and *Swartzia madagascarenensis*. However, even for the most thoroughly studied of these, *Phytolacca dodecandra* (endod), not enough is known about the chemical composition of the active substance, within species variation, methods of preparation and toxicological properties. New saponin compounds derived from *Phytolacca dodecandra* have now been characterized and some are proving molluscicidal.

Biochemistry and chemotherapy

Safe, effective oral drugs have become available in recent years for the treatment of *S. mansoni*, *S. haematobium* and *S. japonicum* infections, and drugs such as oxamniquine and metrifonate have been used in large-scale control programmes. Future control of schistosomiasis will rely largely on popu-

lation-based chemotherapy and repeated drug administration to infected individuals. Differences in drug susceptibilities have been observed in different populations, and drug resistance has been reported, although on too small a scale to affect chemotherapy in large population groups.

Against this background, a future need for new drugs can be anticipated. They could be based on existing drugs or on distinctive features of parasite biochemistry. The schistosome membrane is a particular focus of attention, since it so successfully protects the parasite from damage by the host's immune system that worms may survive in man for as long as 40 years.

The surface membrane of the schistosome in its vertebrate host is made up of a double lipid bilayer. Comparison with other life-cycle stages and with other closely related parasites indicates that this structure may be an adaptation to intravascular parasitism. There is evidence that praziquantel changes the distribution of host antigens in the schistosome membrane, which may explain why it increases expression of parasite antigens.

Differences have been found in phospholipid turnover between the outer membrane bilayer, with a half-time of 1.5 hours, and the inner bilayer, with a biphasic turnover pattern comprising a fast

12-minute and a slow 17-hour half-time.

Schistosomes have been found to incorporate neutral lipids (cholesterol and triglycerides) through a carrier present in serum. Uptake correlates with parasite resistance to immune damage by human serum, which induces the expression on the schistosomula surface of a protein binding to serum low-density lipoproteins (A186).

The parasite tegument has been found to play an important role in regulating ion concentrations within the organism, particularly Ca^{2+} movement and its availability to subtegumental cells. Praziquantel produces a nonspecific increase in permeability to both Ca^{2+} and Mg^{2+} .

Metabolic pathways

Cathepsin B and dipeptidyl peptidases have been localized in lysosome-like organelles of the parasite, and synthetic low-molecular-weight oligopeptides appear to be good substrates for the characterization of dipeptidyl peptidases I and II.

No evidence can be found of *de novo* purine synthesis in schistosomes, although several key enzymes of the metabolic pathway involved appear to be present. In contrast, the parasite was found to be capable of *de novo* pyrimidine synthesis, all six enzymes required for uridine monophosphate synthesis being demonstrable in *S. mansoni* (A68).

In another study, the purine base of the drug tubercidin, which has antischistosomal properties, was found to be incorporated into schistosome nucleic acids. The compound nitrobenzylthioinosine-5-monophosphate (NBMRP-P) inhibits tubercidin transport in mammalian systems but not in *S. mansoni* (A67), and could thus selectively block the otherwise lethal effect of tubercidin on the host without diminishing the compound's antischistosomal effects. The combination of the two compounds has been found in mice to be selectively toxic to both *S. mansoni* and *S. japonicum*.

Neuropharmacology

The nervous system of the schistosome is poorly understood. Some antischistosomal drugs seem to paralyse the worm, but their modes of action and effects on the worm's nervous system are not known. The somatic musculature of the male schistosome is composed for the most part of longitudinal and circular muscles. The longitudinal musculature appears to possess cholinergic receptors, whereas the circular musculature has dopaminergic but not cholinergic receptors. Receptors for serotonin (5-hydroxytryptamine) are present in both sets of muscles.

Drug resistance

Resistance has been induced to the drugs oxamniquine and hycanthon in schistosomes maintained in laboratory animals. Successive generations of the parasite were exposed to "subcurative" therapy, but it has not been possible in this way to induce parasite resistance to praziquantel. Crossresistance to oxamniquine has been demonstrated in parasites previously exposed to hycanthon. Drug resistance is currently not a problem in schistosomiasis, but if the few drugs available are used on a large scale, resistance could develop.

Pharmacokinetics and drug metabolism

The mode of action of the currently available antischistosomal drugs metrifonate, oxamniquine and praziquantel, is not understood. An older drug, niridazole, has been used as a prototype for the study of antischistosomal nitro compounds but since it must be administered in multiple oral doses and is associated with side-effects, it is less than ideal for large-scale chemotherapy. Niridazole's side-effects in the mammalian host and its toxicity to schistosomes have been shown to depend upon its metabolism, but its metabolic fate is fundamentally different in host and parasite (A215). There is evidence that reductive metabolic activation of the drug within the parasite is required for its antischistosomal activity. In contrast, in mammalian liver, niridazole is oxidatively metabolized by the cytochrome P_{450} mixed-function oxidase system, and oxidative metabolism is believed to lead to the formation of a reactive epoxide intermediate possibly related to the drug's putative carcinogenicity. In support of this hypothesis, the oxidative microsomal metabolism of ^{14}C -niridazole also results in macromolecular covalent drug-binding, which does not occur in the presence of carbon monoxide, a specific cytochrome P_{450} inhibitor. Precursor/product studies with ^{14}C -labelled oxidative niridazole metabolites are also consistent with the formation of the putative epoxide. These results suggest that it may be possible to synthesize new niridazole derivatives which retain full antischistosomal activity but are less toxic to the host.

The pharmacokinetics of known niridazole metabolites have been studied in man (A218). Concentrations of niridazole and six of its metabolites have been measured by high-performance liquid chromatography in the sera of four male Filipino patients with mild *S. japonicum* infection given single oral doses of niridazole (15 mg/kg) on two occasions 10 days apart. Of the five oxidative metabolites measured, 4-hydroxyniridazole and 4-ketoniridazole

were the most abundant. Within an hour, niridazole and three other oxidative metabolites, 4,5-dihydroxyniridazole, 5-hydroxyniridazole, and 4,5-dehydro-niridazole, appeared at low levels in the serum, but none of the compounds was detected in 24-hour serum samples. The pharmacokinetics of niridazole and its oxidative metabolites showed marked inter-individual variability but remained constant in the same individual studied after a 10-day interval. 1-Thiocarbonyl-2-imidazolidinone persisted in the serum after a single oral dose of niridazole, a finding consistent with the postulated role of this metabolite in mediating niridazole's prolonged immunosuppressive side-effects.

Immunology and basic sciences

Knowing whether immunity to schistosomiasis infection exists in man, and, if so, whether it is inherent or acquired would enable control programmes, including those based on chemotherapy, to be designed more rationally and would indicate whether protection by vaccination is feasible. More information is required on protective immunity in man and its possible relationship to chemotherapy, on the nature and regulation of the immune response to schistosomes, including identification and isolation of schistosome antigens, and on immunological characteristics of the surface membrane. In addition, immunodiagnostic tests are needed that are cheap, specific and sensitive enough to be used in control programmes.

Immunodiagnostic tests

A collaborative study on antigens of serodiagnostic potential (A154) had previously identified several parasite-derived antigen preparations with high sensitivity and specificity for use in diagnostic tests based on detection of serum antibodies to *S. mansoni*. Subsequently, a collaborative study of *S. japonicum* antigens was begun, involving six laboratory centres in Australia, Japan, Malaysia, the Philippines and the United States.

In addition, test conditions and standard reagents have been studied with a view to improving the performance of an enzyme-linked immunosorbent assay (ELISA). One study, conducted in the West Indies, showed that the ELISA could be used with only minimal laboratory facilities but that it was inadequate as a primary screen for people in the lowest egg-count range and no more sensitive than parasitological methods. This finding has since been confirmed in tests using crude and fractionated egg antigen (CEF-6), which was more efficient in

denoting conversion to seronegativity after treatment of *S. mansoni* infection but, overall, was no better than crude egg antigen and is relatively costly to produce.

Simple techniques, such as thin-layer immunoassay (TIA), diffusion-in-gel TIA and diffusion-in-gel ELISA, have proved impractical for field—as opposed to research—application. Varying the degree of antigen crosslinking increases ELISA sensitivity somewhat, but test specificity is diminished. Cross-linking would have to be optimized for each antigen preparation, making the method impracticable for general use (A184).

The Western blotting technique has been used to define immunoglobulin class-specific antibody responses to different antigens. Individual human subjects and experimental animals were found to vary in their responses to infection, and no clear correlations with stage or intensity of infection have been found. One interesting by-product of this research has been the characterization, by lectin affinity, of a new *S. mansoni* immunogenic glycoprotein antigen of 34 000 MW.

The immune response in man

Studies are being undertaken in Minas Gerais, Brazil, and Machakos, Kenya—areas endemic for *S. mansoni*—to identify humoral and cellular immune responses relating to immunopathology and acquired immunity, and to explore the regulatory mechanisms governing the expression of these responses at different stages of infection and after chemotherapy. Populations were first identified and surveyed for prevalence and intensity of infection.

In Kenya, reinfection was studied in children cured by chemotherapy of established infection. No differences were found in degree of water contact between those who became reinfected and those remaining free of infection. This finding strongly supports the hypothesis that immunity to schistosome infection occurs in man. Attempts to correlate resistance to reinfection with different types of humoral immune responses, and in particular with the presence of antibodies responsible for eosinophil-dependent killing of schistosomulae, have so far been unsuccessful. A significant positive correlation was, however, found between resistance and age. Recently, monocytes were shown to produce a factor which enhances schistosomulae killing by human eosinophils. The possibility is being investigated that this factor may be operative in the group of immune individuals.

Studies conducted in Brazil confirmed a previous finding that lymphocytes from patients with early *S.*

Box 3.2 Immune regulation and vaccination

It is now clear that immune schistosome-host interactions form an extraordinarily complex system. A large number of potential host effector mechanisms have been identified, mainly by *in vitro* studies, and an equally large number of parasite evasion and defence mechanisms have been disclosed. Host-parasite interplay results most often in chronic, long-lasting infection, with generally slow and variable development of resistance. Research has not yet defined a vaccine likely to prevent disease in man, but a safe, potent vaccine would be of such great benefit that this objective should be pursued.

Immune regulation is the subject of several studies using experimental models:

- The immune response of guinea-pigs to irradiated *S. mansoni* cercariae is being investigated to determine

whether guinea-pigs provide a more realistic model for human immunity than mice.

- The immunity induced in rats by vaccination with glutaraldehyde-fixed *S. mansoni* worm particles followed by a low-dose cercarial boost is being assessed, and there are plans to test liposome-incorporated antigens.

- Antibodies and eosinophils from *S. mansoni*-infected rats were found to kill schistosomulae *in vitro* but there was no evidence of *in vivo* killing.

- In mice vaccinated with a high dose of irradiated *S. mansoni* cercariae, cell-mediated immunity seemed to play an important role in resistance to subsequent infection.

- In one study on the genetics of resistance, P-strain mice with deficient macrophage function and IgM antibody responses developed little resistance to infection, but the macrophage

and IgM defects were not genetically linked to the low resistance. In a second study, innate resistance to *S. japonicum* infection was seen in young 129/J mice and appeared to be recessive (A74).

- Vaccination of cattle infected with *S. bovis*-irradiated cercariae has been found to reduce egg production and induce a degree of protection against disease (A13). The antigens responsible for lowered egg production in vaccinated cattle are being studied.

- Resistance acquired by *S. mansoni*-infected mice has been found to diminish rapidly after treatment with several antischistosomal drugs: praziquantel causes a less marked reduction than other drugs but is a less effective antischistosomal drug in immunodeficient mice.

mansoni infection are hyper-reactive to worm and egg antigens in proliferation assays, whereas lymphocytes from some patients with later hepatosplenic-stage infection may not show such hyper-reactivity (A75). Hepatosplenic patients showed abnormal helper-to-suppressor lymphocyte ratios. Such differences in host reactivity may reflect predisposition to infection and disease. Patients infected with *S. mansoni* were shown to be defective in a lymphokine mitogenic factor. In a related study, schistosome infection did not affect the numbers of natural killer cells in peripheral blood. Moreover, the existence of histamine-triggered suppressor cells in patients with *S. mansoni* infection was confirmed.

In both the Brazilian and Kenyan projects, with well-documented patient populations and excellent immunological laboratory facilities, longitudinal studies on immunity and immune regulation are being pursued in collaboration with European and American immunologists, and with several young Brazilian and Kenyan investigators in training.

Membranes and membrane-associated antigens

Antigens on the surface of schistosome blood stream stages are of special interest for vaccine development. The Western blotting technique has

been used to identify surface membrane components reactive with sera from infected or immunized animals, in particular an 18 000 MW protein unique to the surface of the schistosomula, not present in later stages and a potential target for immune attack. Other antigens, including components in the 30 000 - 40 000 MW range, react with antibodies present in infected mouse, monkey and human sera (A55). An IgG_{2a} monoclonal antibody has been produced that reacts with a 38 000 MW parasite component which may also be present in sera of *S. mansoni*- and *S. haematobium*-infected patients.

Embryogenesis

Structure-function relationships have been studied at various stages of *S. haematobium* embryogenesis. Electron microscopy has shown different patterns of organization of the membrane foldings in miracidia, the earliest parasite form, with a "rosette" pattern observed in *S. haematobium* and *S. intercalatum*, and with a "honeycomb" pattern in *S. mansoni* and *S. japonicum*. A higher level of glycogen has been found in *S. japonicum* larvae than in those of *S. haematobium* and *S. mansoni*, possibly because of the longer life-span of *S. japonicum* larvae.



PHOTO. Courtesy of P. Sobhon and Cambridge University Press (A197)

(A): Male *Schistosoma mekongi* indicating the division of the body into anterior, middle and posterior portions. $\times 43$.
 (B): A low-power micrograph of the ventral sucker exhibiting large sharp spines (sp) which are arranged in rows. $\times 2440$.
 (C): A high-power micrograph of the spines (sp) in the ventral sucker. $\times 9930$.
 (D): The dorsal and lateral aspects of the tegument of the anterior part of the body exhibiting round papillae (pa), sensory papillae or bulbs (ps) and perforated ridges (ri). $\times 2510$.
 (E): The ventral tegument of the anterior part showing papillae with crater in the centre (pc), papillae with cilia (pc'), ridges (ri) and a sensory pit (spi). $\times 3000$.
 (Bar: (A) = 500 microns; (B-E) = 2 microns)

Identification of strains and species

S. mekongi, the most recently identified *Schistosoma* species infecting man, has been extensively studied (A197) by scanning electron microscopy (SEM) (Fig. 3.2).

Differences between various other schistosome species have been seen on SEM. Large fungiform papillae (3-4 μ in diameter), mostly without cilia, are more numerous in Indonesian and Malaysian strains than in the other *S. japonicum* strains. Cratered papillae are numerous in the Malaysian strain but absent in the Chinese strain. The Chinese, Indonesian and Philippine strains possess spines on the posterior half of the body surface, whereas in the Malaysian schistosome, spines are observed only in the suckers and the gynecophoral canals.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis of somatic proteins has shown almost identical patterns for adult Chinese and Philippine strains of *S. japonicum*, but both differed from *S. mekongi* and a Malaysian strain of *S. japonicum* for proteins in the 17 000 - 45 000 MW and 94 000 MW regions. The Malaysian strain may be a new species.

Parasite heterogeneity

Evidence has now been obtained that, as has long been suspected by field investigators, *S. mansoni* may not be a uniform species, even within a defined geographical region. Parasites from six different localities in Kenya showed differences in morphology (number of testes), biology (egg-laying behaviour and capacity) and biochemistry (isoenzyme patterns). Such variation may be important in immunological and chemotherapeutic studies.

Mollusc metabolism

Target-specific molluscicides are required for effective control. Research is in progress on respiratory pathways, including the succinate-fumarate pathway, in several snail species; no leads to new, more specific molluscicides have yet been obtained (A166).

Clinical trial of praziquantel

A Phase III clinical trial of praziquantel was conducted by the Tropical Diseases Research Centre in Ndola, Zambia, in 551 patients with *S. mansoni* infection. The drug was given in two daily 20 mg/kg doses or a once daily 40 mg/kg dose. One year after treatment, 467 patients were examined and 153 (32.8%) found to be positive, with little difference in effectiveness between the two regimens.

Box 3.3 The future

- To identify and resolve more easily problems of control operations, close collaboration will be fostered between research and operational programmes. In particular, applied field research will be linked to national control programmes to promote the adaptation of new techniques and operational approaches to field use.
- More research on the basic metabolism of the snail will be encouraged as a basis for the development of new molluscicides.
- The parasite's biochemistry and metabolism will be explored as a basis for the development of new anti-schistosomal drugs and a greater understanding of the modes of action of current drugs.
- The development will be encouraged of new, sensitive, inexpensive diagnostic techniques appropriate for use in developing countries.
- The increasing use of large-scale chemotherapy to reduce morbidity has created unique opportunities to study the effect of drugs on the human immune response. This situation opens new avenues for applied immunological research in endemic countries.

PUBLICATIONS

Schistosomiasis

Publications acknowledging TDR support

- A1. ANDERSON, R.M. & CROMBI, J. Experimental studies of age prevalence curves for *Schistosoma mansoni* infections in populations of *Biomphalaria glabrata*. *Parasitology*, 89: 79-104 (1984).
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- C1. BERQUIST, N.R. Monoclonal antibodies in schistosomiasis research. Document TDR/SCH/BERG/84.3. (English only)
- C2. TDR. Report of the Scientific Working Group on plant molluscicides, Geneva, 31 January—2 February 1983. Document TDR/SCH-SWG(4)/83.3. (English only)
- C3. TDR. Report of the Scientific Working Group on the Biochemistry and Chemotherapy of Schistosomiasis, Geneva, 30 January—1 February 1984. Document TDR/SCH-SWG(5)/84.1. (English only)

Publications from work outside the Programme

- B1. ANDREWS, P., THOMAS, H., POHLKE, R. & SEUBERT, J. Praziquantel. *Medicinal Research Reviews*, 3: 147-200 (1983).
- B2. EDNA MCCONNELL CLARK FOUNDATION. *Schisto Update*. (A quarterly review of the scientific literature on schistosomiasis) New York: Edna McConnell Clark Foundation.
- B3. JORDAN, P. & WEBBE, G. *Schistosomiasis: Epidemiology, Treatment and Control*. London: Heinemann, 1982.
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4 Filariasis

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Note on lists of publications

Publications and other documents relating to the subject of this chapter are listed, at the end of the chapter, under three headings: Publications acknowledging TDR support (list A), Publications from work outside the Programme (list B) and TDR and related WHO scientific reports which became available in 1983-84 (list C). Since most of the work described in the Report is too recent to have appeared in the literature, only a small proportion of publications listed relate directly to the text. List A comprises publications acknowledging TDR support that have not appeared in any previous Programme Report and list B, only those publications pertinent to specific points discussed in the text. Much of the scientific progress made in filariasis during the period under review is summarized in four documents included in the B list (B2-5) and in three documents, including two Onchocerciasis Control Programme reports, referred to in the C list (C1-2, C5). All the documents in list C can be obtained by writing to: The Office of the Director, TDR, World Health Organization, 1211 Geneva 27, Switzerland; requests should carry the full document title and reference number.

4 Filariasis

The context

□ Filariasis comprises several diseases. Most are caused by filarial worms and transmitted by bloodsucking flies. One, dracunculiasis, is a closely related metazoan disease transmitted by water fleas.

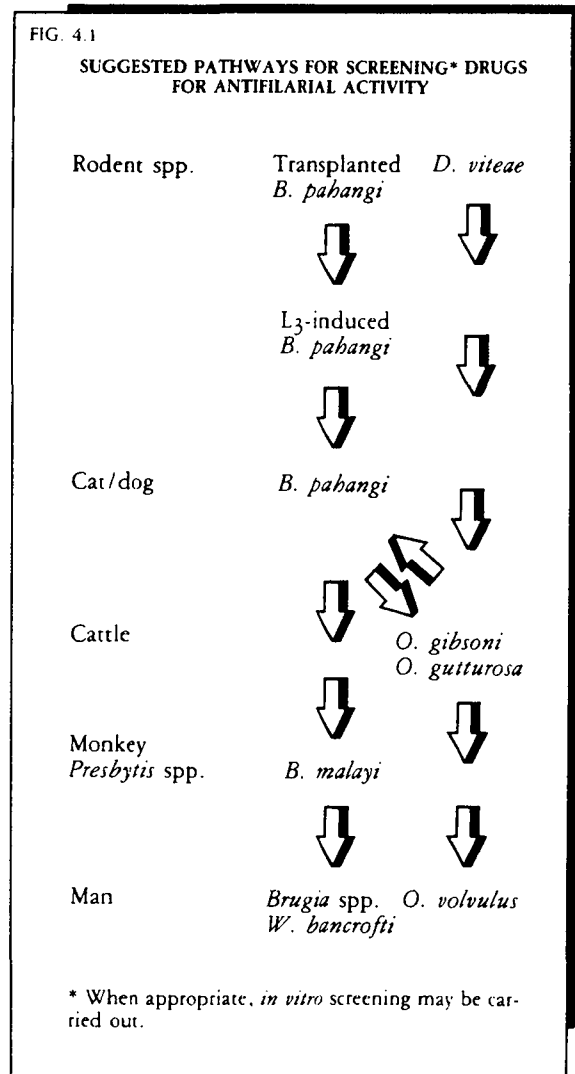
Onchocerciasis, or “river blindness,” which is transmitted by blackflies of the genus *Simulium*, is probably the most serious of the filarial diseases. It affects about 40 million people, mainly in tropical Africa, but also in Central and South America, and foci in the Eastern Mediterranean Region extend to Yemen and the Sudan. The manifestations of onchocerciasis—mainly intense itching and ultimately, in many cases, blindness—are due to the millions of *Onchocerca volvulus* microfilariae scattered throughout the body, especially in the skin and eyes. The adult worms lodge in nodules in subcutaneous and even deeper tissues in various parts of the body.

Treatment of onchocerciasis is still unsatisfactory. There are no safe, effective drugs available for large-scale therapy. Diethylcarbamazine (DEC), which has been in use for the last 35 years, eliminates microfilariae but usually causes an intense reaction (the Mazzotti reaction), consisting of pruritus, rash, lymph-node enlargement, fever, hypotension and occasionally eye damage, and has to be administered under ophthalmological supervision. Suramin, for a long time the only drug known to kill adult worms, has been in use for about 40 years but is toxic, particularly to the kidneys.

Lymphatic filarial diseases, which affect about 90 million people in Asia, Africa and South America, in addition to an estimated 905 million directly exposed to the risk of infection, are responsible for considerable disability and disfigurement, due to acute adenolymphangitis and chronic lesions like elephantiasis and hydrocoele. The parasites that cause human lymphatic filariasis are *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*. Adult worms lodge in lymphatic vessels; the microfilariae circulate in the blood, often in a nocturnally periodic pattern, and are transmitted by various genera of mosquitoes.

Unlike onchocerciasis, lymphatic filariasis can be

relatively safely and effectively treated with DEC, which, although mainly microfilaricidal, may, in large enough doses, be also macrofilaricidal. In lymphatic filariasis, DEC is also not without side-effects, which are related to the rapid destruction of large



numbers of microfilariae in patients with high levels of parasitaemia. On the whole, though, it has been successfully used not only to treat individual patients but also in large-scale chemotherapy programmes.

Overall, the major problem for the control and treatment of filarial infections has been the lack of a completely safe, effective compound that could be used, preferably in a single dose, both for onchocerciasis and other filarial diseases.

In the search for a suitable new drug, the Programme has adopted several approaches: the more or less random selection of compounds from pharmaceutical company stocks and the testing of these compounds in animal filarial screens; lead-directed synthesis of new compounds involving continuous feedback from animal screens; research to identify biochemical characteristics that are unique to the parasite and have potential as chemotherapy targets; pharmacokinetic and toxicological studies on compounds that have shown promise in these early tests; clinical trials of new drugs, as well as of others already in use for other than antifilarial purposes.

To implement these steps, the Programme has set

up drug screening centres in several parts of the world and has established drug screening pathways (Fig. 4.1). Research has also been funded on lead-directed synthesis of new compounds, pharmacokinetic studies have been undertaken on available drugs and clinical trial centres have been set up to test not only new compounds but also better ways of using suramin and DEC.

In addition to drugs, major needs still evident at the beginning of the present reporting period (1983-84) included: immunodiagnostic tests for both onchocerciasis and lymphatic filariasis; information on the biology, bionomics, biting behaviour and transmission potential of filarial worm vectors in different localities; simple tests to identify the origin (human or animal) of filarial parasites in vector flies; methods of distinguishing between savanna and forest strains of the human onchocerciasis parasite; and a better knowledge of the epidemiology of filarial infections in order to identify individuals and population groups at risk of infection and those among them at risk of developing the more serious disease manifestations. □

Box 4.1 Highlights of activities in 1983-84

- Several thousand compounds have now been put through animal screens and a number of them have shown promising activity. Two are almost ready for testing in man.
- Ivermectin, derived from a naturally occurring antibiotic-like substance, has been shown, in Phase II trials, to be capable of clearing patients of *Onchocerca volvulus* microfilariae without causing serious side-effects.
- Advances have been made in attempts to develop a specific, sensitive immunodiagnostic test for filarial infection. Filarial antigens have been detected in body fluids of most microfilaria-positive patients, as well as in a proportion of infected patients without evidence of microfilariae, suggesting the possibility of diagnosing infection serologically at a prepatent or occult stage of infection.

Report of activities in 1983-84

Chemotherapy

Collaboration with the Onchocerciasis Chemotherapy Project

In view of the urgent need to complement successful larvicidal control with effective chemotherapy, in 1982 the Onchocerciasis Control Programme (OCP) in the Volta River Basin area in West Africa established an "Onchocerciasis Chemotherapy Project" (OCT) with two main objectives: to obtain drugs which would kill or permanently sterilize adult *Onchocerca volvulus* worms without causing severe reactions in patients and to identify microfilaricides

which do not produce severe Mazzotti reactions. The OCT has supported mainly clinical trials and basic research on developing leads to novel compounds. Special attention has been given to ivermectin, as described below. The OCT works in close collaboration with the Scientific Working Group (SWG) on Filariasis. The following account refers to progress made under both groups, whose activities complement each other.

Screening, synthesis and clinical trials

Several thousand compounds have now been put through primary, secondary and tertiary screens (a

FIG. 4.2

SWG STUDIES ON FILARICIDAL COMPOUNDS 1980-84

Compound	Indication		Experimental trials	Studies on human pharmacology and pharmacokinetics	Dose-finding trials	Phase III/IV comparative and field trials
	O = Onchocerciasis	Ly = Lymphatic filariasis				
Suramin	O			Pharmacokinetics		
Diethylcarbamazine citrate (DEC)	O	Ly		Pharmacokinetics Pharmacokinetics	Tolerance	Field studies, Prophylactic use
DEC N oxide	O					
Metrifonate	O				Stopped ^a	
Mebendazole	O, Ly			Poor bioavailability	Stopped ^b	
Levamisole/mebendazole combinations	O, Ly				Stopped ^b	
Mebendazole citrate (better bioavailability than mebendazole)	O					
Flubendazole	oral O				Stopped ^b	
	i.m. O		With new galenical formulation		Stopped ^c	
Ivermectin	O					
	Ly					Also for other indications, including loiasis
Amoscanate	O		Stopped ^d	Stopped ^d		
Ciba-Geigy 6140	O			Phase I/IIA trials	Phase II trials	
	Ly					
Ciba-Geigy 20376	O, Ly			Phase I trials		
Ciba-Geigy 24914	O, Ly		Stopped ^d			
RO5-9963	O				Stopped ^e	
Furaprimidone	O, Ly					

Completed In progress Planned Foreseen

^a Mazzotti reaction

^b Poor bioavailability and other factors

^c Poor local tolerance

^d Toxicity

^e Not effective

FIG. 4.3

THERAPEUTIC TRIALS IN FILARIASIS

Compound	Indication	Year								
		1980	1981	1982	1983	1984	1985	1986	1987	
	O = Onchocerciasis									
	Ly = Lymphatic filariasis									
Suramin	O	Optimal dosage/mode of action/pharmacokinetics								
Diethylcarbamazine citrate (DEC)	O	Phase IV trials (standard for comparative trials)								
	Ly	Phase IV field trials: control and prophylaxis								
Metrifonate	O	Phase IV trials								
Amodiaquine	O	Phase IV trials								
Furazolidone	O	Phase IV trials								
Nitrofurantoin	O	Phase IV trials								
Mebendazole (± levamisole)	O	Phase O/I and II trials								
	Ly	Phase O/I and II trials								
Flubendazole	O	Phase I/II trials New i.m. formulation								
Ivermectin	O	Phase I/II trials Phase II/III and field trials								
	Ly	Phase II/III trials								
Amoscanate	O	Phase O/I trials								
Ciba-Geigy 6140	O	Toxicology Phase I/II trials								
Ciba-Geigy 20376	Ly	Toxicology Phase I trials								
Ciba-Geigy 24914	O	Toxicology								
	Ly	Toxicology								
RO5-9963	O	Phase I/II trials								
Furapyrimidone	Ly	Outside TDR ?								

- Trials which have been carried out or are in progress
- Trials which are planned or foreseen

progressive series of tests which use a variety of *in vitro* and *in vivo* models to select compounds effective enough to be tested in man) (Figs. 4.2 and 4.3). They include:

- *two Ciba-Geigy compounds*, CGP 6140 and CGP 20376, whose activity has been confirmed in tertiary screens for onchocerciasis (*O. gibsoni*, in cattle) and lymphatic filariasis (*Brugia malayi*, in leaf monkeys).

Phase I and IIA trials on CGP 6140 in the treatment of onchocerciasis will begin at the Onchocerciasis Chemotherapy Research Centre (OCRC) in Tamale, Ghana, in February 1985. Phase I trials on CGP 20376 in lymphatic filariasis are likely to begin in 1985 at the Tuberculosis Research Centre in Madras, India, which is now a clinical trial centre for lymphatic filariasis.

• *ivermectin*, a macrocyclic lactone antibiotic derived from *Streptomyces avermitilis*. Developed by Merck, Sharp and Dohme of New Jersey, USA, ivermectin has been tested, with Programme support, in primary, secondary and tertiary screens, and open and double-blind Phase II trials have been conducted on man (A8, B1). Given orally in single doses of 50-200 µg/kg, ivermectin has been shown to clear patients of *O. volvulus* microfilariae without precipitating a Mazzotti reaction or eye lesions. Early findings of double-blind studies carried out at the OCRC in Tamale and in three other centres funded by the OCT or by Merck, Sharp and Dohme, suggest that the optimal dose is 150-200 µg/kg and that skin-snip microfilaria counts remain low for 6 to 12 months after treatment. A study of adult worms obtained from nodules of treated patients suggests that microfilariae are prevented, by a poorly understood mechanism, from leaving the female worm's uterus, where they tend to degenerate and die. Ivermectin has been shown, in animal screens (including a cattle screen), to have a prophylactic effect, a finding now being explored in an OCT-funded project involving chimpanzees exposed to *O. volvulus*. The drug may be available some time in 1985 for Phase II therapeutic trials in bancroftian and brugian filariasis that are to be conducted in India, Indonesia and Sri Lanka.

◦ *two benzimidazole carbamates*, mebendazole and flubendazole, already available for use in man, and tested in onchocerciasis patients in Ghana, Mexico (A40-41, A140) and Nigeria. The embryostatic effects of these drugs have been confirmed in the *O. gibsoni* cattle screen (B3, C5). Mebendazole was found also to be microfilaricidal, whereas weekly intramuscular injections of flubendazole, given for five weeks to patients in Mexico, were effective but without an immediate microfilaricidal effect. Because of local pain and inflammation, however, the drug will have to be reformulated before further trials can be undertaken. Mebendazole has been found effective in lymphatic filariasis but has to be given in large doses (1.5 g daily) over long periods (two to three weeks) and is thus unsuitable for large-scale use in its present formulation.

◦ *the Hoffmann-La Roche compound RO5-9963*, which has shown some promise in the cattle screen, but is now known to possess neither micro- nor macrofilaricidal activity in onchocerciasis.

◦ *furapyrimidone*, a compound developed in China for the treatment of filariasis (A20) and found to be effective in the *O. gibsoni* cattle screen.

◦ *a few benzimidazole compounds* synthesized at the University of Michigan, USA, and found to be promising in primary and secondary screens. One, UMF

058, is being tested in the *O. gibsoni* cattle screen and the *B. malayi* leaf monkey screen.

Parasite biochemistry, metabolism and related studies

Metabolic characteristics unique to filarial worms might provide suitable targets for drugs nontoxic to man. Earlier studies disclosed selective inhibitors of folate metabolism that affect the parasite and not the host. Filariae require preformed folate, as do their mammalian hosts. But, unlike man, the parasite can oxidize 5-methyltetrahydrofolate directly to 5,10-methylenetetrahydrofolate, which in turn is converted to other tetrahydrofolate co-factors. This could be a promising target of selective inhibition.

Studies in *B. pahangi*-infected jirds have shown that 5-fluorodeoxyuridine acts synergistically with relatively low doses of methotrexate to cause immediate, long-lasting sterilization of adult female worms. Recent data implicate thymidylate synthase as a major target for chemosterilants, and this lead is being pursued.

Further progress has also been made in delineating phospholipid synthetic pathways of *Dirofilaria immitis*, the key enzymes of which have been isolated.

Studies on the energy metabolism of *O. volvulus* have indicated that the therapeutic effect of the amoscanate derivatives CGP 6140 and CGP 8065 may depend on cyclic AMP-phosphodiesterase inhibition, with accumulation of cyclic AMP and disturbances in regulation of glycogen metabolism, which is controlled by cyclic AMP-dependent protein kinases (A161-162).

Immunology

A knowledge of the mechanisms underlying the host's response to filarial parasites is as critical to an understanding of the clinical manifestations of filarial infections as it is to developing satisfactory methods of diagnosing and possibly preventing these diseases. An apparent lack of responsiveness by the host, for example, can be the result of specific parasite-related immunosuppressive mechanisms. In this context, the Programme gives high priority to research on: immunological reactions responsible for pathological changes occurring in the natural course of infection or as a result of treatment; the identification, detection and isolation of parasite antigens and the production of specific reagents that might be used in immunodiagnostic assays; and host protective immunity to different developmental stages of filarial parasites.

Large quantities of antigens are required for

studies on protective antigens and on immunity against infective larvae and other parasite stages. Techniques developed for the *in vitro* culture and cryopreservation of infective larvae now enable several parasite stages to be transported from areas of infection to highly specialized laboratories.

Immune response to treatment

In one study, immune processes were implicated in the Mazzotti reaction commonly seen after diethylcarbamazine (DEC) treatment. Intense complement activation occurs within two hours of administering the drug, followed by tissue eosinophilia and morphological and biochemical evidence of eosinophil and mast-cell degranulation. High levels of serum IgE and major basic protein (MBP) and of urinary histamine were seen after DEC administration, suggesting that immediate hypersensitivity may be an important component of the Mazzotti reaction. In addition, the severity of most manifestations of the Mazzotti reaction—hypotension, fever, adenitis and pruritus (but not arthralgia or tachycardia)—was found to correlate closely with pretreatment skin microfilaria counts.

Chemical mediators involved in the anaphylactic-like reaction in microfilaraemic hosts given DEC have been studied in *Dirofilaria immitis*-infected dogs, where prostaglandin D₂ (PGD₂) was the most important mediator, and in *B. malayi* *in vitro*, where microfilariae appeared to incorporate and metabolize arachidonic acid to generate prostaglandin, suggest-

ing that DEC affects microfilarial arachidonic acid metabolism.

Two further studies relating to the Mazzotti reaction were conducted during the reporting period. In one, a guinea-pig eye model was established, using *O. lienalis* microfilariae, to determine whether the anti-allergy drug lodoxamide could protect against the Mazzotti reaction, but no significant effect was observed on corneal inflammation. In the second study, conducted on groundhogs infected with skin-dwelling *Ackertia marmotae* microfilariae, a high dose (24 mg/kg) of DEC caused an inflammatory response. This parasite will probably be useful in studying the Mazzotti reaction in jirds.

Mechanisms of parasite destruction

Immunological factors involved in the host-parasite relationship are being investigated, not only to understand the mechanisms of host immunity to filarial parasites, but also to find ways of preventing tissue damage, particularly that associated with treatment.

Studies in *B. malayi* and *B. pahangi* rodent models have confirmed the role of IgG in antibody-dependent cell-mediated cytotoxicity (ADCC) to microfilariae and infective larvae, and have shown that eosinophils are involved in ADCC to sheathed and exsheathed microfilariae. Complement, however, appears to be the major component in the killing process. In the case of exsheathed microfilariae, even complement from normal serum can bring about cell death.

Box 4.2 Understanding the disease

Research on the pathogenesis of filarial diseases may have implications for antifilarial drug use.

- Findings of a study conducted in Cameroon are consistent with the hypothesis that onchocerciasis may be associated with an immune-complex nephropathy.

- *Brugia* antigens and immune complexes were observed in *B. pahangi*-infected dogs, and the antigens are now being characterized. Using antisera to somatic antigens of adult *Brugia* and to microfilarial products, different sets of antigens have been demonstrated in immune complexes

isolated from, respectively, kidney and lymph node tissues of infected dogs.

Differences in the antigen composition of immune complexes also seem to exist between amicrofilaraemic and highly microfilaraemic dogs.

- Xeroradiographic lymphangiography in the *B. pahangi* dog model has made it possible to assess the severity of lymphatic pathology, which has been found to be correlated with microfilaraemia levels.

- Studies in the *B. pahangi* jird model have confirmed earlier findings of a host immune mechanism underlying the development of granulomatous

lesions. The range of lesions, from mild to severe, is similar to that seen in man. Indirect evidence, moreover, points to the involvement of both humoral and cellular events. Similar numbers of adult worms developed in *B. pahangi*-infected jirds, whether or not their mothers had been infected with the parasite. However, the offspring of the infected mothers showed fewer lesions (intralymphatic granulomatous thrombi and lymphatic dilatation) and an increased frequency of microfilaraemia, presumably because of hyporesponsiveness induced *in utero* by the infection.

Immunodiagnosis

Immunodiagnostic tests are needed both for onchocerciasis and for lymphatic filariasis. A test capable of detecting onchocercal infection in the prepatent period would be particularly useful to the OCP, and a test of reinfection would be a valuable adjunct to current parasitological methods (observation of nodules, and skin-snip and slit-lamp examinations), which are inadequate for the diagnosis of early infection.

Microfilaraemia is nocturnal in most forms of lymphatic filariasis. A daytime test of infection is needed to obviate the need for night examination of blood and also, ideally, to detect mild, early infection.

Lack of specificity is the stumbling block to the development of immunodiagnostic tests for filariasis. Filarial antigens are widely shared among different filarial species and even with nonfilarial nematodes. Specific antigens of immunodiagnostic potential are being sought through: the isolation of antigens from human and animal filarial parasites; the fractionation and characterization of surface and somatic antigens, including *in vitro*-released (IVR) and *in vivo* circulating antigens; and the development of filaria-specific monoclonal antibodies. As yet, however, no antigen or epitope specific to human filariae has been discovered.

Several TDR-supported studies conducted during the reporting period have focused on detection of antigens in body fluids (A38, A50, A61, A117). Antigens have been detected in the serum and urine of most microfilaria-positive patients, but also in a number of microfilaria-negative patients, suggesting that serological diagnosis of infection might be possible even in the absence of microfilariae—in other words, when infection is in a prepatent or occult stage. Methods of high specificity and sensitivity are required for these studies, and two radioimmunoassays—the radioimmunoprecipitation polyethylene glycol assay (RIPEGA) and the immunoradiometric assay (IRMA)—have been found especially useful. Nonradioisotopic methods, including the enzyme-linked immunosorbent assay (ELISA), are currently being adapted as immunodiagnostic tests for filarial infection.

Meanwhile, outside the Programme, a monoclonal antibody-based ELISA developed to detect *D. immitis* antigen in sera of infected dogs (B4) effectively denoted the presence of mature adult worms and pointed to a close correlation between adult worm counts and antigenaemia levels.

In Papua New Guinea and Sri Lanka, the filaria-specific Gib-13 monoclonal antibody, developed

from *O. gibsoni* egg antigen for the diagnosis of onchocerciasis, has recently been used in an IRMA to detect circulating antigen in sera of *W. bancrofti*-infected individuals. In Papua New Guinea, 93% of microfilaraemic subjects were antigen-positive. A significant correlation was found between residence in areas of high prevalence, serum antigen levels and blood microfilarial counts. The finding of antigen in blood samples (and, in some cases, also in urine samples) from amicrofilaraemic patients, with or without acute symptomatic disease, but not in controls from nonendemic areas, suggests that Gib-13 will be useful in the diagnosis of occult infections, although it needs to be evaluated in low prevalence areas. Preliminary characterization of the epitope reactive with Gib-13 suggests that it is carbohydrate in nature.

A recent informal workshop held at the Institut Pasteur in Lille, France, to compare reagents and tests highlighted the considerable crossreactivity among the different parasites and the failure as yet to identify any species- or subspecies-specific antigen (C4).

Vaccine development

The immunology of filarial infections in man and animals is at present poorly understood and animal models of human infection are not readily available. Thus, although vaccines could be useful in disease control, their feasibility would have to be demonstrated before they could become a high-priority objective of the Programme. Antigens of infective larvae or of young adult worms are considered likely sources of material for vaccine research.

An important step towards the *in vitro* cultivation of *Onchocerca* was the finding that the Liberian strain of *O. volvulus* and cattle *O. lienalis* will moult from L3 to L4 in a cell-free culture medium. Newly developed techniques for cryopreserving infective larvae and microfilariae are making it possible to transfer these parasite stages from areas of infection to highly specialized laboratories elsewhere. Cryopreserved nodular *O. volvulus* microfilariae injected into Balb/c mice, and thereby "preconditioned" for 17-21 days, can develop into L3 larvae in *Simulium pictipes*. It is possible that the preconditioning corresponds to the coating of microfilariae with host material, as has been demonstrated for *W. bancrofti* (A92) and *O. gibsoni* (A49).

Resistance to infection was assessed in preliminary experiments in jirds vaccinated with ⁶⁰Co-irradiated infective *B. malayi* larvae. Vaccinated animals showed lower worm counts and carried a greater proportion of stunted worms than did control animals.

Field studies

If measures to control transmission of filarial infections are to be improved, more must be learned about the natural history, epidemiology and vectors of these diseases in many parts of the world. The basic data will vary from country to country and within countries, which means that they must be gathered on a local rather than a global basis. The Programme has therefore encouraged both laboratory- and field-based research on: risk factors for development of diseases; population and transmission dynamics for different vector species and strains in given localities; and the identification in vectors of larvae infective to man.

Projects have begun on mosquito vectors (in Egypt and Papua New Guinea), *Simulium* vectors (in Cameroon), DEC dosage (in Fiji, India and the Philippines) and suramin treatment and its effect on onchocerciasis eye lesions (in Zaire).

The Nearctic blackfly, *S. pictipes*, not a natural vector of human onchocerciasis, has been found to be a good laboratory vector for *O. volvulus* and a rich source of L3 larvae, which are now being used in studies on chimpanzees and for the purpose of *in vitro* cultivation (A88).

In southern Sudan, where onchocerciasis vector biting habits and transmission have been monitored for two years, application of the larvicidal insecticide temephos for a nine-month transmission season, in conjunction with vegetation clearance, prevented vector breeding along a 41 km stretch of the Bussere River: transmission fell by 80% at hyperendemic foci and by 50-60% at less severely affected foci.

Other field studies conducted during the reporting period include:

- studies in peninsular Malaysia on the age composition, survival, biting behaviour and transmission patterns of *Mansonia* mosquitoes in two ecotypes: mark-release-recapture experiments suggest that *M. uniformis* has a flight range of 3.5 km, and *Coquilletidia crassipes* was shown for the first time to be a secondary vector of subperiodic *B. malayi* and to transmit a bird filarial worm, *Cardiofilaria nilesi*, previously reported only in Sri Lanka.
- experiments to determine the periodicity and morphological characteristics of *B. malayi* infecting man and animals in different areas of Indonesia: as a first step, human parasites from Kalimantan and Sulawesi were transmitted to cats and jirds, and parasites obtained from wild cats and monkeys were transferred to laboratory jirds, cats and monkeys. These findings could have important epidemiological implications and provide insights into the possible role of zoonotic infection in human disease patterns.

In addition, two meetings took place during the reporting period. One, a workshop on epidemiological research on lymphatic filariasis held in Varanasi, India, in 1984 and organized by TDR's SWG on Epidemiology, developed protocols for case-control studies on risk factors for lymphatic filariasis. The other, an SWG meeting held in Bamako, Mali, examined *Onchocerca - Simulium* relationships with regard to transmission of human onchocerciasis in West Africa and assessed links between laboratory and field research (C5).

Laboratory studies

The same vector flies can carry human and animal parasites, and larvae infective to man have to be distinguished from those infective to animals. In lymphatic infection, for example, it is difficult to distinguish *Brugia malayi* of human origin from the morphologically similar *B. pahangi*, an animal parasite. Similarly, in onchocerciasis, the infective larvae of *O. volvulus* are morphologically indistinguishable from those of the cattle parasite *O. ochengi*. Isoenzyme patterns studied in single adult worms and in pooled infective larvae suggest that *B. malayi* and *B. pahangi* can be distinguished on the strength of their glucophosphate isomerase isoenzyme patterns (A48, A171).

Box 4.3 The future

Chemotherapy:

- continuation of the search for nontoxic macrofilaricidal and embryostatic agents, especially for *Onchocerca volvulus* but also for lymphatic filarial parasites;
- better use of effective compounds.

Immunology:

- continuation of the search for sensitive, specific immunodiagnostic tests, especially for field use, including tests based on antigen detection;
- prevention of disease and the adverse effects of treatment;
- basic studies towards vaccine development.

Field research:

- improved epidemiological studies, including identification of risk factors for disease;
- identification of infective larvae and their differentiation from animal parasites;
- population and transmission dynamics for different vector species and strains.

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5 African trypanosomiases

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Note on lists of publications

Publications and other documents relating to the subject of this chapter are listed, at the end of the chapter, under three headings: Publications acknowledging TDR support (list A), Publications from work outside the Programme (list B) and TDR scientific reports which became available in 1983-84 (list C). Since most of the work described in the Report is too recent to have appeared in the literature, only a small proportion of publications listed relate directly to the text. List A comprises publications acknowledging TDR support that have not appeared in any previous Programme Report and list B, only those publications pertinent to specific points discussed in the text. All the documents in list C can be obtained by writing to: The Office of the Director, TDR, World Health Organization, 1211 Geneva 27, Switzerland; requests should carry the full document title and reference number.

5 African trypanosomiases

The context

□ African trypanosomiasis, or sleeping sickness, is a severe, often fatal disease that occurs widely in the sub-Saharan region of the continent. There are two varieties: one, caused by *Trypanosoma brucei rhodesiense*, is found in East Africa; the other, by *T.b. gambiense*, occurs in West and Central Africa. The *rhodesiense* infection is usually acute, causing severe symptoms and death within a few weeks or months. The *gambiense* form progresses more slowly over several months or years. Both varieties can produce brain damage and a sleeping sickness syndrome, and are fatal if not treated.

About 50 million people in 34 African countries are at risk of developing the disease and of these, only 5 to 10 million have access to some form of protection or treatment. The incidence of reported cases is currently 20 000 a year, but many cases go undetected. Severe outbreaks have occurred over the last 10 years, partly as a result of poor disease surveillance, in Cameroon, the Central African Republic, the Ivory Coast, the Sudan, Uganda and Zaire.

The trypanosomiases are transmitted by a small number of tsetse fly species. An infected bite from the insect causes local inflammation (trypanosomal chancre); the parasites migrate from this site and go on to multiply in lymph and blood. The blood trypanosome count oscillates cyclically, the parasites in each successive parasitaemia wave carrying different surface antigens and thereby evading antibodies raised by the host to previous surface coats of the parasite. Eventually, all organs are invaded, with central nervous system involvement ultimately leading to coma and death.

T.b. rhodesiense trypanosomiasis occurs in a variety of game and cattle. When incidence of human infection is low, transmission occurs mainly through animal hosts, although during epidemics, man-to-man transmission is important. *T.b. gambiense* infection, on the other hand, was thought until recently to be confined to man. Strong evidence, however, has

emerged that domestic and game animals can be infected, although the relevance of this finding to transmission of human disease has yet to be determined.

Control of sleeping sickness, especially of the *gambiense* form, is based on population surveillance, diagnosis and treatment. In view of the wide variability in parasitaemia levels, early diagnosis of infection is not easily achieved under field conditions. The immunofluorescent antibody test is valuable but limited by the need for specialized laboratories. Several simpler tests, notably the Card Agglutination Test for Trypanosomiasis (CATT), are currently being evaluated.

Currently available drugs are grossly inadequate for treatment of these diseases. The earlier stages of infection, which do not involve the brain, can be treated with suramin (for *T.b. rhodesiense* infection) and pentamidine (for *T.b. gambiense* infection), but resistance to pentamidine has been reported. Treatment of late-stage disease is currently based on the arsenical compound melarsoprol, which causes serious side-effects in 5-10% of patients and is fatal in 1-5%. Work is in progress to improve therapeutic regimens based on existing drugs, but less toxic drugs are urgently needed. Treatment is also hampered by inadequate knowledge of the pathology of the disease.

Insecticides can be used to control tsetse vectors but they are expensive, have ecological drawbacks and do not produce a lasting effect. Simple, effective insecticide-impregnated traps and screens developed for use by rural communities have, however, reduced tsetse populations by over 90% in some areas.

In view of the many problems facing those involved in control of the trypanosomiases, the WHO Action Programme on Sleeping Sickness Control recently prepared a Trypanosomiasis Control Manual (partly based on findings of TDR-supported research) which is intended for use by primary health care workers, nurses, medical assistants and doctors. □

Box 5.1 Highlights of activities in 1983-84

- The new Card Agglutination Test for Trypanosomiasis (CATT)—a simple, relatively sensitive field test for African trypanosomiasis—was found, in comparative field trials, to be the most practical test now available for the rapid diagnosis of infection.
- The miniature anion-exchange centrifugation technique (MAECT) has been shown to be the most sensitive method of detecting trypanosomes in blood. The WHO Action Programme on Sleeping Sickness Control is preparing kits to make the test suitable for routine use by sleeping sickness treatment centres.
- DL- α -difluoromethylornithine (DFMO), an ornithine decarboxylase inhibitor, administered in combination with bleomycin, cured mice of berenil-resistant central nervous system *Trypanosoma brucei brucei* infection. In preliminary clinical trials conducted within and outside the Programme and involving about 100 patients with African trypanosomiasis, DFMO achieved cure in 97 patients, including those with melarsopol-resistant infection. Further trials are being conducted in collaboration with the pharmaceutical industry.
- Of 100 compounds put through TDR animal screens for trypanocidal activity, two nitroimidazoles, given with suramin, brought about permanent cure of central nervous system *T. brucei* infection in mice and are now being tested in monkeys.
- In an Ivory Coast study, use of insecticide-impregnated screens in conjunction with selective insecticide groundspraying reduced tsetse population density by 98%.

Report of activities in 1983-84

Trypanosoma brucei gambiense infection

The recent discovery of *T. b. gambiense* infection in animals is an important epidemiological finding. In one study, conducted in the Bouaflé disease focus in the Ivory Coast, infection was found in 283 (58%) of 488 pigs tested and persisted in these animals for up to one year. Infection was also seen to persist in goats: of 65 animals examined, two were infected, and in one, the infection lasted for a year. Another study, however, conducted in the Congo, disclosed a much lower frequency of infection in domestic animals (in only 3 of 229 animals studied), suggesting that the animal reservoir in the Congo is much smaller than that in West Africa.

Research has also focused on the intensity of human exposure to tsetse flies. In the Lobo Valley in the Ivory Coast, differences in exposure to tsetse flies were seen among different tribes and were related to differing agricultural practices. This is a typical transitional forest savanna region, where agricultural encroachment on forest land is associated with increasing man-fly contact. In such situations, growing human population density and the appearance of virulent parasite strains may favour the establishment of the disease.

Trypanosome characterization

Identification of trypanosome strains is essential for an understanding of the epidemiology of sleeping sickness. One method of identification is based

on strain-specific electrophoretic mobilities of enzyme forms (isoenzymes) within the trypanosome. Another, recently developed outside the Programme, is isoelectric focusing (B2-3).

Isoenzyme analysis has brought to light the existence of many different trypanosome strains. Fifty-four clones were grown from one *Trypanozoon* "stock" obtained from a single tsetse fly in the Ivory Coast. Four were found to share an isoenzyme pattern different from that seen in the others, suggesting that the fly had been harbouring a mixed infection.

Isoenzyme analysis has also been used to prepare a computer-based scheme of relationships, or "dendrogram", for 500 *Trypanozoon* stocks derived from human subjects, animals and tsetse flies. Certain isoenzyme patterns can be grouped within the dendrogram and are being studied to determine whether they relate to biological characteristics of epidemiological importance to human disease.

Much of the work on isoenzyme patterns has been carried out in the Luangwa Valley in Zambia, in conjunction with the Tropical Diseases Research Centre at Ndola and the London School of Tropical Medicine and Hygiene in the United Kingdom. A total of 64 different but closely related zymodemes have been identified from 311 trypanosome isolates obtained from human subjects and from game and domestic animals. Five of these zymodemes were found to be associated with human disease and may turn out to be useful markers for human pathogenic strains of the parasite.

DNA probes are being developed to identify

human- and animal-derived *Trypanozoon* stocks and to provide data of use in epidemiological studies. Of 24 trypanosome stocks, 11 were identified as *T. b. gambiense* and of these, four were from domestic animals—two pigs, a dog and a sheep.

Goats have been used to monitor *T. b. rhodesiense* transmission. Natural *Trypanozoon* infection developed in one of six goats left in an area of trypanosomiasis transmission, but resolved spontaneously. This observation reinforces the growing realization that domestic animals can be important reservoirs of infection (A116).

Relationship of *T. b. brucei* to human infection

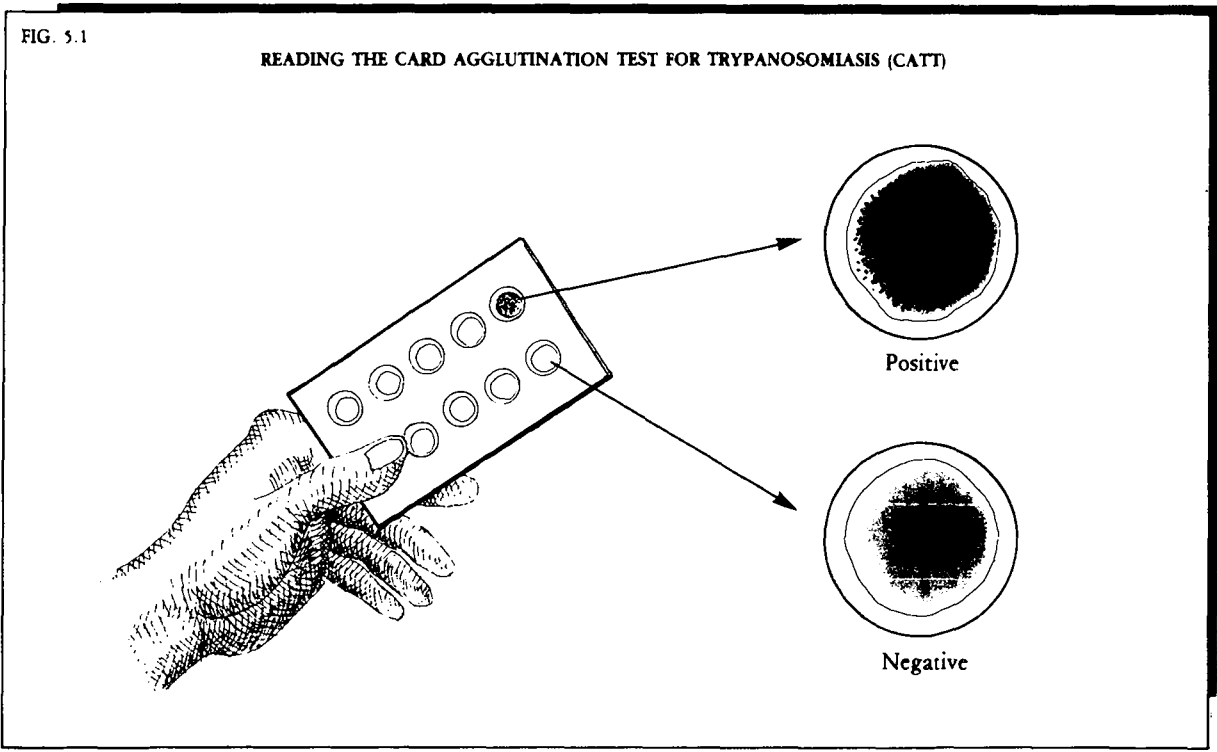
T. b. brucei, one of the few trypanosome species infective to game and livestock animals, is morphologically indistinguishable from the human pathogens *T. b. rhodesiense* and *T. b. gambiense*. There is, in fact, no reliable way of demonstrating human infectivity short of unacceptable human challenge experiments. The blood incubation infectivity test (BIIT), in which test parasites are incubated in human blood and subsequently inoculated into rodents, does provide an indication of human infectivity. Parasites which survive the incubation are considered infective to man (BIIT negative); those which do not survive are noninfective (BIIT positive). It has recently been claimed that the BIIT characteristics of

a trypanosome population are not stable. Tsetse infected with a population of BIIT-positive *T. b. brucei* (putatively noninfective to man) were fed on human serum: the parasites recovered from the flies were BIIT negative (putatively infective). This finding, which is still under study, could imply that the reservoir of trypanosomes potentially infective to man may be much greater than previously suspected (A113). A close relationship between *T. b. brucei* and *T. b. rhodesiense* is also suggested by the findings of enzyme studies conducted outside TDR (B12).

Diagnostic tests

Diagnosing trypanosomiasis under field conditions can be difficult. In *T. b. gambiense* infections, in particular, parasitaemia fluctuates daily and parasites may be difficult to detect even in thick blood films. This is also true of the early stages of *T. b. rhodesiense* infection, although the higher parasitaemia levels associated with this more virulent organism make parasitological diagnosis relatively straightforward.

A miniature anion-exchange centrifugation technique (MAECT), first discovered outside the Programme and subsequently developed with TDR support, concentrates parasites from blood and thereby improves the sensitivity of microscopic examination. A field kit has been prepared and plans are



under way for its mass production. Although the MAECT is now the most sensitive parasitological method available, it is still limited by the fluctuating, inconstant parasitaemia pattern characterizing the disease.

Serological techniques have been devised as adjuncts to direct demonstration of parasites—still the mainstay of definitive diagnosis. One, the Card Agglutination Test for Trypanosomiasis (CATT), derived initially from work conducted outside the Programme, is based on direct agglutination of parasites by human blood (Fig. 5.1). In field studies conducted over the reporting period, the CATT was compared with the other major serological method, the indirect haemagglutination test (IHT). The IHT was found to be more sensitive than the CATT but not so simple to perform under field conditions, giving the CATT the final edge.

Sensitive, serological methods also have their limitations: false-positive results can result from previous exposure to *T.b. brucei* or from an abortive infection with *T.b. gambiense* or *T.b. rhodesiense*. Seropositivity should certainly be taken seriously, as is illustrated by one study in the Ivory Coast, where 48 seropositive subjects were MAECT-tested daily. Within 15 days, 10 subjects were parasitologically positive. Since patients are only given treatment if they are shown to harbour parasites, the question of what action to take in serologically positive but parasitologically negative patients is still an open one. This study underlines the need for repeated parasitological testing in seropositive patients in whom parasites are not seen at the outset.

The vector-parasite relationship

Several studies have been conducted, with Programme support, in an attempt to identify the factors determining a fly's vector potential. One of the most important is the fly's susceptibility to *Trypanosoma* infection. *Glossina* haemolymph has been shown to contain an antitrypanosome factor (A25), and lectin-like substances capable of agglutinating procyclic *T.b. brucei* forms have been found in *Glossina austeni* extracts. (In this connection, extracts of another vector, *Rhodnius prolixus*, have been found to agglutinate *T. cruzi*, the trypanosome responsible for Chagas' disease, the South American form of trypanosomiasis.)

One strain of *G. palpalis*, maintained at the Tsetse Research Laboratories in Bristol, in the United Kingdom, has been shown to be capable of transmitting a West African *Trypanozoon* stock. The parasites infecting the fly achieved maturity (i.e. developed into the form infective to mammalian hosts) in 6%

of exposed flies. The ability of the flies to support parasite maturation appeared to be maternally inherited.

In a study conducted outside the Programme, virus-like particles have been seen in refractory tsetse (in which parasites do not mature). Research is under way to determine whether these findings apply to wild tsetse.

Vector ecology and control

The pig not only acts as a potential reservoir for *T.b. gambiense* but is also associated with high tsetse densities. Studies in Bouaflè (the Ivory Coast) have shown fly densities (numbers of flies trapped per day) 12- to 100-fold higher in villages with pigs than in those without. Peridomestic tsetse breeding sites were characteristic of villages where pigs were kept. Villages with many pigs apparently favour fly breeding and retention, and may be important sites of disease transmission. Drought may also be associated with changes in tsetse population density and may have been partly responsible for the recent increases in density and distribution of *G. tachinoides* in the Bouaflè focus.

Traps have been remarkably successful in controlling tsetse flies. A modified, more durable monoconical trap, costing US\$ 1.50, is being locally produced at the rate of 1000 a month and will be used in a large-scale *Glossina* control project in the Congo, where deltamethrin-impregnated monoconical traps were able to rid two foci of tsetse for a period of six months. The traps—one for every ten villagers—are reimpregnated with insecticide every six months. Their effect on transmission is being assessed serologically over a three-year period. A new trap design, using old rubber tires, was recently developed outside the Programme (B8).

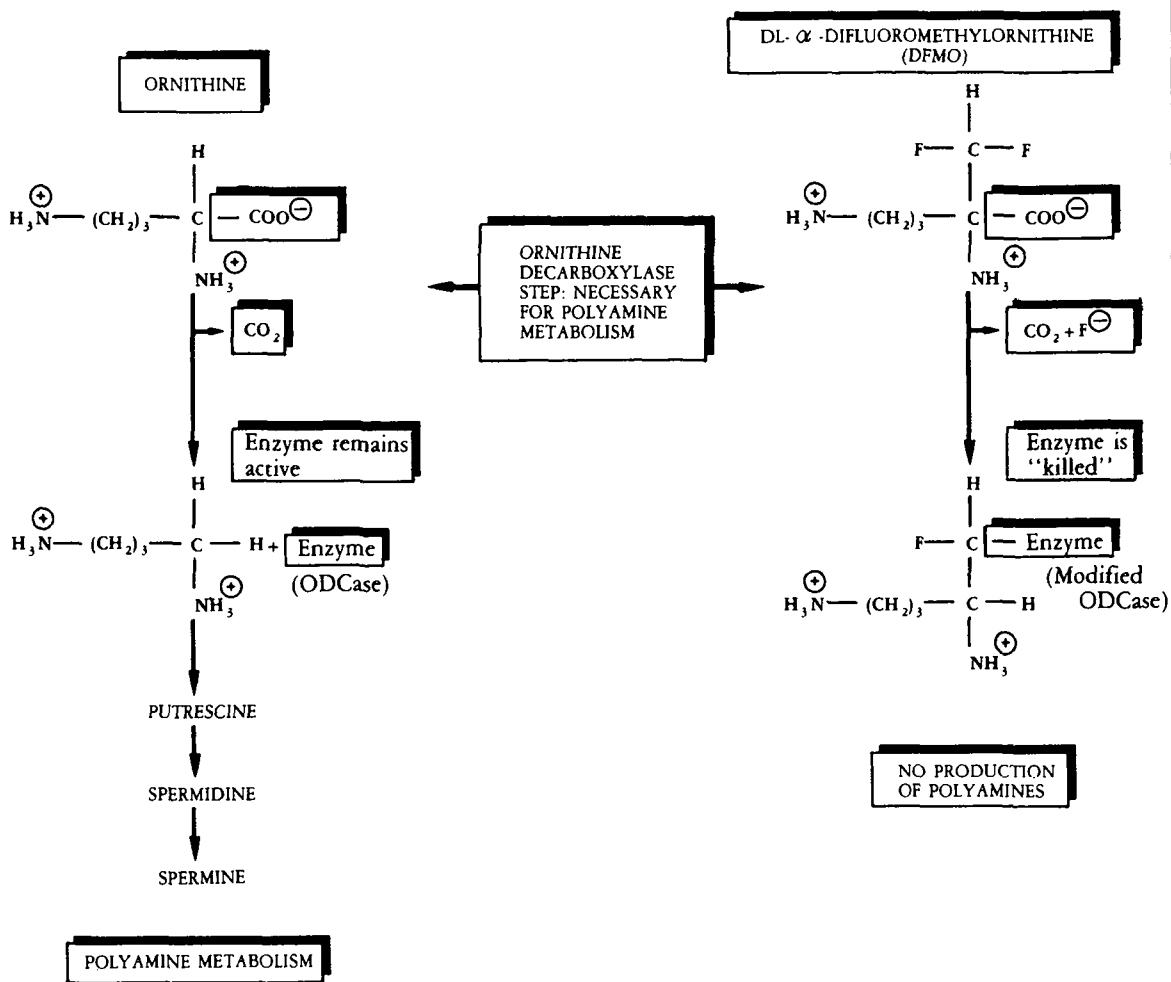
Work is in progress to determine the best types and formulations of insecticide for impregnating traps and screens. Of the different insecticides tested, deltamethrin and alphamethrin, applied for one month during the wet season, were found to have the highest residual activity. Residual effect was also found to depend on the nature of the textile fibres used in the screens and traps. Emulsifiable insecticide concentrates were better than wettable powders.

Host odours have been found extremely effective in attracting tsetse flies (B4-5) and are being assessed as a means of increasing the efficacy of fly traps and screens. Research is being conducted in West and Central Africa on the active components of host odours attractive to flies of the *G. palpalis* group.

Agricultural spraying equipment can be used to apply insecticides like deltamethrin and alphameth-

FIG. 5.2

HOW DFMO BLOCKS POLYAMINE BIOSYNTHESIS



DL-α-difluoromethylornithine (DFMO), a structural analogue of ornithine, covalently modifies and inactivates or "kills" ornithine decarboxylase during catalytic processing. It is therefore a "mechanism-based", specific inactivator of the key target enzyme, ornithine decarboxylase, which generates putrescine at the first step in polyamine synthesis. The use of DFMO for the treatment of African trypanosomiasis illustrates how basic metabolic research can lead to the discovery of a new remedy for disease.

rin (which gave satisfactory control over a five-month trial period). One constraint is toxicity to spraymen: doses of alphamethrin higher than 12 g of active ingredient per hectare cause eye and nose irritation.

Chemotherapy

Melarsoprol, the only drug effective against late-stage African trypanosomiasis, is generally administered in a propylene glycol solvent and, if not given intravenously, can cause severe tissue damage. Bet-

ter formulations are being sought and optimal dosage requirements investigated. Peanut oil, for example, has been found to be a promising solvent for subcutaneous injection and is being further studied for this use. In studies on monkeys, long-term cures have been achieved with 1.8 mg/kg doses, instead of the customary 3.6 mg/kg, with no relapse over a period of up to 600 days. If results continue to be encouraging, trials of lower doses of melarsoprol will be considered in man.

In the late 1970s, two screens for trypanocidal com-

pounds were set up with Programme support: one at the Kenya Trypanosomiasis Research Institute (KETRI) in Nairobi, Kenya; the other in Glasgow, in the United Kingdom. A third, outside the Programme, was established in Berlin (West). By the end of 1984, about 100 compounds had been put through the two TDR screens. Of those obtained from pharmaceutical firms, four 2-substituted 5-nitroimidazoles, given in combination with suramin, brought about permanent cure of central nervous system (CNS) infection in mice. Two of the four are now being assessed in nonhuman primates. One of several compounds tested at KETRI was effective against the EATRO 842 strain, which is resistant to melarsoprol, suramin and pentamidine, and will be submitted to further study.

The ornithine decarboxylase inhibitor, DL- α -difluoromethylornithine (DFMO) (Fig. 5.2), has been found to be effective in acute *T. b. brucei* (animal) infection and to act synergistically with other trypanocidal compounds (A4, A76). In combination with bleomycin, DFMO cured berenil-resistant mice of CNS infection, and used alone in preliminary trials conducted in several centres within and outside the Programme, achieved cure in 97 of 100 patients with African trypanosomiasis (B11), including those with melarsoprol-resistant infection. This drug is now the subject of limited trials being conducted in collaboration with the pharmaceutical industry.

A novel trypanocide, 1,3,5-triacetylbenzene tris(guanyldiazone) (TBG) (Fig. 5.3), has been synthesized and screened in mice: a single 3 mg/kg dose produced permanent cure of early *T. brucei* infection, while a dose of 15 mg/kg given on four occasions, following a single 20 mg/kg dose of suramin, resulted in cure of chronic infection involving the CNS. No relapse was seen over a 95-day follow-up. Cure of infection was also achieved with 2% DFMO in drinking water given for 14 days in conjunction with four 15 mg/kg doses of TBG given from the third day of DFMO treatment. No relapse occurred over an 88-day follow-up. Given alone in doses ranging from 1.25 mg/kg on four occasions to 20 mg/kg on three occasions, TBG was ineffective against chronic *T. brucei* infection, and with four 20 mg/kg doses, evidence of toxicity was seen. Twenty TBG analogues have been tested but none was as effective as the parent compound. Studies on the drug's trypanocidal mechanism of action have shown binding to DNA to be necessary but not sufficient for trypanocidal activity.

Basic trypanosome biology is being explored in the hope of finding new leads to chemotherapy. In research on the trypanosome cytoskeleton, pellicular microtubule stability was found to depend on the

presence of tightly bound polypeptides capable, at low ionic strength, of forming stable complexes with microtubular protein. The neuroleptic drugs phenothiazine and diphenylbutylpiperidine, used alone or in combination with suramin, have been shown to disrupt the *T. brucei* cytoskeleton *in vitro* (A124) but not to affect late-stage *T. brucei* infection in mice. This line of research is being pursued further.

Trypanosomes have an unusual glycolytic pathway, which is packaged into glycosomes within the parasites and may be a potential target for chemotherapeutic intervention (A89). Glycolytic enzymes have been isolated from the glycosomes of bloodstream forms (A92), in which they abound, and specific monoclonal antibodies have been obtained to two glycosomal enzymes. Progress has also been made in identifying and cloning the genes responsible for the synthesis of glycolytic enzymes.

One unusual characteristic of *T. brucei* is a high intracellular concentration of carnitine. O-Bromoacetyl-L-carnitine (BAC) has been found to inhibit carnitine acetyltransferase activity (A66) and a single 50 mg/kg dose significantly prolonged the survival of infected mice. This possible lead is being further explored.

One intriguing laboratory finding in patients with *gambiense* infection is an increase in urinary levels of keto acids (glyoxylate, pyruvate and α -ketoglutarate), which fall following melarsoprol treatment. Reagent sticks used to diagnose phenylketonuria—the only other condition known to produce α -ketonuria in man—have, however, proved insufficiently sensitive for the detection of trypanosomiasis.

Mechanisms underlying antigenic variation of trypanosome surface glycoproteins are also being explored as possible targets of chemotherapy. They are discussed under "Immune mechanisms of disease", below.

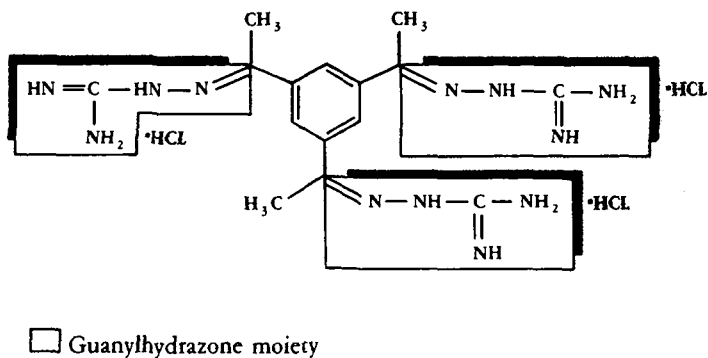
Immunodiagnostic tests

Recently developed serological tests, such as the CATT, are based on antibody detection. Testing blood or other body fluids for the presence of antigen would probably give a more reliable indication of current infection. A systematic search has begun for antigens suitable for such tests. Two-dimensional gel electrophoresis has been used to analyse proteins in extracts of *T. b. brucei*, *T. b. gambiense* and *T. b. rhodesiense* stocks, and body fluids obtained from trypanosomiasis patients are being screened for antigens of diagnostic potential (A108).

The immunodominance of the trypanosomal variant surface glycoprotein (VSG) has so far con-

FIG. 5.3

CHEMICAL STRUCTURE OF 1, 3, 5-TRIACETYLBENZENE TRIS (GUANYLHYDRAZONE) (TBG)



founded all attempts to detect other trypanosome-specific surface antigens. Other surface proteins have been prepared by high-performance liquid chromatography (HPLC) and tested for reactivity with immune sera. One has been found to react with serum from *T. b. gambiense*-infected individuals, and a possible diagnostic test based on this antigen is under study.

Fourteen monoclonal antibodies to *T. brucei* species have shown reactivity with bloodstream and procyclic forms of all subspecies of the parasite and are being further studied for their diagnostic potential.

Immune mechanisms of disease

Immune mechanisms may be involved in several aspects of African trypanosomiasis, especially those involving the brain. Immune complexes and elevated IgG and IgM levels have been demonstrated in cerebrospinal fluid (CSF) from patients with late-stage (encephalitic) sleeping sickness, and might provide a diagnostic marker of cerebral involvement and of drug resistance arising during treatment. Human infection is associated with intense polyclonal B-lymphocyte activation and the consequent production of a wide variety of antibodies and autoantibodies, including some reactive with DNA, striated and smooth muscle, immunoglobulins and, occasionally, red blood cells (A135).

Antigenic variation

The ability of pathogenic trypanosomes to change their surface antigens appears to be the key to their evasion of the mammalian host's humoral immunity.

The phenomenon has been extensively studied as a model for control of gene expression and there is good reason to believe that some of the mechanisms underlying it might be vulnerable to chemotherapeutic attack. A knowledge of the VSG repertoire is certainly relevant to the development of possible vaccines and of sensitive, specific serodiagnostic tests.

Antigenic variation has been the subject of several studies:

- Six types of gene rearrangement have been described for *T. brucei* (A11, A75, A79). Each variant surface glycoprotein is encoded separately, and there is evidence that there are 1000 VSG genes occupying about one-tenth of the parasite's genome. Research on the genetics of VSG production is also being actively pursued outside the Programme (B6-7, B13).
- Studies in the *T. b. rhodesiense* focus near Lake Victoria, Uganda, suggest that the repertoire of metacyclic variable antigens (M-VATs) consists of no more than 12 types, some of which occur frequently and are stable during tsetse transmission. Others, however, are not stable, implying the need for caution in considering possible vaccines based on mixtures of M-VATs.

In its present form, the CATT uses frequently occurring *T. b. gambiense* VATs. Comparable *T. b. rhodesiense* VATs are being sought. Eleven isolates from Busoga, Uganda, and two from Akagera, Rwanda, have been cloned: all the VAT repertoires of the Busoga isolates are similar, whereas those of the Akagera isolates differ both among themselves and from the Busoga VATs, of which those most commonly expressed are being assessed for diagnostic potential. Several VATs, selected from repertoires identified in different localities, may be necessary

Box 5.2 The future

Future plans and areas of interest of the Scientific Working Group on the African Trypanosomiases include:

- further clinical trials on DL- α -difluoromethylornithine (DFMO), using a revised protocol and initially involving centres in Belgium, the Ivory Coast, Kenya, Uganda and Zaire and possibly, at a later stage, other centres in Cameroon, the Congo, Ethiopia and the Sudan. Although the use of DFMO for the treatment of African trypanosomiasis is currently restricted to patients participating in these trials, it will also be given, on humanitarian grounds, to other patients, who do not respond to melarsoprol.
- long-term multidisciplinary studies on the epidemiology of human trypanosomiases, to be continued in the Congo (*Trypanosoma brucei gambiense*) and in Ethiopia and Zambia (*T.b. rhodesiense*);

biense) and in Ethiopia and Zambia (*T.b. rhodesiense*);

- epidemiological studies on cryptic infections in man, to be followed by long-term studies on seropositive individuals, using intensive parasitological examination at three-monthly intervals;
- further simplification of the miniature anion exchange centrifugation technique (MAECT);
- exploration of *in vitro* culture systems for diagnostic use;
- field assessment of the cDNA "Dot" test;
- a search for safe, effective drugs, through synthesis and screening;
- continuation of studies on the pharmacokinetics and pharmacodynamics of melarsoprol and suramin, and on drug resistance;

- tests on combinations of known trypanocidal drugs with new compounds and with anti-inflammatory agents;
- development of serodiagnostic tests for *T.b. rhodesiense* infection;
- continued research on *T.b. gambiense* and *T.b. rhodesiense* antigenic repertoires with a view to the development of more sensitive and specific diagnostic tests;
- studies on pathological manifestations of African trypanosomiases, including inflammatory processes and immunological factors;
- identification of simple, inexpensive vector control methods suitable for use by rural communities;
- a search for odour attractants for *Glossina palpalis* vectors, to improve vector trapping and control.

for a VAT-based serodiagnostic test of *rhodesiense* infection.

Vaccine development

The Programme is not at present directly supporting research on vaccine development. Basic research on antigens, including VAT repertoires and mechanisms of antigenic variation, is being conducted in Africa, Europe and the United States, and the Programme keeps close contact, on this subject, with the International Laboratory for Research on Animal Diseases (ILRAD) in Nairobi, Kenya.

Reference banks

Monoclonal antibodies

Monoclonal antibodies have become important tools for research on the trypanosomiases and a registry of antitrypanosomal antibodies has been set up at the University of Victoria, BC, Canada.

Serum and cerebrospinal fluid

A central bank of sera and CSF from patients infected with *T.b. gambiense* and *T.b. rhodesiense* has been established at the "Prince Leopold" Institute

of Tropical Medicine in Antwerp, Belgium, and will provide investigators with samples for research on: the development, evaluation and standardization of diagnostic tests; the pathogenesis of the disease; and VAT repertoires.

Pathology

Trypanosome products may be toxins responsible for some of the pathological manifestations of the disease. One product, tryptophol, has been shown to be neither immunosuppressive in mice nor haemolytic for human, rabbit or mouse erythrocytes. A trypanosomal lipopolysaccharide has also been isolated. Using trypanosomes growing in implanted millipore chambers, studies have been conducted on a trypanosomal platelet aggregating factor (PAF): trypanosomes in chambers caused reductions in platelet counts, and PAF was shown to pass through 0.5 μ millipore filters.

Post-mortem studies have been conducted at the Institute of Neurological Studies, University of Glasgow, UK, in collaboration with the Daloa Clinic in the Ivory Coast. The histopathological features of the meningoencephalitis and carditis associated with trypanosomiasis confirm the existence of a reactive encephalopathy, which takes the form of an acute

haemorrhagic leukoencephalopathy. Hypoxic brain damage also seems to play an important part in causation of death, suggesting that anticonvulsants might be useful as preventive treatment in patients with sleeping sickness.

Research on experimental infection conducted out-

side the Programme (B1, B9-10) has shown that trypanosomes may lodge within host cells, particularly the ependymal cells of the choroid plexus, suggesting that drugs would have to be capable of penetrating these cells to be effective in patients with CNS involvement.

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African trypanosomiases

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TDR scientific reports which became available in 1983-84

- C1. TDR. Report of the second Scientific Working Group on African Trypanosomiasis, Arusha, United Republic of Tanzania, 26-30 October 1981. Document TDR/TRY-SWG(2)/81.3. (English and French)
- C2. TDR. Serotyping of African trypanosomes: A meeting of the Scientific Working Group on African Trypanosomiasis, Glasgow, UK, 7-9 September 1982. Document TDR/TRY-SWG(SERO)/82.3. (English and French)

6 Chagas' disease

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Note on lists of publications

Publications and other documents relating to the subject of this chapter are listed, at the end of the chapter, under three headings: Publications acknowledging TDR support (list A), Publications from work outside the Programme (list B) and TDR scientific reports which became available in 1983-84 (list C). Since most of the work described in the Report is too recent to have appeared in the literature, only a small proportion of publications listed relate directly to the text. List A comprises publications acknowledging TDR support that have not appeared in any previous Programme Report and list B, only those publications pertinent to specific points discussed in the text. All the documents in list C can be obtained by writing to: The Office of the Director, TDR, World Health Organization, 1211 Geneva 27, Switzerland; requests should carry the full document title and reference number.

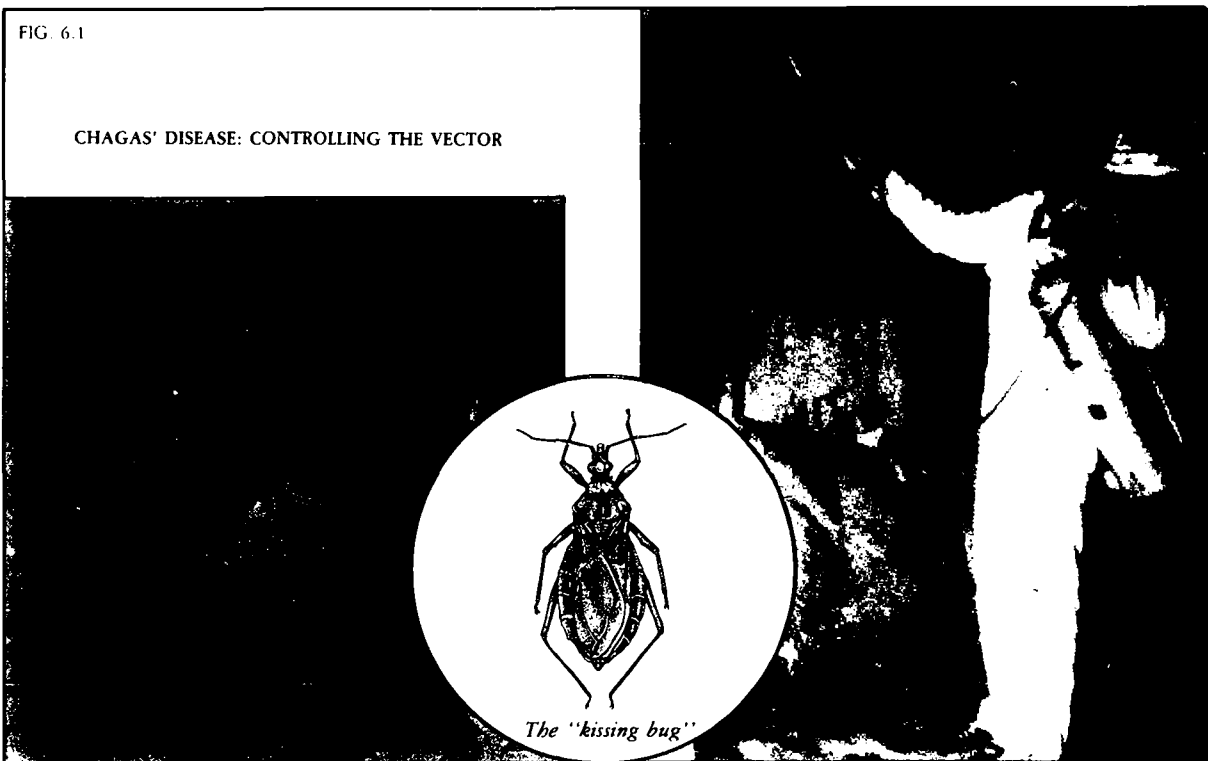
6 Chagas' disease

The context

□ Chagas' disease, a chronic illness caused by the flagellate parasite *Trypanosoma cruzi*, was first described in 1909 by the Brazilian physician Carlos Chagas. It is confined to the American continent, particularly to Latin American tropical and subtropical countries, although indigenous cases have been reported from temperate areas of North America (B9). There are two stages of the disease: an acute stage, occurring shortly after the initial infection, and a chronic stage, in which the heart, oesophagus, lower intestine and peripheral nervous system are chiefly affected. Fifteen to 20 years or more may elapse bet-

ween the two stages. During this "interim" period, infection is present without overt illness. Definitive diagnosis of infection is based on the demonstration of parasites in the blood and is achieved by xenodiagnosis: patients are exposed to the bites of laboratory-reared triatomine bugs, the vectors of *T. cruzi*, which are then examined for the presence of parasites.

Triatomines, commonly known as "kissing bugs" because they often bite their victims on the face, infest and breed in substandard mud-wall dwellings (Fig. 6.1). *T. cruzi* infection may originally have



PHOTOS: Courtesy of A M. Oliveira Filho

Left: Triatomine bugs, vectors of the parasite causing Chagas' disease, live and breed in the cracks of mud walls of substandard dwellings. Right: Insecticidal paint being applied on mud walls of a house. New slow-release insecticidal paint formulations have been tested in the field and shown to be effective against triatomines for up to nine months.

been a zoonosis of sylvatic mammals, with man becoming involved through exposure to the triatomines.

It has been estimated that up to 20% of blood donors in nonendemic urban areas are infected with *T. cruzi*, and transmission by transfusion has now become a serious problem (B1). About 65 million people are directly exposed to the risk of *T. cruzi* infection, a further 15 to 20 million are actually infected, and of infected individuals, approximately 10% develop chronic Chagas' cardiopathy. According to recent evidence, chronic Chagas' disease may be responsible in some areas for up to 10% of deaths among the adult population (B6).

Many countries recognize Chagas' disease as a public health problem and a number of control programmes have been in operation since the early 1950s. Designing and evaluating such programmes requires a knowledge of the prevalence of infection.

Current prevalence estimates are based largely on evidence from serodiagnostic tests, which are validated and standardized in a continental network of Collaborating Laboratories sponsored by TDR (Fig. 6.2).

Research topics being given high priority by the Programme include: the immunopathogenesis of chronic Chagas' lesions; prevalence rates and geographical variations in prevalence; improvement of control programmes; development of improved, long-acting insecticides; improvement of housing (to prevent transmission); standardization of serodiagnostic reagents and tests; improvement of serodiagnostic tests by the use of defined antigens; development of tests suitable for screening transfusion blood; development of trypanocidal compounds for sterilizing transfusion blood; and development of drugs to cure disease and not merely clear blood of parasites. □

Box 6.1 Highlights of activities in 1983-84

- Prevalence studies being undertaken with the Ministries of Health of Ecuador, Honduras, Paraguay and Uruguay are providing information for the implementation and evaluation of national control programmes.
- The continental network of Collaborating Laboratories for the standardization of serodiagnosis in Chagas' disease now covers 11 countries: Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Ecuador, Honduras, Panama, the United States and Uruguay. The central Reference Laboratory in Sao Paulo, Brazil, has distributed 2800 samples of reference sera to other Collaborating Laboratories for calibration of serodiagnostic tests. Quality control is undertaken by the central Reference Laboratory, and high indices of agreement have been obtained between the different laboratories in assessment of sera as positive or negative for infection.
- Slow-release insecticidal paint formulations have been field-tested and found effective for up to nine months. The new paints can be easily applied and are well accepted by the community (A103).
- A new, more cost-effective formulation for the insecticide deltamethrin has been developed and field-tested, and has now been adopted for routine control activities by the Brazilian Chagas' Disease Control Programme (SUCAM).
- A fumigant insecticide canister has been tested for indoor use and initial results are encouraging.
- Twenty-one active compounds potentially suitable for sterilizing transfusion blood have been identified in *in vitro* screens. Two have been selected for further study (A63).
- A new serological monoclonal antibody-based assay is being field-tested in Bolivia and Venezuela (A106).

Report of activities in 1983-84

Epidemiological studies

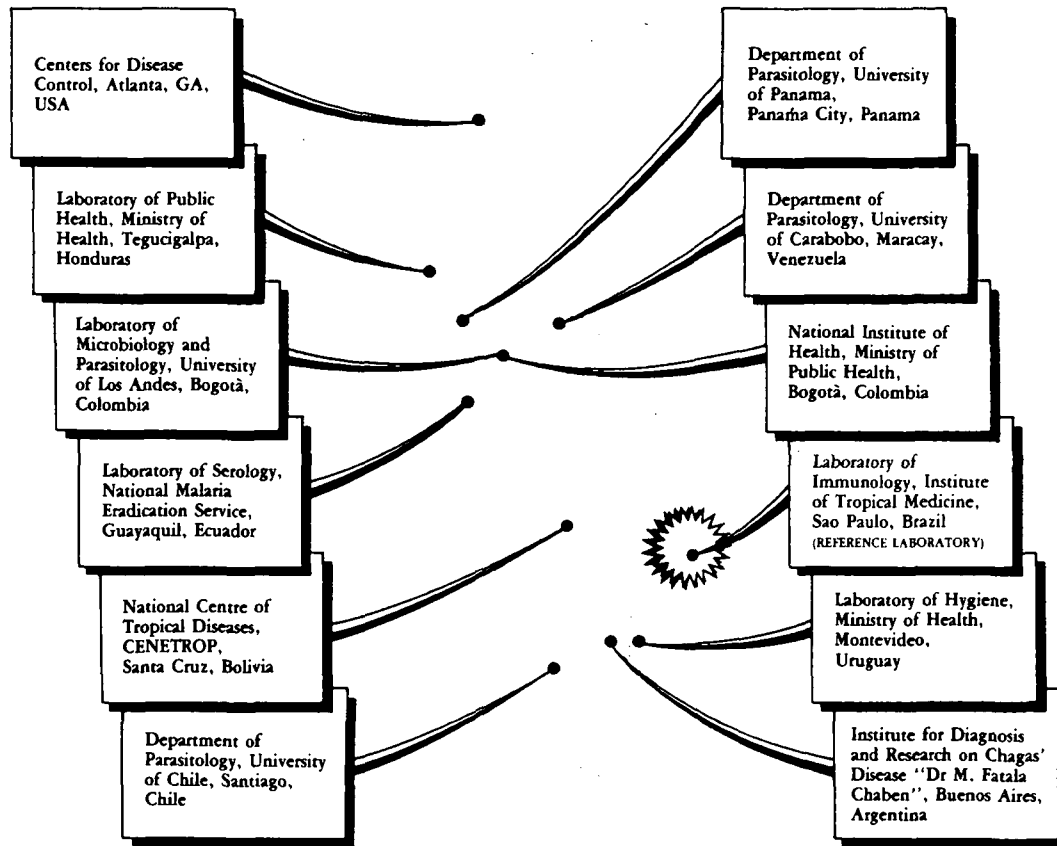
Prevalence of Trypanosoma cruzi infection and house triatomine infestation

Investigators, field personnel and laboratory technicians have been trained, and forms designed, for the collection of prevalence data. Country-wide entomological and serological surveys have begun in Ecuador, Honduras, Paraguay and Uruguay, follow-

ing a standard protocol devised by the Scientific Working Group (SWG) on Chagas' Disease, and serological quality control is being carried out by the Reference Laboratory in Sao Paulo, Brazil. Human infection and house infestation rates have been found to vary widely, both between and within countries (Table 6.1). Information of this type will make it possible to concentrate control measures on high prevalence areas.

FIG. 6.2

TDR'S CONTINENTAL NETWORK OF COLLABORATING LABORATORIES FOR THE STANDARDIZATION OF SERODIAGNOSIS IN CHAGAS' DISEASE



Longitudinal studies

Longitudinal studies, using a standard protocol, are being carried out in selected areas of Bolivia, Chile, Colombia and Panama to analyse factors influencing disease transmission, prevalence of infection and morbidity patterns (Table 6.2). Since serological tests are used for these studies and since serology does not necessarily indicate active infection (which can only be definitively diagnosed by demonstration of parasites in the blood), frequency figures for these studies refer only to seropositivity.

◦ **Bolivia:** The study area is in a low-income rural region in south-eastern Bolivia. Prevalence of seropositivity to *T. cruzi* differs between the three distinct climatic and ecological zones of the region, ranging from 35.3% in a cold, dry zone to 70.4% in a hot, humid zone. Prevalence was greater in older age-groups throughout the study area. The lowest seropositivity rate was observed in the highest mountain locality within the cold, dry zone: the vector may only infest dwellings at lower altitudes, as in other countries, transmitting infection to workers and their families when they migrate from higher, colder areas

TABLE 6.1

Prevalence of seropositivity and triatomine house infestation, 1983-84

Country	Seropositivity			Triatomine house infestation		
	No. of samples	Positive	%	No. of houses	Positive	%
Honduras						
Zone A	991	151	15.2	1862	277	14.9
Zone B	2015	93	4.6	3536	124	3.5
Zone C	401	1	0.2	615	0	0.0
Ecuador (excluding Guayaquil)	532	57	10.7	3602	45	1.2
Paraguay	4037	863	21.4	2003	1192	59.5

(In Honduras, zone A is a high mountain region near the Pacific Coast, zone B is a lower mountain region and includes the Caribbean coastal area, and zone C includes a low-altitude, swampy region in the north-east of the country and several islands in the Caribbean.)

to lower altitudes in response to seasonal work patterns. This study will be completed by entomological surveys to measure house infestation and vector infection rates.

• **Colombia:** The study area is in a rural settlement in north-eastern Colombia and covers 100 square km: one-third is mountainous and the remainder, intensively cultivated plains. Thirty-three percent of the inhabitants were found to be seropositive. No sex differences were observed in prevalence, but there was a correlation between seropositivity and age ($r = 0.958$). In a cross-sectional study of the entire population, electrocardiograms (ECGs) were obtained from 1236 persons: 4.3% of tracings were abnormal and compatible with Chagas' disease. Among these, the most frequent abnormalities were right bundle-branch block (40.7%) and left anterior hemiblock (24.7%). A high correlation ($r = 0.980$) was observed between the frequency of ECG changes and age. Considered together with the correlation between seropositivity and age, this finding implies that the longer infection lasts, the greater is the likelihood of Chagas' cardiopathy developing.

• **Chile:** Studies were carried out in the northern and central parts of the country, including metropolitan Santiago, and covered a wide range of geographical settings, from desert in the north to fertile inter-Andean valleys in the centre of the country. The overall seropositivity rate was 20.3%. No sex difference was seen for prevalence rates, but there was an association between increasing seropositivity rates and age, as was found elsewhere. In a study of 1000 mothers and their newborn infants in metropolitan Santiago, xenodiagnosis was positive in seven seropositive mothers and in three seropositive neonates, again pointing to the importance of congenital transmission (A114, A152). Of the 1130 seropositive in-

dividuals living in the study areas, 26.3% were positive on xenodiagnosis (34.5% of those living in metropolitan Santiago), as were 20% of domestic animals (cats and dogs) tested. Nineteen percent (215) of the seropositive individuals had abnormal ECG tracings compatible with Chagas' disease: right bundle-branch block in 6.6% of the 215 individuals, atrioventricular block in 0.4%, ventricular extrasystoles in 1.2% and sinus bradycardia in 2.6%. The frequency of ECG abnormalities was found to increase significantly with age and to be more common in males than females. There was also a significant correlation between seropositivity and ECG abnormalities (relative risk = 2.01, $\chi^2 = 78.8$).

• **Panama:** Two ecologically and epidemiologically different areas were studied: Chorrera District, Panama Province, in central Panama, and Boquete and Gualaca Districts, Chiriqui Province, in western Panama, each with a distinct parasite transmission cycle. *Rhodnius pallescens* is the vector in central Panama and *Triatoma dimidiata*, a domestic species associated with wooden houses and earthen floors and with domestic animal reservoirs, in western Panama. In Chorrera District 22.3% of inhabitants tested were seropositive and in Boquete and Gualaca Districts, 2.7%. Isoenzyme analysis of parasite strains recovered from man, wild mammals and *R. pallescens* vectors has identified isozymic Types I and III, which were previously associated with domestic transmission in Brazil. Type II, characteristic of sylvatic transmission, has not been found. In a recent study of 25 human *T. cruzi* isolates from rural areas of central Panama, all were identified as Type I. Some findings, such as the correlation between infection and age, clearly apply to all the study areas, whereas others—for example, the seropositivity rate—differ considerably from area to area. Future work will seek the reasons

TABLE 6.2

Initial findings of ongoing longitudinal studies on Chagas' disease

Location	No. of individuals examined	No. sero-positive	Prevalence rate of seropositivity (%)	Frequency of ECG abnormalities in seropositive individuals (%)	Correlation between seropositivity and age	Correlation between abnormal ECGs and age	Evidence of congenital transmission
Bolivia	1414	737	52.1	24.0	+	+	+
Cold, dry zone	573	202	35.3	ND*	+	ND	ND
Hot, dry zone	389	217	55.8	ND	+	ND	ND
Hot, humid zone	452	318	70.4	ND	+	ND	ND
Colombia	1236	408	33.0	4.3	+	+	ND
Chile	5566	1130	20.3	19.0	+	+	+
Panama	1770	314	17.7	ND	+	ND	ND
Santa Cruz**	101	11	10.9	ND	+	ND	ND
Alto Del Jopo**	367	137	37.3	ND	+	ND	ND
Jaramillo Arriba***	302	9	2.9	ND	+	ND	ND
Bajo Mendez***	46	2	4.3	ND	+	ND	ND

* ND = No data

** Gualaca District

*** Chorrera District

for these differences, with special emphasis on relationships between different *T. cruzi* strains and the course of the disease.

Network of Collaborating Laboratories for the standardization of serodiagnosis in Chagas' disease

Serodiagnostic techniques for *T. cruzi* infection had until recently been neither sensitive nor specific enough for epidemiological research. Test results from different laboratories have often not been comparable: an initial, previously reported comparison of test results from laboratories in Argentina, Brazil and the United States showed a low kappa index (ranging from 56 to 86%) in diagnosis of infection. (The kappa index denotes the degree of concordance between pairs, in this case, of laboratories.) Techniques have since been standardized and threshold titres for diagnosis established, and a second comparative study showed a higher kappa index range (70-92%).

The Reference Laboratory in Brazil now provides other laboratories with samples of infected and noninfected sera to enable them to evaluate their own tests: during the past two years 2800 samples have been distributed to 12 laboratories (Fig. 6.2).

Insecticides and other vector control measures

Rural houses and periurban shanty town dwellings are often built with materials which favour triatomine

infestation and breeding. Ways have been sought to improve the use of insecticides in controlling triatomine vectors:

- During the past two years, deltamethrin, a pyrethroid insecticide, has been field-tested in three different formulations: wettable powder, emulsifiable concentrate and flowable formulation. Use of the flowable formulation, which keeps its insecticidal activity for at least one year, may make it possible to reduce the frequency of house spraying to once a year and considerably cut the cost of field operations.

- Another insecticide, malathion, was found to have a much longer-lasting effect when latex was added. The formulation—a colourless paint—can be easily prepared in the field, is well accepted by the community and will be used by the Brazilian Chagas' Disease Control Programme (SUCAM) in a large-scale trial involving 3000 houses in Posse, State of Goias (A103).

- An insecticide fumigant canister for indoor use that is easy to operate and harmless to man and domestic animals is undergoing initial field tests in Argentina in collaboration with the National Chagas' Disease Control Programme.

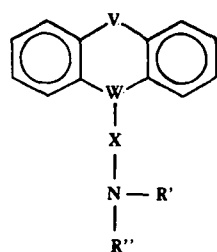
- In a recent Costa Rican study, the cementing of floors in rural dwellings was found effective in preventing *T. dimidiata* reinfestation (A159).

Prevention of transmission by blood transfusion

With massive rural-to-urban migration, transmission of Chagas' disease by infected transfusion blood

FIG. 6.3

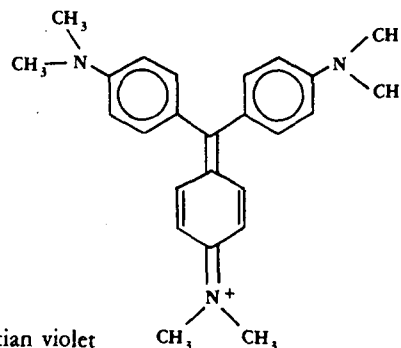
CHEMICAL STRUCTURES OF GENTIAN VIOLET AND AMPHIPHILIC DRUGS ACTIVE *IN VITRO* AGAINST *T. CRUZI*



Amphiphilic drugs



Gentian violet



W may be C, N or O. The chain length *X* = 0-5 atoms. *R'*, *R''* = usually short-chain neutral groups. *V* may be a single atom (C, N, S or O) or two atoms in a chain (C-O, C-C or C-S). (See D. J. Hammond et al. [A 63].)

has become a serious problem in many Latin American cities. Two approaches to solving this problem have been adopted:

Testing blood for infection

Blood banks require simple, reliable and sensitive screening tests. A test for anti-*T. cruzi* antibodies, the G-agglutination test, uses epimastigote fragments coated onto graphite particles. Visible agglutination of the black particles denotes a positive result. This test is now routinely used in a Sao Paulo hospital, in Brazil.

Identification of trypanocidal compounds for the treatment of blood

A simple, rapid *in vitro* method, devised with Programme support, has been used to screen more than 700 compounds for trypanocidal activity in stored blood. All these compounds are already registered for therapeutic use in man. Twenty-one have shown trypanocidal activity at low concentrations. All of them share a similar amphiphilic chemical structure and are related to gentian violet (Fig. 6.3) (A63). (Two compounds also show *in vitro* activity against *Leishmania* [B11].)

A group of experts has selected two of the most promising of these compounds for field study in endemic areas and for comparative assessment with gentian violet.

New serodiagnostic tests

A new enzyme-linked immunosorbent assay (ELISA), based on a monoclonal antibody reactive with a 72 000 MW glycoprotein antigen of *T. cruzi* epimastigotes, has been used to detect serum antibody and appears to be specific for *T. cruzi* infection. It is now being field-tested in Bolivia, where serological differentiation from leishmaniasis is important, and in Venezuela, where differentiation from infection with nonpathogenic *T. rangeli* is a problem (A105). Another stage-specific *T. cruzi* epimastigote antigen (of 25 000 MW) has been characterized and its serodiagnostic potential is being assessed at the Reference Laboratory in Sao Paulo, Brazil (A134). In another study, a radioimmunoassay using biotinylated antibodies and ³H-avidin has been shown to be specific for *T. cruzi* infection in cases of mixed infection involving *T. rangeli* (A145).

In research conducted outside the Programme, a new test, the "Staphylococci adherence test", uses agglutination of staphylococci incubated with *T. cruzi* antigens as an indicator of infection (B2).

Drug development

Basic biochemistry

Different *T. cruzi* strains have shown quantitative differences, *in vitro* and *in vivo*, in the metabolism of several nucleic acid metabolites, notably allopu-

riinol, allopurinol riboside and formycin B. Allopurinol riboside shows the greatest potential for chemotherapy.

9-Deazainosine (9-DINO), an analogue of another nucleic acid metabolite, inosine, has been found to have low toxicity for mammalian cells but to be highly active *in vitro* against *T. cruzi* (A87), a finding that is being further explored as a promising chemotherapeutic lead.

In other studies conducted with Programme support, the *T. cruzi* receptor for cyclic AMP was purified and characterized. Cyclic AMP analogues will be assessed for their ability to prevent parasite multiplication (A119).

Mechanisms of drug action

Electron spin resonance spectroscopy has shown that the nitro compounds currently used in the treatment of Chagas' disease act through the formation of free radical intermediates. Moreover, nitrofurantoin reductase activity, which plays a key role in the action of nitrofurantoin compounds, has been shown to be localized at the mitochondrial membranes of mammalian cells (A38).

The role of light in the trypanocidal action of dyes has also been investigated. Oxidation of rose bengal, for example, has been shown to cause ultrastructural changes in *T. cruzi*—notably, increased cell membrane permeability—that are responsible for parasite lysis (A25).

Screening for anti-T. cruzi activity

A total of 618 new compounds have been screened in mice for suppressive activity against *T. cruzi*. Of these, 95 have been found worthy of further study in chronically infected mice.

Parasite classification

Restriction endonuclease analysis of kinetoplast DNA is providing a new tool of clinical and epidemiological value for the identification of different *T. cruzi* strains: *T. cruzi* schizodemes—populations of the parasite sharing a common restriction endonuclease kinetoplast-DNA pattern—have been identified as markers of parasite strains involved in specific clinical and epidemiological situations (A61). (Schizodemes identify groups of parasites which may be different from those identified by isoenzyme analysis or monoclonal antibody-based assays.) Research conducted outside the Programme has revealed a high degree of correlation between *T. cruzi* classification based on monoclonal antibody reac-

tivities and that based on isoenzyme (zymodeme) patterns (B3-5).

Immunology

Animal models

Research on Chagas' disease has for many years suffered from uncertainty over the appropriateness of different animal models for the study of human infection and disease. At a meeting held in 1984 to evaluate animal models of *T. cruzi* infection and Chagas' disease, it was agreed that different strains of inbred mice could provide a variety of models for experimental chemotherapy and research on the pathology and immunology of infection and disease. Other animal models (rabbits and monkeys, for example) might be useful for the study of certain aspects of chronic Chagas' disease.

The mouse model has also been used to study the interaction of *T. cruzi* with macrophages: parasite surface components involved in this interaction have been identified and their chemical structures defined. Different macrophage receptors have been shown to play a part in epimastigote and trypomastigote recognition (A93).

Immunoprophylaxis

Research is being pursued on the identification of *T. cruzi* mutant strains that induce protection rather than cause disease, and that might therefore form the basis for future vaccines. Variant *T. cruzi* strains that are completely eliminated by an experimental animal host have been produced by successive mutagenic treatments and are currently being studied for their immunoprotective potential (A85, A124).

Several studies have been conducted on the characterization of antigens and other structures of importance in the host-parasite relationship, particularly the parasite's penetration of host cells:

- Antigens present in the immunoprotective flagellar fraction of *T. cruzi*, for example, are being purified and characterized with the help of monoclonal antibodies.
- Oligosaccharides linked to parasite surface proteins have been shown to play an important role in the ability of *T. cruzi* to adhere to and penetrate host cells, and could be a possible target of immunoprophylaxis.
- Antigens that are present on the parasite surface and involved in its penetration of host cells have been characterized and appear to be specific to the trypomastigote stage (A9). Attempts to block penetration

with antibodies to these antigens are currently being made in experiments conducted with TDR support.

- A monoclonal antibody, CE5, which recognizes a crossreacting antigenic determinant on mammalian neurons and *T. cruzi*, has been found not to react with the 90 000 MW glycoprotein that had been credited, on the strength of vaccination experiments, with protective properties (B10).
- In a study conducted outside the Programme, fibronectin, a high molecular-weight glycoprotein present in blood and connective tissue and also on cell surfaces, was found to bind specifically to *T. cruzi* trypomastigotes and may be a recognition site for parasite attachment to host cells (B8).

Immune mechanisms

Murine T-cell lines specifically reactive with *T. cruzi* antigens are being established and their capacity to induce disease will be studied.

In research conducted outside the Programme (B4), lymphocytes from Chagas' patients were found to stimulate heart tissue contractility *in vitro*, possibly through a mechanism involving increased production of arachidonic acid metabolites. In another study conducted outside the Programme, the addition of β -interferon to mouse peritoneal macrophages in culture was found effective in preventing *T. cruzi* bloodstream forms from adhering to and infecting the cells (B7).

Box 6.2 The future

Short-term:

- large-scale trials with insecticide-containing paints and new insecticide formulations, to be conducted with the financial and technical participation of national control programmes;
- field assessment of new monoclonal antibody-based serological tests;
- assessment in endemic areas of the G-agglutination test for routine screening of transfusion blood;

- efficacy, toxicity and safety studies on selected trypanocidal compounds for routine use in endemic areas as additives to transfusion blood;
- cost-effectiveness and operational analyses for the planning and operation of control programmes.

Long-term:

- development of a curative drug for all stages of Chagas' disease;

- identification and characterization of parasite antigens for safe and effective immunoprophylaxis;
- studies on the relations between different parasite subpopulations, and different clinical forms and geographical varieties of Chagas' disease;
- studies on the immunopathogenesis of irreversible chronic Chagas' lesions.

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Chagas' disease

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7 The leishmaniases

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Note on lists of publications

Publications and other documents relating to the subject of this chapter are listed, at the end of the chapter, under three headings: Publications acknowledging TDR support (list A), Publications from work outside the Programme (list B) and TDR scientific reports which became available in 1983-84 (list C). Since most of the work described in the Report is too recent to have appeared in the literature, only a small proportion of publications listed relate directly to the text. List A comprises publications acknowledging TDR support that have not appeared in any previous Programme Report and list B, only those publications pertinent to specific points discussed in the text. All the documents in list C can be obtained by writing to: The Office of the Director, TDR, World Health Organization, 1211 Geneva 27, Switzerland; requests should carry the full document title and reference number.

7 The leishmaniasis

The context

□ The leishmaniasis, diseases caused by infection with the flagellate protozoan parasite *Leishmania*, occur in three major forms: visceral, mucocutaneous and cutaneous.

Visceral leishmaniasis (VL), in which the parasite invades internal organs (spleen, liver, bone marrow, etc.), is usually lethal if untreated. It is endemic in several parts of Africa, the Indian subcontinent and Latin America, and occurs sporadically in China, the Mediterranean Basin, South-West Asia and southern parts of the Soviet Union. In 1977, an epidemic of VL, or kala-azar as it is called in India, occurred in Bihar State, India, affecting 100 000 people (according to a survey of the Indian National Institute of Communicable Diseases (B8)), and for 1978 a conservative estimate of 40 000 cases was reported.

Mucocutaneous leishmaniasis (MCL) is primarily found in South America, although cases have also been reported in Africa, notably Ethiopia and the Sudan. The disease begins with a primary skin lesion, followed several years later by metastasis to the oronasal or pharyngeal mucosa. These degenerative mucocutaneous lesions can be mistaken for leprosy and carry something of the stigma and socioeconomic problems experienced by many leprosy patients.

Cutaneous leishmaniasis (CL), the most prevalent form of leishmaniasis, is found in Africa, Latin America, the Indian subcontinent, South-West Asia, and parts of the Mediterranean Basin and of the Soviet Union. Uncomplicated cutaneous lesions heal within nine months (in the Middle East) to two years (in Central and South America). However, non-healing lesions occur, as in diffuse cutaneous leishmaniasis (found in Ethiopia), leishmaniasis *recidivans* (in the Middle East) and post-kala-azar dermal leishmaniasis (PKDL) (in East Africa and India). Even uncomplicated CL is associated with high morbidity, a disfiguring lesion and life-long scarring. Loss of worktime due to CL, especially in new development projects, can have important economic consequences. (Projects in forest areas of Brazil and desert

areas of Saudi Arabia, for example, were curtailed due to the high incidence of CL in these areas.)

Leishmaniasis are transmitted by sandflies of the *Phlebotomus* (Old World) and *Lutzomyia* (New World) genera. They require humid but not wet conditions for breeding and live on a wide range of organic materials. Females generally require a vertebrate blood meal for development, although autogenous development has been reported.

Since larval growth and adult activity are depressed at temperatures well below 20°C, sandflies are generally found only in areas with a mean temperature of 20°C for at least one month of the year (the time it takes for the insect to develop from egg to adult). They have also been reported in areas where leishmaniasis does not occur.

The ecological systems maintaining the different *Leishmania* species in different areas vary from place to place. In India and in central Kenya man is thought to be the reservoir host for *L. donovani*, the species responsible for kala-azar. In almost all other endemic areas of the world, i.e. north-western Africa, South America and southern Europe, the dog is the reservoir. Other animals—foxes (in France and the Islamic Republic of Iran), rats (in Italy) and jackals and wolves (in the Soviet Union)—have also been implicated as *L. donovani* reservoir hosts.

At present there is no diagnostic test for early *Leishmania* infection. Definitive diagnosis depends on isolation and identification of the parasite from biopsy material. In visceral leishmaniasis this is a serious problem, since biopsies of bone marrow, spleen or liver are required. Serological tests give a presumptive indication of infection and are positive in advanced disease.

The only drugs available to treat the leishmaniasis are pentavalent antimony compounds, which are toxic and require repeated injections given under close supervision for three weeks or more. Common side-effects include vomiting, nausea, malaise, headache, lethargy and electrocardiographic (ECG)

changes. Current treatment schedules are, moreover, not always successful.

A form of vaccination has long been practised against CL in parts of the Middle East: an infective dose of the parasite is administered at a chosen site of the body in order to prevent naturally occurring lesions from developing on the face or in multiple sites. Following this "leishmanization" procedure, the disease runs its normal course and, after the lesion has healed, leaves the patient with strong immunity to the parasite.

Vector control is not always feasible in zoonotic leishmaniasis. However, in anthroponotic forms (such as kala-azar or some forms of CL) vector control programmes directed primarily against malaria have also been very effective against leishmaniasis. Indeed, cessation of antimalarial insecticide application has been suggested as the cause of the 1977-78 outbreak of leishmaniasis in Bihar (B7).

Scientific interest in the leishmaniasis has increased significantly in recent years. Over the past decade, the number of published scientific papers on these diseases—of which those arising from TDR support constitute a considerable proportion—has risen markedly (Fig. 7.1). The availability of animal models and the ease with which *Leishmania* can be grown *in vitro* are two reasons why experimental leishmaniasis is now widely used as a model for studying the host-parasite relationship in general. Research on the leishmaniasis, however, is still in its infancy, despite the fact that the diseases have been known for centuries and their causes for more than 70 years. There is little doubt, though, that if adequate support is now provided for a concerted, sustained research effort, effective agents to treat and control these diseases, including drugs, diagnostic tests and even vaccines, could be developed within a reasonably short period of time. □

Box 7.1 Highlights of activities in 1983-84

- Studies on nucleotide metabolism in *Leishmania* have led to the development of allopurinol riboside, a nucleic acid analogue now undergoing a limited Phase II clinical trial.
- Monoclonal antibodies and cloned kDNA probes specifically recognizing various *Leishmania* species have facilitated the identification and characterization of the parasite and are being used to develop new diagnostic tests.
- It has been found that some types of T-lymphocytes can aggravate leishmanial lesions in mice, a finding pertinent to research on new approaches to vaccine development.
- Fourteen strains of *Leishmania* have been selected as reference strains to be used by all laboratories involved in the identification and characterization of these parasites.

Report of activities in 1983-84

Disease distribution

An initial goal of the Scientific Working Group (SWG) on the Leishmaniasis was to assess the distribution and public health importance of leishmaniasis throughout the world. It soon became apparent that the public health importance of these diseases was far greater than had been previously suspected (C3).

Reliable estimates of the prevalence of the leishmaniasis are not available for all countries reporting cases of the diseases. New disease foci have been discovered, through TDR-supported projects, in Brazil and Ethiopia (A6) and, through work conducted outside the Programme, in Algeria (B1) and Egypt. Other foci have spread: from Bihar, India, VL has moved into Nepal, south of the Ganges River, and eastwards into West Bengal (according to the National Institute of Communicable Diseases in

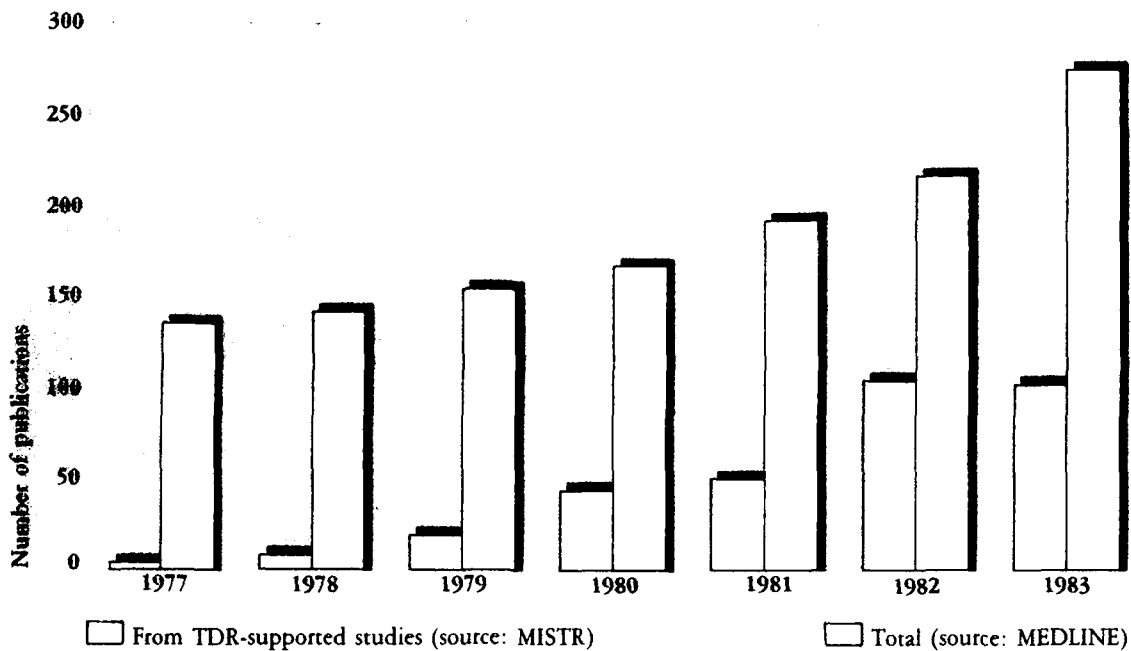
Patna, India). Incidence rates, too, have changed, reportedly rising on both sides of the border between the Islamic Republic of Iran and Iraq, and in some parts of the Jordan Valley. The Programme is supporting field research on in-depth assessment of transmission foci in selected areas with a view to improving control of the disease.

The parasite

Parasite identification is important for several reasons (A123). First, from an epidemiological and disease control standpoint, it is important to know whether an organism causing disease in a given area is of the same biotype as that found in local sandfly vectors or in putative animal reservoirs. Second, the cutaneous lesions marking the early stages of the disease do not necessarily indicate its final outcome, particularly in areas where CL and MCL coexist, so

FIG. 7.1

PUBLICATIONS RELATING TO THE LEISHMANIASES



that early treatment and subsequent management must be decided on the basis of the causative organism. Finally, different vaccines may be required against leishmanial infections caused by different parasites, which must therefore be differentiated as precisely as possible. (This is pointedly illustrated by the recent finding, in CL lesions, of a parasite, *L. infantum*, previously associated only with infantile VL.)

In 1984, a meeting was held in Montpellier, France, to set up a means of coordinating the activities of centres involved in the identification and characterization of *Leishmania* and to select international *Leishmania* reference strains (A151). There are now three major techniques for the identification of *Leishmania*: isoenzyme electrophoresis, kinetoplast DNA (kDNA) hybridization and monoclonal antibody-based assays. The meeting adopted a list of reference strains to be used by all centres (Table 7.1). These strains will be deposited at the American Type Culture Collection (ATCC) in Rockville, MD, in the United States, for wide distribution to scientists and will be exchanged and preserved in laboratories designated as regional/subregional identification and cryobank laboratories. The activities of all International Reference Strain Laboratories (IRSL) will be coordinated through the use of a standard questionnaire, a common database and compatible

computer systems and should yield valuable information on the relationship between parasite characteristics and the clinical manifestations of leishmanial infections.

In several countries—Algeria, Bolivia, Brazil, Colombia, Peru and the United States—laboratories have received TDR support to set up or expand facilities for identifying and characterizing *Leishmania*, using isoenzyme analysis, monoclonal antibody-based assays (A62) and kDNA hybridization (A152).

Three laboratories in, respectively, Peru, the United Kingdom and the United States, are receiving TDR support to study the use of kDNA probes in parasite characterization. The results of these studies are already being applied to field diagnosis of the leishmaniasis, and suitable test systems are being developed.

The vector

A greater understanding of vector biology is needed in order to improve disease control. There have been several developments in this area:

- Sandfly colonies have been raised in a number of

TABLE 7.1

International reference strains designated by TDR's Scientific Working Group on the Leishmaniases, and other well-characterized *Leishmania* reference strains

Species	Strain designation	Analysis by:			Comments
		kDNA	Isoenzyme	Monoclonal antibodies	
International reference strains					
<i>L. donovani donovani</i>	MHOM/IN/80/DD8	+	+	-	Infective in hamsters
<i>L. d. infantum</i>	MHOM/TN/80/LEM235	-	+	-	Infective in hamsters
<i>L. d. chagasi**</i>	MHOM/BR/74/M2682	+	+	+	Infective in hamsters
<i>L. major</i>	MHOM/SU/73/5-ASKH	+	+	-	Infective in hamsters
<i>L. tropica</i>	MHOM/SU/74/K27	-	+	-	
<i>L. aethiopica</i>	MHOM/ET/72/L100	+	+	+	
<i>L. mexicana mexicana</i>	MHOM/BZ/82/BEL21	+	+	+	Infective in hamsters
<i>L. m. amazonensis</i>	MHOM/BR/73/M2269	+	+	+	Infective in hamsters
<i>L. m. pifanoi**</i>	MHOM/VE/57/LL1	+	+	-	
<i>L. m. garnhami**</i>	MHOM/VE/76/JAP78	+	+	+	
<i>L. m. venezuelensis</i>	MHOM/VE/00/H17	-	+	+	Infective in hamsters
<i>L. braziliensis braziliensis</i>	MHOM/BR/00/LTB0014(L)	+	+	+	Infective in hamsters
<i>L. b. panamensis</i>	MHOM/PA/71/LS94	+	+	-	
<i>L. b. guyanensis</i>	MHOM/BR/75/M4147	+	+	+	Infective in hamsters
Other well-characterized <i>Leishmania</i> strains*					
<i>L. donovani</i> s. sp.	MHOM/ET/67/L82	+	+	+	Infective in hamsters
<i>L. major</i>	MHOM/IL/67/Jericho II	+	+	+	Infective in hamsters
<i>L. major</i>	MRHO/SU/59/P	+	+	+	Infective in hamsters
<i>L. tropica</i>	MHOM/SU/60/LRC-L39	+	+	+	
<i>L. m. mexicana</i>	MNYC/BZ/62/M379	+	+	+	Infective in hamsters
<i>L. m. amazonensis</i>	IFLA/BR/67/PH8	+	+	+	Infective in hamsters
<i>L. b. braziliensis</i>	MHOM/BR/75/M2903	+	+	+	Infective in hamsters
<i>L. enriettii</i>	MCAV/BR/45/L88	+	+	+	Infective in hamsters and guinea-pigs
<i>L. hertigi hertigi</i>	MCOE/PA/65/C8	+	+	+	
<i>L. h. deanei</i>	MCOE/BR/74/M2674	-	+	-	Infective in hamsters
<i>L. aristidesi</i>	MORY/PA/69/GML3	+	+	+	
<i>L. gerbilli</i>	MRHO/CN/60/GERBILLI	+	+	-	

* Strains which do not meet all criteria for designation as international reference strains

** Controversy exists as to whether these represent distinct taxa

laboratories and a complete experimental transmission cycle has been established.

- A sandfly pheromone has been discovered which may lead to insights on sandfly behaviour and ultimately to better vector control (A139).
- An association has been found between fungal (yeast) or bacterial infection in the sandfly and the absence of *Leishmania* in the vector, a finding which may have potential for vector control with microorganisms such as yeasts and which is therefore being further explored in TDR-supported studies.

Host reservoirs

Results of field research include the identification of possible host reservoirs, notably jackals in Algeria,

opossums in the Amazonas State of Brazil and rats in Peru.

Chemotherapy

Improved therapeutic drug regimens

Previous TDR-supported research indicated that for the treatment of VL in Kenya longer regimens and higher doses of pentavalent antimony compounds are needed than are currently used. This research has continued and is now about to begin in India, where there is evidence that a drug regimen different from that required in Kenya may have to be used. Findings of the Kenya studies formed the basis of the recommendations of a WHO Expert

Committee on the Leishmaniases (B9).

Drug screening

Most pharmaceutical firms are reluctant to invest in the costly development of new antileishmanial drugs for a market that is not commercially attractive. The Programme is therefore seeking alternative approaches. Support is being given, for example, to university laboratories to screen compounds obtained from the pharmaceutical industry or other sources. If a compound with the desired activity is identified, further development is discussed with the supplier. TDR has entered into two agreements, one with the Walter Reed Army Institute of Research in Washington, DC, USA, and the University of Georgia, Athens, GA, USA, the other with Merck, Sharp and Dhome of Rahway, NJ, USA, and the University of Georgia. One highly active compound against VL has been identified through the first contract and is being further developed. In all, several thousand compounds have been assessed, two of which have been selected for clinical study. With the aim of saving the time and cost of developing a completely new drug, drugs already in use for other diseases are also being screened.

Basic research

In a more fundamental approach to the development of new drugs, parasite physiology and biochemistry are being studied with a view to identifying parasite-specific metabolic pathways and enzymes that could serve as chemotherapeutic targets. Parasite-specific 3'-nucleotidase and acid phosphatase activities have been discovered (A40-41) and are being studied for their possible application to diagnosis and assessment of parasite infective load, and as possible drug targets.

Whereas mammalian cells depend primarily on *de novo* purine synthesis, *Leishmania* have been shown to possess a purine salvage pathway (A12, A57, A92). This finding led to the discovery that some purine analogues, such as allopurinol, a compound used in the treatment of gout, have strong antileishmanial activity (A93). Research on allopurinol metabolism in *Aotus* monkeys showed allopurinol riboside, an allopurinol metabolite, to be the active component. It is now being tested against cutaneous leishmaniasis in a collaborative TDR-supported study involving the pharmaceutical industry, the University of Colorado (Denver, CO, USA) and the Gorgas Memorial Laboratory (Panama City, Panama). Meanwhile, studies on nucleic acid metabolism are continuing and new purine analogues are being tested *in vitro*.

Other activities receiving TDR support in this area include:

- The development of an *in vitro* screen to test drugs for activity against amastigotes: two laboratories, in the United States and the United Kingdom, respectively, are using macrophage-like cell lines and primary mouse macrophage cultures to screen compounds.
- An ointment has been developed for the topical treatment of CL (A30) and is being clinically tested in collaboration with industry.
- Sinefungin, a nucleotide analogue produced by a fungus, and its derivatives are being studied as part of the search for a nontoxic antileishmanial drug.

Outside the Programme, local heat treatment has been reported to be effective against American CL, liposome-encapsulated antileishmanial drugs are being studied and two analogues of the antidepressant imipramine, 3-chloroimipramine and 4-nitroimipramine, have been shown to have leishmanicidal activity *in vitro*.

Immunology and biochemistry

Vaccine development

Several studies, both within (A103) and outside the Programme, have shown that avirulent *Leishmania* clones or, in some cases, killed organisms have immunizing potential in various mouse strains, although they must be given intravenously or intraperitoneally in high doses. Subcutaneous injections are detrimental and block subsequent, otherwise protective, vaccination in mice (B4).

The mechanism whereby protection is induced is also being actively studied. Two studies on the characterization of protective immune responses in the mouse model, one carried out within (A148), the other outside the Programme (B3), have shown that certain *Leishmania*-specific T-cell clones can enhance the disease or abrogate protective immunity.

In two studies on CL being conducted outside the Programme, one in Brazil (B5), the other in the Islamic Republic of Iran, human subjects were vaccinated with, respectively, leishmanial extracts and live virulent organisms. The results of the first study were encouraging but inconclusive, as incidence rates were low in both control and vaccinated groups. In the second, the vaccine produced a lesion similar to those of the natural infection. In contrast to findings in the mouse model, neither of these human studies indicated a deleterious effect of exposure to leish-

manial antigen administered by the cutaneous route. A meeting on CL is planned for 1985, at which a workplan and specific protocols for the next stage of vaccine development will be established.

Diagnostic tests

TDR is giving support to several studies on possible diagnostic tests based on monoclonal antibody techniques (A60) or an enzyme-linked immunosorbent assay (ELISA) using leishmanial parasite fractions. These techniques have been tested in the laboratory with some success and will be simplified for field use. In another study, a simple, sensitive slide test is being developed for serodiagnosis of leishmaniases.

Basic research on immunology and biochemistry

The establishment of *Leishmania*-specific T-cell clones and the possibility of studying their functions *in vitro* and *in vivo* have advanced research on the host-parasite relationship (A33, A87). A number of findings have emerged from TDR-supported research in this area. Lymphokines have been shown to induce

macrophage-mediated killing of *Leishmania in vitro* (reviewed in A11). Moreover, interleukin-1 (IL-1) production has been found to be greater in macrophages from resistant than in those from susceptible mice, and mouse macrophage IL-1 production is diminished by *L. major* infection and increased by *L. braziliensis braziliensis* infection (a finding consistent with *in vivo* susceptibility and resistance, respectively, to the two organisms, at least in the mouse model used for these studies).

Several studies on *Leishmania* antigen and membrane composition have been conducted with TDR support (A49, A116). Membrane proteins and lipophosphoglycans have been shown to possess unique components, some of which might be useful for diagnostic tests and as chemotherapeutic targets.

The genetic control of susceptibility to *Leishmania* in mice (B2) and the mechanism of parasite killing by macrophages have been extensively studied in work conducted outside the Programme. Doubt has been cast on the correlation between delayed hypersensitivity and protective immunity, and between T-cell phenotypes and their immunoprotective role in mice (B4).

Box 7.3 The future

Epidemiology, parasitology, vector biology and control:

- in-depth studies on selected disease foci to elucidate transmission cycles and develop simple control measures, and to identify sites suitable for trials of vaccines or new diagnostic tests as they are developed;
- increased efforts to isolate, characterize and cryopreserve parasites with a view to relating different characteristics of *Leishmania* to different disease manifestations in various parts of the world and thereby providing detailed information of importance for disease control;
- studies on the vector-parasite relationship for the development of simple disease control measures;
- epidemiological studies in selected, potentially high-risk areas.

Chemotherapy:

- further trials of allopurinol riboside (depending on the outcome of current trials) and continuation of the search for other, related compounds;
- *in vitro* followed by *in vivo* screening of selected drugs already available for human use and of other compounds;
- development of compounds found in TDR screening tests and elsewhere (as in the case of antidepressants) to have antileishmanial activity;
- improvement of regimens using currently available antileishmanial drugs.

Immunology and biochemistry:

- development of new, simple tests, and simplification of currently available diagnostic tests based on monoclonal antibodies or kDNA probes and their adaptation for field trials;
- studies on mechanisms of protective immunity in man and experimental animals;
- assessment of the protective role of avirulent parasites and parasite fractions;
- continued studies on the metabolism of *Leishmania* with a view to the identification of unique pathways or components for drug targeting and diagnosis;
- review of previous experience of vaccination in man and development of plans and protocols for vaccine studies.

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The leishmaniasis

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- C4. TDR. Report of the workshop on chemotherapy of old world cutaneous leishmaniasis, Jerusalem, 14-16 February 1983. Document TDR/LEISH/CL-JER/83.3. (English only)

8 Leprosy

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Note on lists of publications

Publications and other documents relating to the subject of this chapter are listed, at the end of the chapter, under three headings: Publications acknowledging TDR support (list A), Publications from work outside the Programme (list B) and TDR scientific reports which became available in 1983-84 (list C). Since most of the work described in the Report is too recent to have appeared in the literature, only a small proportion of publications listed relate directly to the text. List A comprises publications acknowledging TDR support that have not appeared in any previous Programme Report and list B, only those publications pertinent to specific points discussed in the text. All the documents in list C can be obtained by writing to: The Office of the Director, TDR, World Health Organization, 1211 Geneva 27, Switzerland; requests should carry the full document title and reference number.

8 Leprosy

The context

□ About 1.4 billion people—nearly a third of the world's population—live in leprosy-endemic areas, mostly in Africa and Asia, and more than a third of the (conservatively) estimated 10.6 million leprosy patients in the world face the threat of permanent, progressive physical disability, often with its concomitant of social rejection. Indeed, in many countries the social dimensions of the disease carry its public health impact far beyond the extent suggested by prevalence rates.

A major problem in leprosy treatment has been the growing resistance of *M. leprae* to dapsone, the only cheap, safe and effective antileprosy drug and one that has been used widely for almost 40 years as monotherapy for leprosy. Dapsone resistance is now widespread and increasing: alternative drugs are urgently needed. Over the past 15 years, secondary resistance (appearing during the course of treatment) has been reported with increasing frequency. Whenever dapsone resistance has been sought among

treated or relapsed lepromatous leprosy (LL) patients, it has been found, and its prevalence has been steadily increasing in many countries.

Primary resistance (confirmed before treatment) presents an even more disturbing picture and seems to be increasing more quickly than secondary resistance. As a countermeasure, the use of combined drug regimens has been proposed by a WHO study group, on the strength of research conducted under the Scientific Working Group (SWG) on the Chemotherapy of Leprosy (THELEP). Based primarily on intermittent administration of rifampicin, these regimens are simple, operationally practicable, and effective, and have been widely accepted for use in control programmes. Before 1983, controlled clinical trials of combinations of existing bactericidal drugs were begun in Bamako (Mali) and Chingleput (India), and plans were drawn up for a field trial of combined therapy in patients with previously untreated paucibacillary leprosy. In the long run, though, cur-

Box 8.1 Highlights of activities in 1983-84

• Leprosy vaccines are now being studied in human subjects. A Phase I trial of a killed-*Mycobacterium leprae* vaccine, involving 31 Norwegian volunteers, was completed and showed the preparation to be safe, as well as effective in inducing sensitization. Another study, conducted in Malawi in over 2000 volunteers, compared three vaccine preparations (BCG, killed *M. leprae* and a mixture of both) and showed the mixed vaccine to be capable of inducing sensitization in 73 to 87% of vaccinated individuals (depending on dose), vs. only 63% for killed *M. leprae* alone and 42% for

BCG alone. A vaccine prophylaxis trial, using a combination of killed *M. leprae* and BCG, involving over 60 000 household contacts of leprosy patients, began in Venezuela. Other trials are shortly to begin in other countries.

• Ten *M. leprae*-specific monoclonal antibodies were prepared and will be used to develop immunodiagnostic tests for field use.

• Alarming high prevalence rates of primary resistance to dapsone were reported in some areas. Generally, primary resistance (in previously untreated patients) is becoming increas-

ingly widespread. Arising against a backdrop of widespread secondary resistance (following drug treatment), this development calls for the use of combination or "multidrug" therapy for all leprosy patients.

• Preliminary findings of field trials conducted in India on multidrug regimens suggest that combination therapy is operationally feasible: the regimens tested were well accepted and found to be free of serious side-effects. Although it is too early to assess the efficacies of the different regimens, none has so far been associated with clinical deterioration.

rently available drugs may lose their effectiveness. The search for new and better drugs must therefore continue. In spite of some progress in chemotherapy, a method of primary prevention is urgently needed. BCG vaccination has been tested in large-scale prospective trials and found to give only a modest degree of protection. The development of a more effective vaccine is clearly of high priority.

Report of activities in 1983-84

Immunology of leprosy

Vaccine development

There are two approaches to leprosy vaccination being considered by the Scientific Working Group (SWG) on the Immunology of Leprosy (IMMLEP): immunoprophylaxis, which is designed to protect people at risk, and immunotherapy, which, in combination with chemotherapy, is designed to rid lepromatous leprosy (LL) patients of their disease through stimulation of specific cell-mediated immunity. Potential vaccines could be of three types: 1) a killed-*Mycobacterium leprae* vaccine designed exclusively for immunoprophylaxis on the assumption that an immunologically naïve population could be primed against *M. leprae*-specific antigens; 2) a killed or live vaccine prepared from cultivable mycobacteria and providing crossreactive immunity against *M. leprae* (the first tests of such a vaccine, using BCG, gave varying rates of protection in different parts of the world); 3) a vaccine consisting of killed *M. leprae* and live BCG (such a vaccine has already been used for immunotherapy, although its immunological rationale remains unclear [B2]).

Before field trials can be carried out on a vaccine preparation, it must first be shown to be capable of inducing long-lasting, delayed-type hypersensitivity or cell-mediated immunity, as well as skin-test conversion, in healthy individuals from nonendemic countries (i.e. in a non-exposed population) and of doing so at doses causing minimal side-effects. Similar studies would later be required in leprosy-endemic areas.

It is widely accepted that untreated polar lepromatous patients manifest no sensitivity to *M. leprae* antigens and have negative skin reactions to lepromin. Despite effective chemotherapy, most remain lepromin-negative throughout their lives and have a significant risk of relapse. It is believed that such patients would benefit if they could be sensitized to

Epidemiological research on leprosy is currently based on the study of well-established cases. There is now good evidence that only a proportion of infected individuals actually develop overt disease. A test for early, subclinical infection would undoubtedly facilitate epidemiological study of the disease, which would in turn provide data essential for future control efforts. □

M. leprae. Recent work by Convit and co-workers indicates that lepromatous patients given intradermal injections of a mixture of live BCG and killed *M. leprae* acquire delayed-type hypersensitivity to soluble *M. leprae* antigen and Mitsuda reactivity to integral lepromin. These results have stimulated renewed interest in immunotherapy: a standard protocol has been developed to compare the effectiveness of BCG alone, killed *M. leprae* alone and a mixture of the two in converting to positivity skin reactions to soluble *M. leprae* antigen in smear-negative multibacillary patients.

Vaccine development and M. leprae production

The infected armadillo is currently the only practical source of *M. leprae*. Four armadillo colonies maintained in the United States and one in the United Kingdom supply infected tissues to the *M. leprae* bank at the National Institute for Medical Research in London, where nearly 14 kg of infected tissue, with *M. leprae* counts greater than 10^9 per g of tissue, was in stock by December 1984. Sixteen investigators have received a total of nearly 3 kg of infected tissue and another 52 investigators, nearly 2 g of purified *M. leprae* and cell-free extract equivalent to 138 mg of protein. The bank has also supplied nearly 4 kg of tissue to the Wellcome Research Laboratories in Beckenham, in the United Kingdom, for production of skin-test antigens and a killed-*M. leprae* vaccine preparation. The Programme continues to be the most important, if not the sole, source of *M. leprae* for scientists throughout the world.

Human sensitization studies

The first Phase I vaccination trial, involving intradermal inoculation of $1.5 - 5 \times 10^8$ killed

TABLE 8.1

Skin-test conversion rates* following vaccination with BCG and BCG + killed *M. leprae* (B2)

Vaccine	Initial conversion rate (% at 60 days)	Initial mean skin-test diameter (mm)	% still positive at:	
			8 mo.	14 mo.**
BCG	92 (166 of 180)	15.0	51 (60 of 118)	58 (40 of 69)
BCG + <i>M. leprae</i>	98 (302 of 308)	21.2	86 (176 of 204)	81 (75 of 93)

* Positivity denoted by reaction of ≥ 10 mm in diameter

** Evaluated in only one of the two study areas

M. leprae in 31 volunteers previously vaccinated with BCG, has been completed in Norway. The preparation caused no serious systemic or local side-effects. Local reactions (ulceration) were acceptable and of the same magnitude as those produced by smallpox vaccination. A subsequent skin-test using soluble *M. leprae*-derived antigens showed that all doses of vaccine had induced sensitization in a vaccine dose-related manner. Similar studies on two volunteer populations not previously vaccinated with BCG are due to begin in the United States early in 1985.

A sensitization study of 2100 healthy volunteers in Malawi, comparing the effects of BCG, killed *M. leprae* and a mixture of both, was completed late in 1984 and showed that: side-effects were generally well tolerated, although there was a high rate of ulceration; the response to a soluble skin-test antigen prepared by the Convit method appeared to be more specific than that to an antigen prepared by the Rees method; sensitization occurred more frequently with killed *M. leprae* plus BCG (in 73% of individuals receiving a low dose and in 87%, a high dose) than with killed *M. leprae* alone (42% and 63%, respectively) or with BCG alone (39%—only one dose).

Trials in Venezuela

A trial of immunoprophylaxis involving about 69 000 leprosy contacts began in May 1984 in three states in Venezuela. The contacts, both household and non-household, are initially skin-tested with a soluble *M. leprae* antigen. All household contacts are included in the trial, whatever their skin-test reaction, as are all non-household contacts with a skin-test reaction of less than 10 mm in diameter and a sample of non-household contacts with stronger reactions. A total of 28 000 subjects is anticipated, one-half to receive a mixture of BCG with *M. leprae* and the other half, BCG alone. Follow-up will consist of repeat skin-testing of a proportion of subjects at two months, re-testing of all subjects at one year and periodic examination of all subjects for evidence of

leprosy. No significant adverse reactions to the vaccine have been seen in the few hundred subjects vaccinated so far.

An earlier study conducted by Convit and co-workers (B3) assessed the ability of BCG alone and of BCG plus killed *M. leprae* to convert to skin-test positivity (10 mm or more) individuals reacting negatively (0-9 mm) to the soluble skin-test antigen. The addition of *M. leprae* to BCG increased the conversion rate, the magnitude of skin-test reactions to *M. leprae* antigen and the duration of sensitization (Table 8.1).

Samuel and co-workers obtained similar findings in another study (B9). Vaccination with killed *M. leprae* plus BCG induced skin-test positivity to the Rees antigen, which was sustained for up to eight months.

Vaccine studies using M. leprae-related mycobacteria

Studies in India (conducted outside the Programme) have continued on two vaccines derived from cultivable mycobacteria: "ICRC" bacillus (B4) and mycobacterium "W" (B10). Results to date have been similar to earlier skin-test conversion findings in patients and patient contacts, with an improvement in polar lepromatous (LL), borderline lepromatous (BL) and borderline leprosy (BB) patients. However, in a recent study using the mouse footpad model, neither vaccine was effective in preventing *M. leprae* infections.

Future vaccine research

The armadillo is expected to remain the only major source of *M. leprae* antigen for the next few years and armadillo colonies will have to be maintained at a cost accounting for about half of the IMMLEP budget. Human sensitization studies in nonendemic and endemic areas should be completed in 1985-86 and ought to provide more information on optimal

vaccine dose, side-effects and duration of sensitization. Different types of vaccines will also be studied in attempts to sensitize smear-negative LL patients. If successful, these studies will be extended to include a trial of vaccines used therapeutically in smear-positive patients.

Large-scale vaccine trials are planned, in Africa and Asia, and will take into account the hitherto unexplained geographical variability in the protective efficacy of BCG against leprosy and tuberculosis. Pre-vaccination studies will be carried out to determine

which vaccine should be used (killed *M. leprae* with BCG or killed *M. leprae* alone). Vaccine trials are also being considered in highly endemic areas, using seroconversion to monitor transmission among vaccinated individuals. Trials to determine the protective efficacy of vaccines against multibacillary leprosy will eventually require studies conducted over long periods of time on large populations. Advantage will be taken of all vaccine trials and related studies to obtain more information on the natural history of the disease.

TABLE 8.2

Characterization of monoclonal antibodies to *M. leprae* submitted to the IMMLEP monoclonal antibody bank

<i>IMMLEP monoclonal antibody reference</i> ^a	<i>Mycobacterial reactivity</i> ^b	<i>Antigen recognized</i> ^c
1. MC-2924	Crossreactive	Carbohydrate/lipid
2. MC-4311	Crossreactive	Carbohydrate/lipid
3. MC-5205	Crossreactive	Protein(s)
4. MC-6225	Crossreactive	Carbohydrate/lipid
5. MC-0401	Crossreactive	Protein(s)
6. MC-1433	<i>M. leprae</i>	Phenolic glycolipid
7. MC-2009	Crossreactive	Protein(s)
8. MC-2404	<i>M. leprae</i>	Protein(s)
9. MC-2817	<i>M. leprae</i>	Phenolic glycolipid
10. MC-3131	<i>M. leprae</i>	Phenolic glycolipid
11. MC-3316	<i>M. leprae</i>	Protein(s)
12. MC-3607	<i>M. leprae</i> (?)	Protein (35 KD)
13. MC-3906	Crossreactive	Carbohydrate/lipid
14. MC-4027	<i>M. leprae</i>	Protein (200 KD)
15. MC-4220	Crossreactive	Protein(s)
16. MC-4732	"Sticky"	"Sticky"
17. MC-5802	<i>M. leprae</i>	Phenolic glycolipid
18. MC-5828	<i>M. leprae</i>	Protein (36 KD)
19. MC-6630	Armadillo liver homogenate	Protein (95 KD)
20. MC-7029	Armadillo liver homogenate	Protein (95 KD)
21. MC-8026	<i>M. leprae</i>	Protein (18 KD)
22. MC-8610	Crossreactive	Carbohydrate/lipid
23. MC-8908	<i>M. leprae</i>	Protein (12 KD)
24. MC-9215	<i>M. leprae</i>	Protein(s)

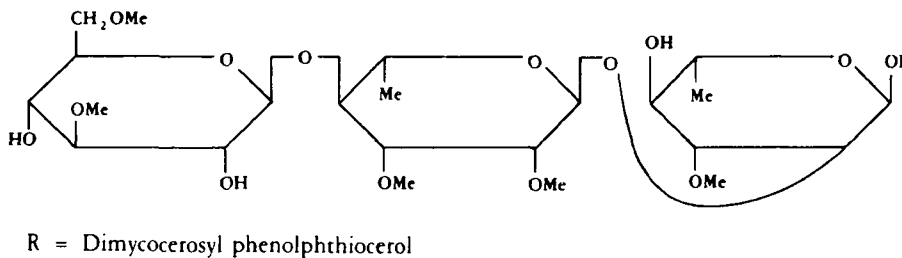
^a Monoclonal antibodies were sent "blind" for analysis to seven laboratories which used a variety of methods, including enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), electrophoretic and immunoblotting techniques, crossed immunoelectrophoresis and indirect immunofluorescence assay (IFA). Samples 8 and 11 were aliquots of the same monoclonal antibody, as were samples 9 and 17.

^b Crossreactivity was tested by ELISA, dot blots and IFA using 25 different species of mycobacteria.

^c Antigens were classified as protein in nature if they were sensitive to proteolytic enzymes and as carbohydrate/lipid if they were resistant to proteolysis. Phenolic glycolipid reaction was assayed by ELISA with deacylated phenolic glycolipid.

(For further details on certain of these monoclonal antibodies and methods of analysis, the reader is referred to the following publications: A22, A40, A76, A77, B5, B7-8 and Abe *et al.*, *International Journal of Leprosy*, 48: 109-119 [1980]).

FIG. 8.1

STRUCTURE OF PHENOLIC GLYCOLIPID-I TERMINAL TRISACCHARIDE SPECIFIC TO *M. LEPRAE***Molecular biology**

Recombinant DNA technology could be usefully applied to three main aspects of vaccine research and immunodiagnosis: taxonomic assignment, based on DNA characteristics, of *M. leprae* and related organisms; identification and isolation of genes coding for antigens with protective and diagnostic potential; and large-scale production of such antigens in relatively pure form (although polypeptide antigens produced in this way may not themselves be highly immunogenic). Much current work on vaccine development, including research on other vaccines conducted under the newly established WHO Programme for Vaccine Development, is based on these new approaches and is reviewed annually through joint meetings of IMMLEP and of scientists working on the immunology of tuberculosis.

Taxonomic studies on *M. leprae* have shown a 40% DNA sequence homology with *M. tuberculosis*, the highest degree of homology found among mycobacteria. No significant similarities have been observed between *M. leprae* and corynebacteria isolated from leprosy lesions.

M. leprae-derived clone banks are being established and cosmids containing relatively large DNA fragments are being used to infect *Escherichia coli* (A33). Although *M. leprae* DNA has been expressed and some new proteins have been synthesized, no synthetic macromolecule has yet been found to react with antisera to *M. leprae*. In other studies, a λ gt 11 vector known to ensure DNA expression in a variety of microorganisms has been used. Fusion proteins have been expressed and studies to identify *M. leprae* antigens are in progress.

Immunodiagnosis**Serology**

Specific immunodiagnostic tests are required to detect infection and also, ideally, risk of disease. Until recently the immunochemical basis for the development of such tests was lacking. Careful searching had turned up no *M. leprae*-specific proteins or glycoproteins. A number of monoclonal antibodies directed against *M. leprae*-specific epitopes have now been obtained. A workshop, held in 1984 to test

Box 8.2 Antibodies to *M. leprae* phenolic glycolipid-I (PGL-I)

Serum anti-PGL-I antibody levels in leprosy patients are proportional to bacillary load and fall following therapy. High antibody levels were found in 5% of household contacts in Sri Lanka and in 10% of the general population in Micronesia. Serum obtained from patients in the same areas showed high antibody levels three months to two years prior to the

development of overt disease. Anti-PGL-I antibodies are not permanent markers of infection, since a proportion of positive individuals become negative over time.

The predictive value of anti-PGL-I antibodies will be further studied in high-risk groups in China, Cuba, Mexico, Sri Lanka and other countries, using PGL-I from supernatants ob-

tained during the purification of *M. leprae*. Cheap and simple tests, based on PGL-I and other potentially *M. leprae*-specific fragments, will be developed to: detect *M. leprae* infection; study its mode of transmission; measure the latent period from time of infection to appearance of clinical disease; and, hopefully, predict the form of clinical disease.

24 monoclonal antibodies produced by seven laboratories using a variety of techniques, found 10 to be *M. leprae*-specific (Table 8.2) and to react with several different epitopes, including those on a phenolic glycolipid (PGL-I), a recently identified *M. leprae*-specific macromolecule (Fig. 8.1). The 10 monoclonal antibodies are now being used to develop specific tests for immunodiagnosis and for the detection of *M. leprae*-specific DNA products. They will also be used to determine whether the antigens against which they are directed are capable of inducing cell-mediated immune responses.

PGL-I has been fully characterized and synthesized, and has been used as the basis for an enzyme-linked immunosorbent assay (ELISA) (B6). Molecules with the same terminal trisaccharide as PGL-I have not been found on other mycobacteria, and three laboratories have now reported (A7, A76, B1) that antibodies to PGL-I are found only in leprosy serum, not in that from patients with other mycobacterial infections. Monoclonal antibodies have revealed the presence of PGL-I on the surface of *M. leprae* and in large quantities in infected tissues.

Skin tests

Skin tests are widely used in leprosy research, particularly to evaluate potential vaccines. Antigens in current use are in the form of crude extracts of *M. leprae* and hence not only lack specificity and sensitivity but also show variability between batches and over time. Work is in progress to standardize test antigens and prepare sufficiently large batches to ensure comparability of skin-test results from different field studies.

Immunological unresponsiveness

Suppression of *M. leprae*-specific T-cell reactivity is a feature of LL and BL leprosy and appears to be essential to the development of progressive disease. Evidence of T-cell suppression was seen in a study in which suppressor T-cells (of the TH2⁺ and OKT8⁺ subsets) from LL or BL patients were found to be capable, after exposure to lepromin, of suppressing *in vitro* mitogenic responses of lymphocytes from patients or normal donors (A6). However, other attempts to find disease-related suppression in lepromatous leprosy have produced varying results. T-cell suppression was not detected in one study (A54) and another study (A67) failed to show suppression in lymphocytes obtained from lepromin-positive HLA-A, -B, and -D matched donors exposed to lymphocytes from LL patients.

Haregewoin and co-workers (A27) have recently

shown that T-cell responsiveness could be restored by the use of T-cell-conditioned medium rich in interleukin, implying that LL patients have *M. leprae*-reactive circulating T-cells and that the defect in such patients may be related to a failure of production of interleukin or other lymphokines rather than to defective antigen presentation.

Chemotherapy of leprosy

Drug resistance

With *secondary* resistance so widespread, it is not surprising that prevalence rates of *primary* resistance are alarmingly high in some areas (Table 8.3): dapsone therapy presumably cures susceptible infections, leaving resistant cases as sources of new infection. Multidrug therapy is clearly needed for all categories of leprosy patients.

Studies on dapsone resistance conducted under the SWG on the Chemotherapy of Leprosy (THELEP) have been completed, but research on resistance to other drugs is being considered. In a recent study (B16), resistance was seen in nine of 45 relapsed patients who had been treated with rifampicin, a powerful antileprosy drug that has been widely used during the past decade either alone or in combination. The absence of reported resistance to clofazimine is regarded as a valuable asset of this compound, but too few studies have specifically looked at clofazimine resistance to warrant complacency about its use. Indeed, a recent study conducted in Ethiopia (B22) disclosed one case of clofazimine resistance.

Improved use of existing drugs and combined chemotherapy regimens

Clinical trials

The purpose of these trials is to study, among other things, the effectiveness of different antileprosy drug regimens in eliminating "persisters" (drug-susceptible, viable organisms that are found, in a supposedly dormant state, following chemotherapy) (A117). Trials in Chingleput (India) and Bamako (Mali) have continued. Biopsies taken 3, 12 and 24 months after the start of therapy showed no clear association between clinical response and the presence of persisters. Persistence was diagnosed on the basis of a mean harvest of at least 5×10^5 acid-fast bacilli (AFB) from the footpads of thymectomized and irradiated

TABLE 8.3

Prevalence of resistance to dapsone

Location	Rate per 1000 cases
Secondary Resistance	
Burkina Faso	70
Burma	200 ^a
Burundi	37 ^b
China	
Shanghai municipality	86
India	
Gudiyatham Taluk	95
Trivillon Taluk	30
Mali	57 ^b
Primary Resistance	
China	
Shanghai municipality	500 ^c
India	
Chingleput	320 ^d
Gudiyatham Taluk	420 ^e
Mali	
Bamako	340 ^d
Martinique and Guadeloupe	700 ^{b, f}
Philippines	
Cebu	33
Republic of Korea	220 ^b
United States of America	
San Francisco	18 ^b

^a Preliminary result

^b Study conducted outside the Programme

^c Based on 20 specimens studied

^d Data from controlled clinical trials

^e Based on 12 specimens studied

^f Based on 17 specimens studied

(TR) mice inoculated with material obtained from leprosy lesions or on the finding of *M. leprae* multiplication in non-immunosuppressed mice. Improved drugs or drug regimens may be needed to deal with the problem of persisters, although persistent bacilli may not always cause relapse.

Field trials

Several THELEP-supported field trials are in progress:

◦ Trials designed to assess the effectiveness of multi-drug therapy under field conditions are now taking place in Gudiyatham Taluk and Polambakkam, in India, where two regimens are being studied. In both trials, periodic, supervised drug administration is supplemented by daily self-administration. More than 1200 patients have been recruited at Gudiyatham Taluk (of whom more than 700 smear-negative) and more than 1100 patients at Polambakkam (almost all smear-negative). Preliminary findings show high

acceptability of both regimens, with an attendance rate of over 93% in both trials. The regimens are well tolerated and side-effects minimal and rare. In no case has clinical deterioration been noted. The 2300 patients already recruited into these trials will have completed intensive treatment by the end of 1986. Since many were released from treatment by mid-1984, relapse rates for the first two-and-a-half years following the end of treatment should be available by the end of 1986 and provide the first data available on relapse rates in lepromatous patients following cessation of combined therapy. Any cases of relapse appearing either during or after treatment will provide an opportunity of studying multidrug-resistant *M. leprae*. Critical drug concentrations defining resistant mutants will be determined, using the mouse footpad model, and informal surveys on multidrug-resistant *M. leprae* will be conducted in other endemic countries, especially where rifampicin and/or clofazimine have not been used in the correct manner.

• A field trial of combined therapy for paucibacillary leprosy began in Malawi in April 1983. By the end of 1984 about 400 patients had been recruited. Acceptability has so far been high. Patient intake into the trial should be completed by early 1985. Since patients are treated for six months and since relapse may occur early in nonlepromatous leprosy, significant data on relapse during the first year after cessation of treatment may be available by the end of 1986. At least one additional trial will be started in another country in 1985.

◦ A short-term clinical trial began in Cebu, in the Philippines, to compare the efficacies—judged by *M. leprae* killing rates—of two different doses of ethionamide/protioneamide. No results from this trial are yet available.

Protioneamide hepatotoxicity

In research conducted in Guadeloupe, outside the Programme, approximately 15% of multibacillary patients treated with a combination of rifampicin (10 mg/day), dapsone (100 mg/day) and protioneamide (5 or 10 mg/kg/day) developed jaundice and laboratory evidence of hepatotoxicity (B13). Liver damage occurred with similar frequency for the two doses of protioneamide. Similarly, in China, some 30% of patients demonstrated elevated alanine transferase (formerly, serum glutamic pyruvic transaminase) activity during a three- to four-month course of treatment with protioneamide (5 to 6 mg/kg/day) plus dapsone daily and rifampicin monthly (B17). In a multicentre study, hepatotoxicity was observed in 28 (4.7%) of 596 patients given rifampicin (600 mg),

Box 8.3 Antileprosy antimicrobials

- Some rifamycin SV derivatives have longer half-lives and have shown more potency and a longer-lasting effect, in the mouse footpad test, than rifampicin itself. These encouraging findings are being explored, and it is hoped that some compounds may reach the clinical trial stage.
- Screening of structurally diverse β -lactam antibiotics has identified five active compounds. Three have an inhibitory effect on *M. leprae* growth that lasts beyond the period of drug administration, suggesting a bactericidal effect. The parent compound, 7-aminocephalosporanic acid, is also active.
- A number of aminoglycosides known to be active against *M. tuberculosis* are being assessed. In these studies, for which results are not yet available, β -lactam - aminoglycoside combinations will be assessed on the hypothesis that the β -lactam may increase bacterial cell-wall permeability to the aminoglycoside.
- Fluorinated quinolone derivatives that are active against gram-positive organisms and penetrate readily into tissues have been found to be active against rapidly growing mycobacteria, including *M. tuberculosis*, and preliminary findings in mice suggest that at least one quinolone compound is highly active against *M. leprae*. Further work is required to confirm the importance of this very promising new lead.

ethionamide (500 mg) and dapsone or clofazimine (100 mg) daily. Seven of the 28 patients died (B21). In view of these results and the limited number of antileprosy drugs available, THELEP is exploring ways of using protionamide without causing hepatotoxicity. The carefully supervised, short-term clinical trial currently being conducted in Cebu in the Philippines is expected to give some indication of the value of low-dose therapy with this drug.

New leads

Folate inhibitors

The earlier discovery of a highly dapsone-susceptible mycobacterium, but one that, unlike *M. leprae*, is readily cultivable *in vitro*, has given considerable impetus to studies on folate metabolism inhibitors. With "*M. lufu*", as this slow-growing organism has been provisionally named, the minimal inhibitory concentration (MIC) of dapsone is only ten times that with *M. leprae*. In addition, the two enzymes of "*M. lufu*" strongly inhibited by dapsone—dihydropterotic acid synthase and dihydrofolate reductase—have been shown to be similar to those of *M. leprae*. "*M. lufu*" may therefore be a good model for the development of inhibitors of these two enzymes (A111-113).

Several dapsone analogues synthesized on the basis of quantitative structure-activity relationships (QSAR) have been found to be more potent than dapsone itself against "*M. lufu*" and to show pharmacokinetic and toxicological differences from the parent compound. Three of these compounds, however, showed little or no activity in the mouse footpad model against strains of *M. leprae* normally susceptible to dapsone. The inhibitory action of dap-

sone on folate-synthesizing enzyme did not differ significantly, whether the enzyme was derived from dapsone-sensitive or from dapsone-resistant "*M. lufu*" and *M. leprae* mutant strains, suggesting that resistance is not due to structural or functional changes in this enzyme. Moreover, resistance was not associated with an increase in *p*-aminobenzoic acid (PABA) receptor affinity, in intracellular PABA concentration or in cell-wall permeability, three possible causes of resistance.

Ribonucleotide diphosphate reductase inhibitors

A new series of 2-acetylpyridine thiosemicarbazone (P2TSC) compounds have been studied. These drugs are thought to act by inhibiting the iron-containing bacterial enzyme ribonucleotide diphosphate reductase (RDR). On the strength of research conducted outside the Programme, it has been claimed that a number of P2TSC compounds are active against *M. smegmatis* and several other slow-growing mycobacteria, and that their anti-*M. leprae* activity is greater than that of thioacetazone (B19). However, four of these compounds tested in mice showed very little activity—certainly less than that seen with thioacetazone (A115).

A series of hydrazones have also been investigated. They are also probably RDR inhibitors but less toxic than the P2TSC compounds. A hydrazone/dapsone combination showed synergism of antibacterial effect against "*M. lufu*". However, when the drug was given in the diet to mice, blood levels were scarcely detectable, due either to poor absorption or to rapid degradation. Cyanimino analogues of ethionamide/protionamide showed no detectable activity in the mouse footpad test.

Several studies have been conducted on anti-

microbial drugs effective in the mouse footpad model against *M. leprae* (Box 8.3).

The mouse footpad model, at present the only reliable screen for potential antileprosy compounds, has many disadvantages, including the need for a considerable quantity (10 to 20 g) of test substance and the impossibility of controlling its pharmacokinetics. A number of rapid screening systems have been prepared but none is totally satisfactory. *M. leprae* isolates still have to be obtained from

human or animal biopsies, which contain variable proportions of viable organisms. Genetic engineering approaches to this problem are being explored.

A test to determine *M. leprae* viability would be useful in evaluating the efficacy of different chemotherapeutic agents. Immunochemical assays of PGL-I may provide such a test, and ELISA and immunofluorescent techniques are being assessed for this purpose.

Box 8.4 The future

Future plans and expectations of IMMLEP include:

- large-scale studies to be conducted in Africa and Asia on leprosy vaccines
- research on immunotherapy as a means of promoting cure of disease and prevention of relapse
- expression of *M. leprae*-specific antigens in *Escherichia coli* and other cultivable hosts for use in vaccines and diagnostic tests
- development of phenolic glycolipid derivatives for immunodiagnosis
- improvement and standardization of skin-test reagents

- elucidation of the role of suppressor T-cells in immunoregulatory mechanisms underlying leprosy
- development of animal models for the study of nerve damage
- application of serological tests to epidemiological research.

THELEP's future plans and expectations include:

- development of new antileprosy drugs
- development of rapid *in vitro* methods for screening drugs, including potentially feasible techniques

for cultivating *M. leprae in vitro*

- improved methods of determining *M. leprae* viability *in vitro*
- research on *M. leprae* cell-wall chemistry, especially on the synthesis of precursors, cell-wall assembly, *M. leprae*-specific lipid synthesis, and iron uptake, metabolism and membrane transport
- trials of immunotherapy, alone and combined with multidrug therapy
- study of the impact of multidrug therapy on disease transmission.

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- C5. TDR. Report of the fourth meeting of the Scientific Working Group on the Chemotherapy of Leprosy, Geneva, 18-19 April 1983. Document TDR/THELEP-SWG(4)/83.3. (English only)
- C6. TDR. Standard protocol for chemotherapy trials in non-lepromatous leprosy. Document TDR/THELEP/PROTOCOL/82.1. (English only)

9 Biomedical sciences

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9 Biomedical sciences

The context

□ The recent remarkable growth of concepts and techniques in fundamental biological sciences, especially in molecular biology, genetics and immunology, has had a major impact on research on tropical diseases. This development was foreseen when the Special Programme was established: a Scientific Working Group (SWG) on Biomedical Sciences (BIOS) was given the task of identifying promising leads and introducing them into the

Programme's research activities. Since then, many of TDR's Scientific Working Groups have exploited approaches made possible by advances in fundamental biological sciences, especially in relation to vaccine development.

BIOS has explored and promoted new possibilities through pilot research projects, symposia, seminars, workshops and the publication of reviews and books. □

Box 9.1 Highlights of research in TDR

- Development of vaccines, particularly malaria vaccines, has been greatly accelerated by the use of new technology, including gene cloning, construction of genomic libraries, monoclonal antibody identification of immunoprotective epitopes and immunogenic antigens, and experimental recombinant virus research.
- The difficulty of obtaining reagents for serological tests specific for *Mycobacterium leprae* has been overcome through the use of monoclonal antibodies.
- Isoenzyme and kinetoplast-DNA restriction endonuclease analyses of *Trypanosoma cruzi* are being used to identify which parasite species or subspecies is associated with a particular clinical form of Chagas' disease in a given geographical area.
- DNA probes are being studied for their ability to differentiate between

pathogenic and nonpathogenic species of *Onchocerca* isolated from blackfly vectors.

- A monoclonal antibody-based immunoassay — the Zavala test — has been developed for the detection of species-specific plasmodial circumsporozoite antigens in mosquitoes and should be very useful to malaria control programmes in assessing the vectorial capacity of mosquitoes.

- Polytene chromosomal analysis is being used to provide markers of mosquito behaviour related to the disease transmission potential of different mosquito species and subspecies.

- *Leishmania* have been found to possess a purine metabolic salvage pathway. Since man is capable of *de novo* purine synthesis, this pathway could be a possible target of chemotherapy. One purine analogue, allopurinol, has been found to be

active against *Leishmania*, and its metabolite, allopurinol riboside, is now undergoing a Phase II clinical trial.

- A histidine-rich protein of *Plasmodium falciparum* has been shown to be associated with the knob-like projections seen on *P. falciparum*-infected red blood cells. Several L-histidine analogues have exhibited activity *in vitro* against knob-producing *P. falciparum* strains.

- High intracellular carnitine and carnitine acetyltransferase (CAT) levels have been found in *T. brucei*. Although the biological role of the carnitine-CAT pathway is not yet clear, CAT can be inhibited by O-bromoacetyl-L-carnitine (BAC), which has been found to prolong the lives of mice infected with *T. brucei* and may provide a lead to a new class of trypanocidal compounds.

Report of activities in 1983-84

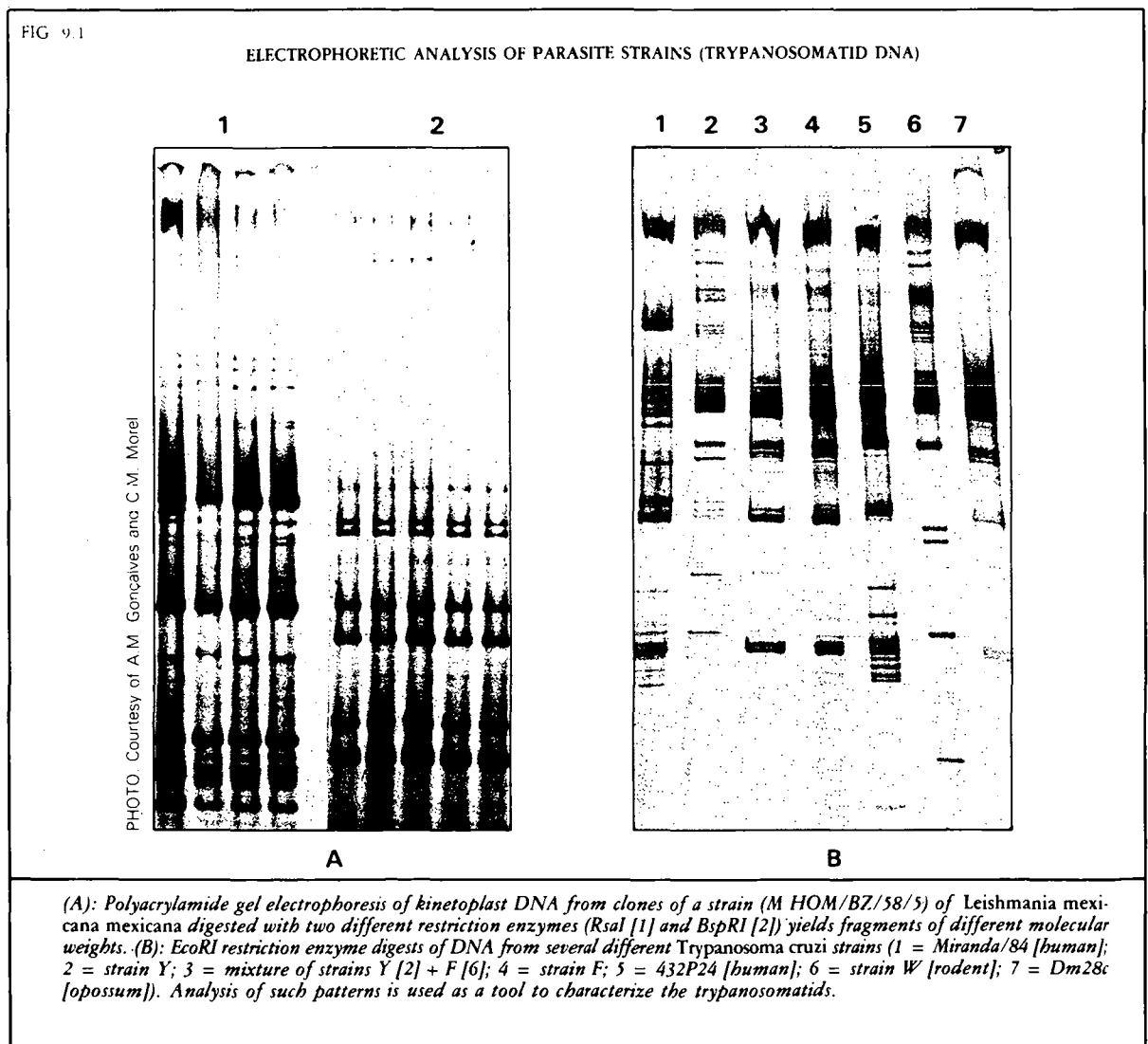
Research projects

Features of biochemistry and cell biology peculiar to the parasite but absent from the host have been of special interest to BIOS: during the past two years, an unusual calcium-stimulated RNase has been identified in *Trypanosoma brucei*; *T. brucei* phosphofructokinase has been isolated as a 270 000 Mr hexamer with 45 000 Mr subunits; DNA replication in kinetoplasts (organelles unique to haemoflagellates) has been shown to be inhibited by nalidixic acid, which has been found to inhibit *Leishmania* and *T. cruzi* growth *in vitro*, raising the possibility that compounds of the quinolone series, potent agents against gram-negative bacilli, might also be active against protozoa; a study of the molecular basis for

attachment of malaria merozoites to red blood cells has implicated red cell glycophorin as a merozoite "receptor", a finding being further developed under TDR's Scientific Working Group on the Immunology of Malaria (IMMAL).

The advent of monoclonal antibodies has made it possible to focus on the role of humoral immunity in the host-parasite relationship. Methods for the study of cellular responses are now becoming available. An analysis of T-lymphocyte function in experimental leishmaniasis in mice has shown that T-cell subsets differ in their ability to protect against or aggravate disease, a finding of fundamental importance to the selection of antigens and adjuvants appropriate for incorporation in vaccines.

Fine structural analysis — to the 6 Å level — has



been made of *T. brucei* variant surface glycoproteins by combining monoclonal antibody methods and X-ray crystallography in order to define antigenic structures and ultimately to produce synthetic peptides capable of inducing the production of antitrypanosome antibodies. Monoclonal antibodies to crossreacting trypanosome epitopes are also being sought for potential diagnostic and protective uses.

Leishmania strains which differ in pathogenicity and virulence have been analysed using panels of monoclonal antibodies. Differences in reactivities revealed by these panels may point to avirulent strains which could be considered for development as possible vaccines.

Work on DNA analysis for parasite identification has included the development of probes for *Leishmania* and kinetoplast-DNA (kDNA) analysis of *T. cruzi* digests using different restriction enzymes (Fig. 9.1). Different strains show different DNA fragment patterns ("schizodemes"), which do not necessarily correlate with the findings of isoenzyme analysis. Schizodemes are now being studied in relation to the epidemiology and pathology of *T. cruzi* infection.

Publications

A total of 52 publications arising from BIOS research projects were recorded for 1983-84. These have been included in the publications lists of the chapters of this Report which deal with specific diseases. In addition, the following books were prepared under the aegis of BIOS:

◦ *Basic Biology of Microbial Larvicides of Vectors of Human Diseases*. Proceedings of a Consultation Convened in Geneva, 26-27 April 1982. Geneva: UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases, 1983, 188 pp.

• *Genes and Antigens of Parasites: A Laboratory Manual*. Proceedings of a Course Sponsored by the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases and the Fundação Oswaldo Cruz (FINEP - CNPq - FIOCRUZ), 2nd ed. Rio de Janeiro: Fundação Oswaldo Cruz, 1984, 580 pp.

◦ *Tropical Diseases Research Series No. 5: New Approaches to the Identification of Parasites and Their Vectors*. Proceedings of a Symposium on Application of Biochemical and Molecular Biology Techniques to Problems of Parasite and Vector Identification, held in Geneva, Switzerland, 8-10 November 1982. Basel: Schwabe & Co., 1984, 466 pp.

Training courses

Three courses were organized in 1983-84:

• *An International Laboratory Training Course on Genes and Antigens of Parasites*, held at the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, from 14 November to 17 December 1983.

The 16 participants in this practical laboratory course used *T. cruzi* and *Leishmania* as models to perform recombinant DNA techniques, gene cloning, DNA sequencing and chemical oligonucleotide synthesis, and to produce monoclonal antibodies to parasite antigens. Specific tasks were set in the course and during their performance, homologies and differences were demonstrated between kDNA sequences from *T. cruzi* and a closely related haemoflagellate isolated from bats; *T. cruzi* tubulin genes were cloned and partially characterized; and several trypomastigote- and epimastigote-specific antigens were identified.

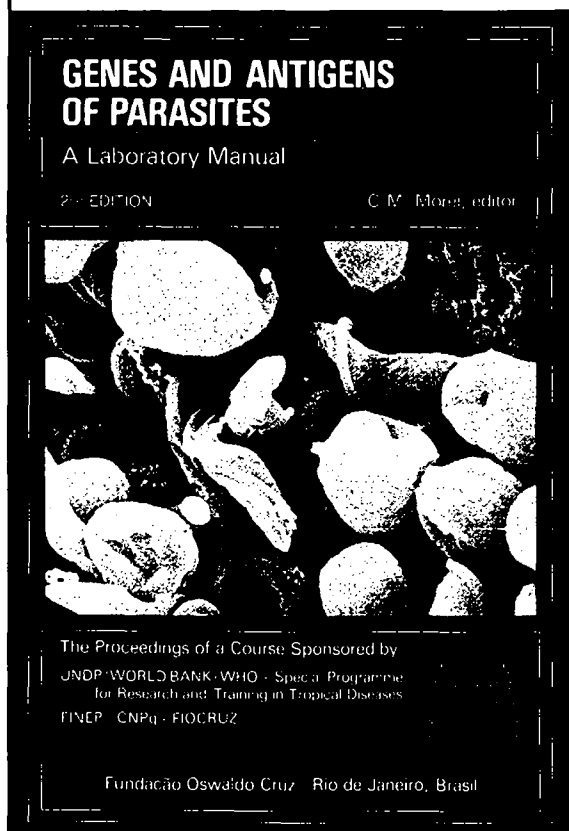
The comprehensive manual, *Genes and Antigens of Parasites: A Laboratory Manual*, prepared for the course (Fig. 9.2), includes detailed protocols for hybridoma and recombinant DNA techniques and other molecular biology techniques directly applicable to research on tropical diseases. The manual was revised in the light of experience gained during the course.

◦ *An International Training Programme on Molecular Approaches to Research in Tropical Diseases: Filariasis and Biological Control of Insect Vectors*, held in the Department of Molecular Biology, School of Biological Sciences, Madurai Kamaraj University, Madurai, India, from 24 September to 12 October 1984.

Twenty scientists from developing countries participated in the programme, which provided practical experience in recombinant DNA and immunochemical techniques, and in gene cloning and small-scale biotechnological production methods. Antigens common to different strains of filarial parasites were demonstrated and monoclonal antibody-based immunochemical techniques were used to detect filarial antigens in biological material. Restriction enzyme filarial DNA patterns were obtained which showed similarities and differences within several parasite strains. Several nick-translated DNA probes were prepared; at least one showed potential as a possible tool for parasite identification. *Bacillus sphaericus* strains were screened for the presence of the gene coding for the organism's larvicidal factor. Certain repeated DNA sequences, identified in different strains of *B. sphaericus*, correlated with high levels of bacterial biocidal factors. (The proceedings and laboratory protocols of this advanced training

FIG. 9.2

BENCH MANUAL RESULTING FROM
THE TDR-SUPPORTED INTERNATIONAL TRAINING
COURSE HELD AT THE OSWALDO CRUZ INSTITUTE
IN RIO DE JANEIRO, BRAZIL



programme will be published in due course.)

- *An International Laboratory Workshop: Genetic Engineering Techniques in Tropical Diseases Research*, held at the Faculty of Sciences, Mahidol University, Bangkok, Thailand, from 13 to 30 November 1984.

Eighteen scientists from developing countries participated in this workshop and learned about techniques in genetic engineering, with emphasis on *Plasmodium* genotype and phenotype characterization, endonuclease fingerprinting, DNA sequencing, gene cloning and related technologies.

Support of selected groups

In 1983, two grants were awarded to laboratories in developing countries to strengthen their resources for the application of modern biological research to disease control:

- *The Department of Molecular Biology, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil*, for a project on new biotechnology tools in the study of parasite genes and antigens. The grant should help

to strengthen scientific links with other laboratories, allow urgently needed information and data processing technology to be established, and broaden the scope of the department's activities and potential. Researchers in the department will be able to undertake oligonucleotide synthesis and automate trypanosomatid schizodeme analysis by the introduction of gel scanning and computer analysis, and the department will be able to increase training activities for scientists in Latin America.

- *The Department of Molecular Biology, School of Biological Sciences, Madurai Kamaraj University, Madurai, India*, to find ways of producing bacterial larvicides, including the use of physicochemical and genetic engineering approaches. The department's scientists have expertise in gene cloning and in the development of biotechnological tools for the production of microbial larvicides, such as those from *B. thuringiensis* and *B. sphaericus*. The grant should enable them to extend their activities to: basic biochemistry of bacterial toxins; the study of naturally occurring mutants; development of sensitive, reliable techniques for evaluating larvicidal potency, including DNA probes and immunochemical assays; research on the molecular aspects of filariasis and establishment of a filarial DNA (gene) bank; and development of nucleic acid probes for diagnostic parasite identification.

Box 9.3 The future

Research and training related to basic biological sciences

In 1984, TDR's Scientific and Technical Advisory Committee (STAC), reiterating the importance of basic biological research in generating new approaches to disease control, concluded that the work of BIOS could effectively be handed over to other SWGs and hence that BIOS itself should be dis-established at the end of 1985. The work of BIOS in supporting basic research in tropical developing countries through courses and projects will therefore be taken over by TDR's Research Strengthening Group.

The following are examples of fundamental biological science activities likely to receive special attention by TDR during the next few years:

- identification of areas of biological research of potential application to the control of tropical diseases;
- exchange of information between scientific disciplines through symposia, workshops, consultations, advanced training courses and other meetings;
- links with relevant WHO units and programmes, such as the newly established WHO Vaccine Development Programme.

10 Biological control of vectors

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Note on lists of publications

Publications and other documents relating to the subject of this chapter are listed, at the end of the chapter, under three headings: Publications acknowledging TDR support (list A), Publications from work outside the Programme (list B) and TDR scientific reports which became available in 1983-84 (list C). Since most of the work described in the Report is too recent to have appeared in the literature, only a small proportion of publications listed relate directly to the text. List A comprises publications acknowledging TDR support that have not appeared in any previous Programme Report and list B, only those publications pertinent to specific points discussed in the text. All the documents in list C can be obtained by writing to: The Office of the Director, TDR, World Health Organization, 1211 Geneva 27, Switzerland; requests should carry the full document title and reference number.

10 Biological control of vectors

The context

□ Of the six diseases within TDR's mandate, all but leprosy are transmitted by vectors. Programmes aimed at controlling these vectors have up to now relied heavily on chemical pesticides. But increasing costs, vector resistance, low target specificity and ecological concerns have prompted the search for better, more "natural" vector control approaches.

One way is to exploit the existence of organisms whose pathogenicity to other members of the ecosystem acts as a natural "regulator" of the system. This approach has several advantages, not the least being a shift of emphasis from the not always realistic aim of vector eradication to the ecologically more acceptable, and often more attainable, one of vector control. Another advantage is that, unlike most chemical pesticides, natural regulators tend to have fairly narrow ranges of targets, so that their effects are more amenable to ecological containment.

One difficulty, though, is that the more efficient the pathogen is in "regulating" (i.e. destroying) its host, the rarer it becomes. In addition, many natural regulators have succumbed to the widespread, often indiscriminate, use of chemical pesticides.

The aims of the Scientific Working Group (SWG) on the Biological Control of Vectors (BCV) are: to

identify natural, biological regulators that have survived these hazards; to test them not only for efficacy and field applicability against vectors of the five vectorborne diseases of interest to the Programme — malaria, schistosomiasis, filariasis, the trypanosomiasis and the leishmaniases — but also for safety; to stimulate the development and production of those that emerge from efficacy and safety tests as promising agents; and, if possible, to improve the effectiveness of naturally occurring regulators. An ultimate aim is the inclusion of agents of proven efficacy into integrated vector control programmes.

Of the over 40 agents so far investigated with TDR support, some were discovered in research supported by TDR, others by investigators initially working outside the Programme but later receiving Programme support for subsequent testing and development. These agents include: viruses, bacteria, fungi, protozoa, worms (nematodes), invertebrate predators, biological competitors and fish. Of these, certain larvicidal bacteria and larvivoracious fish have shown greatest promise (Box 10.2 and Table 10.1). But all the agents selected for further development have proved remarkably "target-specific" and safe for non-target species, including man. □

Box 10.1 Highlights of activities in 1983-84

• *Bacillus sphaericus*, a larvicidal microorganism capable of recycling even in heavily polluted waters, has been shown in several countries — Israel, the Ivory Coast, Nigeria, the Soviet Union, Thailand and the United States — to be an effective

agent against both *Anopheles* and *Culex* vector mosquitoes.

• A method of mass-producing oospores of the larvicidal fungus, *Lagenidium giganteum*, is in the early stages of development and is showing great promise. The oospore is

the stage during which the fungus is most resistant to natural hazards and one which provides a rich source of infective zoospores. Control of mosquito larvae should be possible for up to two years with this agent, with a cumulative effect over time.

Report of activities in 1983-84

The most effective biological vector control agents have been discovered mainly in countries with a tropical or desert-like climate, including Indonesia, Israel, Nigeria, the Philippines, Romania and Sri Lanka. TDR has supported efforts by scientists in these countries to identify hitherto unrecognized disease vectors and their natural pathogens. The list of countries in which BCV-related projects have been supported has been extended in this biennium to include Argentina, Bolivia, China, Colombia, Cuba, the Dominican Republic, Ghana, India, the Islamic Republic of Iran, Mexico and the Sudan. TDR has been successful in enlisting an increasing number of scientists throughout the world in research on biological vector control, with 51 projects funded in 32 countries to a total of US\$ 1.2 million in the reporting period (compared with 43 projects in 25 countries, amounting to US\$ 0.95 million for 1981-82).

Training of scientists in BCV-related research also continues, and grants totalling nearly US\$ 200 000 were made in 1983-84 to institutions in India and Malaysia to develop BCV-related research facilities.

Bacillus thuringiensis H-14

Several strains of *Bacillus thuringiensis* have been used over the past two decades as agricultural pesticides. One strain, *B. thuringiensis* H-14 (*B.t.* H-14), discovered in Israel in 1976 and subsequently studied there and in a number of other countries, had been found, by 1982, to be an extremely promising vector control agent with activity against blackflies and mosquitoes, although data on the degree and extent of its efficacy against the different vector species were meagre.

During the reporting period, 26 projects in 19 countries received from BCV a total of US\$ 614 000 for work related to *B.t.* H-14, mainly on local production in endemic areas. Some of these studies have been completed, and it is now clear that *B.t.* H-14 is effective in the field against all the major mosquito and blackfly vector species (A35, A59, A68).

One problem with this agent, however, is that its spores do not usually replicate in the environment to an extent sufficient for long-term larvicidal purposes, so that its residual effect is of short duration. Different formulations of *B.t.* H-14 have been tested, including powders, liquids, prolonged-release granules, pellets and briquettes, but so far none has increased the duration of residual efficacy by more than two weeks (A34, A55, A70). Preliminary data from Thailand, however, suggest that under certain conditions efficacy can persist for up to eight months

(A76), but the organism's residual efficacy is still far from optimal for vector control in tropical countries.

Second, with all *B.t.* H-14 formulations so far tested, large quantities of the product are required for control purposes, which creates problems of logistics and cost.

Third, despite the widely confirmed safety of *B.t.* H-14, reservations have been expressed by some health officials about the extensive use in rivers and other sources of drinking water of an organism whose spores are naturally "designed" to last under the most adverse conditions.

A fourth problem with sporogenic *B.t.* H-14 is that the larvicidal toxin is present only within the spores. The fermentation process involved in the large-scale production of this agent may well be more complex than it would be with a non-spore-producing (asporogenic) organism.

The Special Programme is looking at several ways of overcoming these problems. One is to find, through biochemical, genetic or other means, asporogenic strains of equal or greater pathogenicity with the desired toxin-producing potential. Early results in this direction have produced promising products, and two strains are being studied intensively by industrial concerns in several countries. A second approach exploits some of the recent advances in molecular biology. Great progress has been made in 1983-84 in isolating the gene coding for the larvicidal toxin of *B.t.* H-14, and attempts are being made to splice it into the genome of a naturally recycling and readily available aquatic organism. Toxin production has been achieved in *Escherichia coli* carrying the inserted gene, but gene expression has so far not been sufficiently stable. Meanwhile, work continues on characterization of the "natural" toxin and the mechanisms underlying both its formation and its release (A12).

One serious drawback with conventional insecticides is, of course, the relative rapidity with which many vectors develop resistance to these chemicals. A study to investigate whether resistance could be expected with *B.t.* H-14 was undertaken, with Special Programme support, in the United States (at the University of California, Riverside, CA). One population of the vector mosquito, *Culex quinquefasciatus*, was submitted over 36 generations to high levels of *B.t.* H-14 "pressure" — ensuring over 90% mortality; a second population of the same species was submitted to gradually increasing levels of *B.t.* H-14 pressure, as would occur under field conditions. There was no evidence from the study that mosquitoes, at least of the species and over the

number of generations studied, would develop resistance to *B.t.* H-14 to a degree that would significantly impair vector control operations. Nor was there any indication that resistance to conventional insecticides could confer crossresistance to *B.t.* H-14. These findings are in line with over 20 years' experience with other strains of *B. thuringiensis*, which have been used against agricultural pests without producing resistance or crossresistance. All in all, these results justify an optimistic view of the long-term usefulness of the organism in the field.

Bacillus sphaericus

B. sphaericus, another larvicidal bacterium, is toxic to several genera of mosquito but not to the black-fly species against which it has so far been tested. Unlike *B.t.* H-14, though, it persists or recycles actively, even in heavily polluted waters.

At the end of 1982, little was known about the spectrum of activity of this organism or its residual efficacy under different ecological conditions. Moreover, strains then available were not suitable for large-scale fermentation.

Spectrum of activity

The pathogenicity of *B. sphaericus* to vector mosquitoes has been assessed mainly in relation to *C. quinquefasciatus*, a common filariasis vector in many countries. Research conducted during the reporting period has confirmed its effectiveness against this species and shown it to last for up to four months. In addition, evidence was obtained of its effectiveness against *Anopheles* and *Mansonia* larvae. In particular, two strains recently discovered — within the Programme, in Nigeria, and outside the Programme, in Sri Lanka (strains 2362 and 2297, respectively) — were found to be more active than *B.t.* H-14 against *Culex* mosquito larvae (A84).

Residual efficacy

The residual efficacy of an organism depends on its ability to survive in the environment and multiply in the host. A considerable effort was made in 1983-84 to determine the residual efficacy of *B. sphaericus* under a wide variety of ecological conditions. Samples of the organism were produced (with TDR support) and sent to researchers in Israel, the Ivory Coast, Nigeria, the Philippines, the Soviet Union (southern areas), Thailand and the United States (subtropical areas of Florida, notably). On the whole, the organism was effective, but the duration of its efficacy varied widely according to the degree

of water pollution: from up to three weeks in clean water to four months in highly polluted water (A57). As with *B.t.* H-14, the greater the degree of water pollution, the higher the dosage required, but with greater residual efficacy than for *B.t.* H-14.

Further studies conducted in 1983-84 have shown that the activity of *B. sphaericus* against the different vector mosquito species depends on larval gut enzyme activity, which varies from species to species and prevents the *B. sphaericus* toxin from entering the vector's haemolymph. The Special Programme is supporting research on ways of neutralizing these enzymes and hence widening the organism's spectrum of activity, which ideally would also include blackflies (A53). One approach being explored is genetic manipulation.

As with *B.t.* H-14, it is hoped that recombinant DNA methods could be used to ensure toxin production by more easily handled organisms, like *E. coli*. To this end, studies are under way on characterization of the toxin and isolation of the relevant structural gene (A30).

Lagenidium giganteum

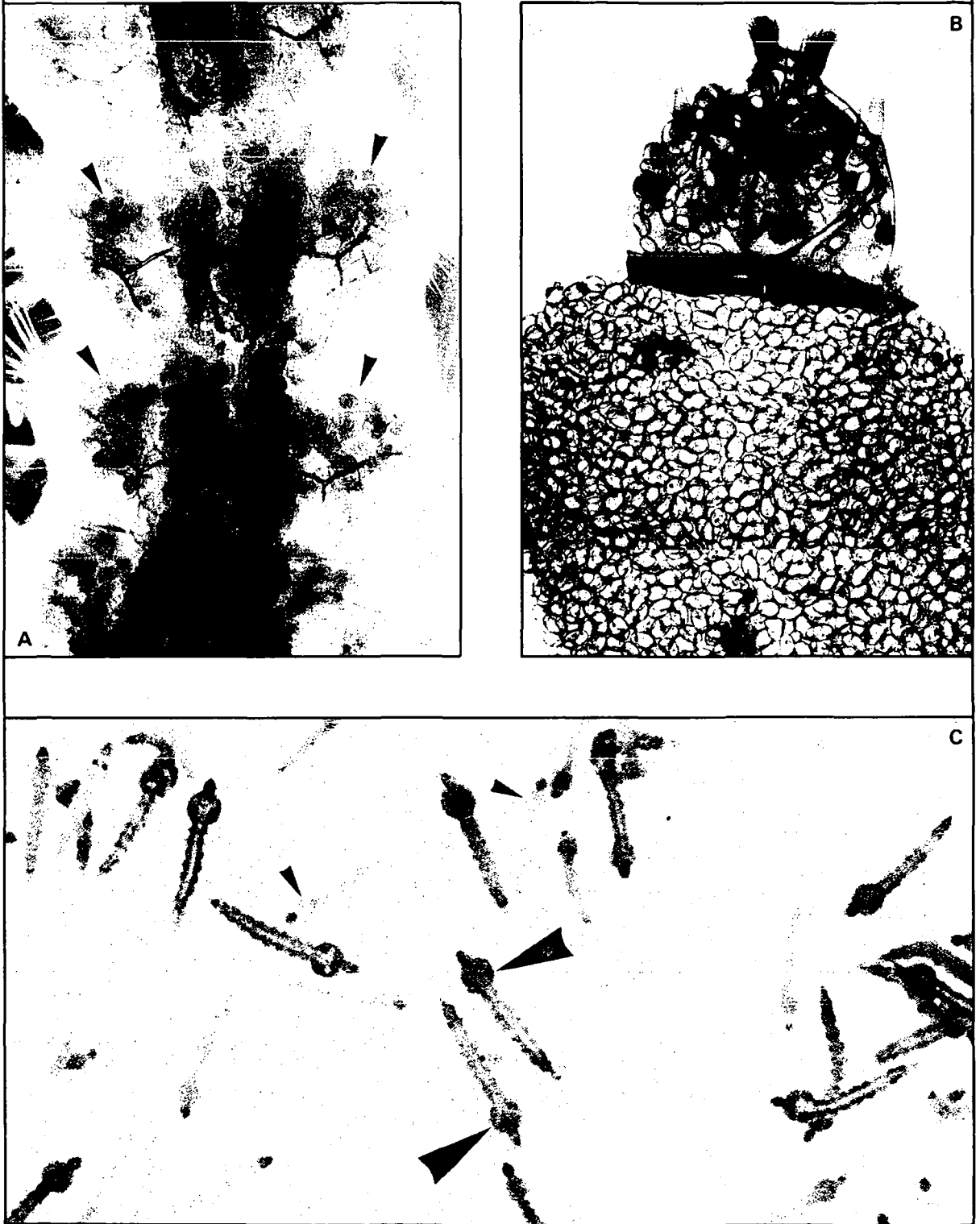
Discovered in the United States, this fungus of the Oomycetidae subclass has been known for the past decade and a half to be a natural regulator, and several attempts have been made to produce it in sufficient quantities for field application. In nature, it may recycle for periods of at least two years, which could be long enough for effective vector control. Up to now, production methods have focused on zoospores, which are not very resistant to natural hazards and have a short shelf-life. In 1983-84, work began on developing a method for the mass production of oospores, the stage of the fungus which is most resistant to natural conditions and itself constitutes a long-lasting, rich source of infective zoospores (B2). Early results with this method have been very promising and justify hopes of achieving mass production of *L. giganteum* oospores that would provide residual activity for up to two years, and thus (like *B. sphaericus*) cumulative vector control from one mosquito breeding period to the next.

Coelomomyces

Coelomomyces, a fungus of the Chytridiomycete subclass, comprises over 80 known species: most of them are obligate mosquito parasites and most are specifically pathogenic, each to a single species complex of *Anopheles* mosquitoes (Fig. 10.1). Several years ago, almost an entire *Anopheles* population in

FIG. 10.1

LARVAE OF MALARIA MOSQUITO VECTOR INFECTED WITH FUNGAL BIOCONTROL AGENT



PHOTOS: Courtesy of C.E. Bland (B) and B.A. Federici (A & C)

(A): Sporangia (spore-containing sacs) of the parasitic fungus *Coelomomyces* in the body of a larva of the malaria mosquito vector *Anopheles quadrimaculatus*.
(B): *An. quadrimaculatus* larva filled with *Coelomomyces* at an advanced stage of infection.
(C): Healthy (small arrows) and diseased (large arrows) *An. quadrimaculatus* larvae infected with *Coelomomyces*, which grows throughout the body of the mosquito larva and causes larval death.

Box 10.2 Five-stage review system

Biological agents of vector control potential go through five stages or levels of investigation and development, starting with initial identification and culminating in field use (Table 10.1). Stage I comprises identification, characterization, assessment against selected targets and preliminary evaluation of cultivation possibilities; Stage II, initial "risk tests" for mammals and assessment of activity against non-target organisms; Stage III, preliminary field trials of efficacy under natural conditions; Stage IV, thorough safety tests in mammals, ef-

fects on non-target organisms and formulations; Stage V, production, pilot plant and established plant development, and field use in cooperation with local epidemiologists. The table below shows the different biological control agents being considered by the Scientific Working Group on the Biological Control of Vectors (BCV) and the stage of investigation and development of each.

Two agents have reached the operational stage: the sporogenic strain of the bacterium *Bacillus thuringiensis* H-14 (*B.t.* H-14), registered as a mos-

quito and blackfly larvicide, and currently being used, albeit to a limited extent, for blackfly (*Simulium*) control in the Onchocerciasis Control Programme (OCP) in West Africa; and the larvivoracious fish of the *Aphanius*, *Aplocheilus*, *Poecilia* and *Tilapia* species, which have been found (in work conducted both within and outside the Programme) to be highly effective, either alone or with *B.t.* H-14, in the control of several mosquito species in Cuba, India, Somalia, Sri Lanka and the Soviet Union.

TABLE 10.1

Relative positions in the five-stage review scheme and priorities assigned to candidate biological control agents affecting vectors of human disease

Organism	Stage of investigation					Priority*	Comments on results to date
	I	II	III	IV	V		
Bacteria:							
<i>Bacillus thuringiensis</i> H-14 (sporogenic strains)	+	+	In progress	In progress	In progress	2	Improvement of formulations still first priority
	+	+	+	+	Operational	A	
<i>Bacillus thuringiensis</i> H-14 (asporogenic strains)	—					—	Recently developed mutants
	+	+				B/1	
<i>Bacillus sphaericus</i>	+	+	In progress	In progress	Planned	1	Evaluation of first commercial samples
	+	+	+	In progress	Projected	B/1	
Fungi:							
<i>Coelomomyces</i> spp.	In progress	Planned	Planned	Projected		3	Complicated life-cycle, no industrial interest
	In progress	In progress	Planned	Projected		B/3	
<i>Culicinomyces clavosporus</i>	+	In progress	In progress	Projected		2	Activity restricted to moderate temperatures
	+	+	+ ¹	—	—	C	
Entomophthoraceae	In progress					4	New aquatic strains to be studied
	In progress	Planned				B/3	
<i>Lagenidium giganteum</i>	+	In progress	In progress	Projected		3	Industrial interest expected
	+	+	In progress	Projected	Planned	B/1	
<i>Leptolegnia</i> sp.	+	In progress				3	Loss of activity in culture
	+	+ ¹	—	—	—	C	
<i>Metarhizium</i> spp. and <i>Beauveria</i> spp.	+	+ ¹	—	—	—	4	Loss of activity in culture
	+	+ ¹	—	—	—	C	

□ As of October 1980; priorities as established by the fourth SWG.

□ As of March 1984; priorities as established by the seventh SWG: category "A" includes agents available for operational use; category "B", potential agents at different stages of evaluation; and category "C", agents which have been studied intensively and, at present, appear to possess little potential for development as operational vector control agents.

* Ranked in descending order from 1 to 5.

¹ = Until better strains are found; + = Completed; — = Not feasible.

Organism	Stage of investigation					Priority*	Comments on results to date
	I	II	III	IV	V		
<i>Tolypocladium cylindrosporium</i>	+	In progress	Planned	Projected		3	Loss of activity in culture
	+	+	In progress			B/4	
Protozoa:							
Dimorphic (polymorphic) microsporidia	In progress					4	New infectious isolates
	+	In progress				B/3	
<i>Lambornella</i> spp.	In progress	In progress	In progress			4	No active isolates
	+					B/4	
Monomorphic microsporidia	+	+	Projected			4	New active isolates needed
	+	+ 1				B/4	
<i>Nosema algerae</i>	+	+	Planned			4	High dosage required
	+	+	+			B/4	
Snail/schistosome microsporidia	+	Planned				4	No laboratory cultures available for study
	+	Planned				B/4	
<i>Vavraia culicis</i>	+	Planned				4	Low activity
	+	+				B/4	
Nematodes:							
<i>Romanormis culicivora</i> x	+	+	Projected			2	Limited use; possible local application
	+	+	+	+	Planned	B/3	
<i>Neoapectana</i> spp.	-	-	-			5	No evidence of efficacy against vectors
	+	+	+ 1			B/3	
<i>Octomyormis muspratti</i>	In progress	Planned	Planned			4	More data needed
	In progress	Planned	Planned			B/3	
<i>Romanormis iyengari</i>	+	Planned	Planned			3	Limited use; possible local application
	+	+	In progress	Projected	Planned	B/2	
Viruses:							
Baculoviruses	In progress					4	More data needed
	-	-	-	-	-	C	
Densonucleosis viruses	In progress					5	More data needed
	-	-	-	-	-	C	
Iridescent viruses	-	-	-	-	-	5	More data needed
	-	-	-	-	-	C	
Fish:							
<i>Gambusia affinis</i>	+	+	In progress	+	Operational	1	Limited use due to effects on non-target organisms
	+	+	+	+	Operational	A	
<i>Aphanius dispar</i>	+	In progress	In progress	Planned		3	More local studies needed
	+	In progress	In progress	Planned		B/3	
<i>Aplocheilus</i> spp.	+	+	Planned			2	More local studies needed
	+	+	In progress	Planned		B/2	

As of October 1980; priorities as established by the fourth SWG.

As of March 1984; priorities as established by the seventh SWG: category "A" includes agents available for operational use; category "B", potential agents at different stages of evaluation; and category "C", agents which have been studied intensively and, at present, appear to possess little potential for development as operational vector control agents.

* Ranked in descending order from 1 to 5.

1 = Until better strains are found; + = Completed; - = Not feasible.

Organism	Stage of investigation					Priority*	Comments on results to date
	I	II	III	IV	V		
<i>Nothobranchius</i> spp.	+	In progress	Planned			3	More local studies needed
	+	In progress	Planned			B/3	
<i>Ctenopharyngodon idella</i>	+	In progress	Planned			4	More local studies needed
	+	+	+	In progress	Planned	B/3	
<i>Oryzias (Aplocheilus) latipes</i>	+	Planned				4	More local studies needed
	+	+	+	In progress	Planned	B/2	
<i>Poecilia reticulata</i>	+	+	In progress	Planned		2	More local studies needed
	+	+	+	In progress	Planned	B/2	
<i>Tilapia</i> spp.	+	In progress	Planned			4	More local studies needed
	+	+	+	+	Planned	B/2	
Invertebrate predators and parasites:							
<i>Dugesia dorotocephala</i>	+	+	Planned			4	More local studies needed
	+	+	Planned			B/4	
<i>Exhyalanthrax</i> spp.	In progress	Planned	Planned			3	More local studies needed
	In progress	Planned	Planned			B/4	
<i>Hellobdella</i> sp.	+	In progress	In progress	Planned		4	More local studies needed
	+	+	+	-	-	B/4	
<i>Limonogeton feiberi</i>	+	+	-	-	-	5	More local studies needed
	+	+	-	-	-	B/4	
<i>Lutzia</i> spp.	+	In progress	Planned			4	More local studies needed
	+	+	-	-	-	B/4	
<i>Mesocyclops</i>	+	+	In progress	Planned		4	More local studies needed
	+	+	+	Planned		B/4	
Mutillidae	In progress	Planned	Planned			4	More local studies needed
	In progress	Planned	Planned			B/4	
<i>Nesolynx</i> spp.	+	+	+	+	-	5	More local studies needed
	+	+	+	+	-	B/4	
Sciomyzidae	+	Planned				4	More local studies needed
	+	Planned				B/4	
<i>Toxorhynchites</i> spp.	+	+	+	In progress	Planned	2	Data on field efficacy to be confirmed
	+	+	+	In progress	Planned	B/3	
Competitors of snails:							
<i>Marisa cornuarietis</i>	+	In progress	Planned			3	Safety aspects to be confirmed
	+	In progress	Planned			B/3	

As of October 1980; priorities as established by the fourth SWG.

As of March 1984; priorities as established by the seventh SWG; category "A" includes agents available for operational use; category "B", potential agents at different stages of evaluation; and category "C", agents which have been studied intensively and, at present, appear to possess little potential for development as operational vector control agents.

* Ranked in descending order from 1 to 5.

1 = Until better strains are found; + = Completed; - = Not feasible.

the Philippines and several African countries was destroyed by a *Coelomomyces* epizoonosis, an event that strengthens the hope that several species of the fungus might be suitable for the control of a number of mosquito species.

Only a few species of the fungus have been studied in detail, but all have been shown to have a complex life-cycle involving an obligate alternate crustacean host, commonly the water flea, *Cyclops*. It is the sexual stage (zygote) of the fungus which infects the mosquito, and the asexual stage (gametophyte) which infects and destroys the crustacean host. The sexual stage only develops in *Cyclops*, which until recently defied attempts to cultivate it in the laboratory (A27). During 1983-84, however, methods of cultivating *Cyclops* were developed. One prerequisite to subsequent production of the fungus — both for research and field requirements — was therefore achieved (B1). An additional benefit deriving from the use of *Coelomomyces* would be the control of *Cyclops* in areas of dracunculosis transmission.

Larvivorious fish

Work is in progress on the use of fish to control mosquito malaria vectors in rice paddies.

Mass production of vector control agents

Several industrial firms in a number of countries, including Belgium, Cuba, Czechoslovakia, France,

the Soviet Union, the United Kingdom and the United States, have agreed to undertake production of *B.t.* H-14 and make it available, wherever needed, in formulations suitable for field application.

The results of preliminary field trials of *B. sphaericus* have encouraged companies in Belgium and the United States to produce experimental samples of different strains of the organism. Some of these samples have already been sent to collaborating centres to be tested for duration of efficacy under tropical conditions. There are indications that other companies might also be interested in producing this agent.

Following the development of a method of mass-producing *L. giganteum*, several industrial concerns have expressed interest in producing this fungus on an industrial scale.

At a meeting held in Geneva towards the end of 1982, guidelines were established for the local production of *B.t.* H-14 and *B. sphaericus* in endemic countries. During 1983-84, five projects were undertaken to develop local production facilities in, respectively, India, the Islamic Republic of Iran, Nigeria, the Philippines and Thailand. If local production does turn out to be practicable, it would certainly lower the foreseeable operational costs of these agents, notably for transportation, trained personnel and basic materials.

Box 10.3 The future

A number of biological agents are being accorded highest priority for future development. These include: *Bacillus thuringiensis* H-14, development of asporogenic mutants and long-acting formulations for mosquito control; *Bacillus sphaericus*; *Lagenidium*

giganteum; larvivorious fish; and nematodes, notably of the *Romanomermis iyengari* species (see Table 10.1 for priorities assigned to other agents).

In addition, emphasis is being placed on methodological approaches, such as genetic engineering techniques

and their application to toxin production by bacilli, methods for developing formulations of biological control agents specifically adapted for use in tropical developing countries, and field trials of the effectiveness of biological control agents in disease control.

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Biological control of vectors

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- C3. VANDEKAR, M. & DULMAGE, H.T. *Guidelines for the Production of Bacillus thuringiensis H-14: Proceedings of a Consultation held in Geneva, Switzerland, 25-28 October 1982*. Geneva: UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases, 1983, 124 pp.

11 Epidemiology

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Note on publications

Since the work of TDR's Scientific Working Group (SWG) on Epidemiology concerns several diseases, publications arising from projects supported by this SWG have been listed in the "disease-specific" chapters of this Report. The only publication noted for this chapter is a TDR document (C1) which reviews in depth the work of the SWG from mid-1978 to mid-1983. It can be obtained by writing to: The Office of the Director, TDR, World Health Organization, 1211 Geneva 27, Switzerland; requests should carry the full document title and reference number.

11 Epidemiology

The context

□ Knowledge about the distribution of the six major diseases of concern to TDR has been accumulating steadily over the years, and some of the factors underlying the occurrence and distribution of these diseases are now better understood. But further epidemiological research is needed: to measure the disease burden so as to provide national health services with guidelines on priorities and to enable the impact of specific control measures to be monitored; to identify specific risk factors for infection and disease, with a view to improving disease control; to develop techniques for testing the efficacy of new control tools, such as drugs and vaccines, so that they might be used most effectively; and to test alternative approaches and determine the best strategies for their use in the field.

The increasingly vigorous application of basic biomedical sciences to tropical diseases is now yielding powerful tools for diagnosis, prevention, treatment and control. The new diagnostic tests must not only be epidemiologically validated for sensitivity and specificity in the field but also standardized to facilitate comparison of results. Vaccines must be carefully tested for safety and protective value. Well-designed, epidemiologically sound studies are needed to ensure the effective deployment of these new tools and their integration into control operations. All branches of epidemiology must be marshalled to meet these requirements — from simple descriptive studies to complex computer simulations, from case-control studies of causal factors to community trials of vaccines and therapeutic agents. With the limited resources available to it, however, TDR has had to concentrate only on those aspects of research most

likely to lead rapidly to effective control of the six tropical diseases within its mandate.

Important epidemiological research is conducted with the support of TDR's "disease-specific" Scientific Working Groups. Studies describing disease distribution patterns have been completed in areas of the world where little information had previously been available. Baseline studies, for example, of Chagas' disease (see Chapter 6, under "Epidemiological studies") and the leishmaniasis (see Chapter 7, under "Disease distribution") are now providing the foundations on which national control programmes can be undertaken and on which analytical studies have been started with a view to establishing risk factors and designing community-based disease control strategies.

In many respects, the factors underlying disease distribution in the community are still obscure. Some diseases, such as schistosomiasis, Chagas' disease and lymphatic filariasis, show a common pattern: infection may be highly prevalent in the community but only a minority of those infected have serious clinical disease. Identifying risk factors for infection, for disease and for disease complications is an important task of TDR's Scientific Working Group (SWG) on Epidemiology and one that is being accomplished through the application of epidemiological techniques originally developed for the study of chronic, nonparasitic diseases like cancer and heart disease. For other diseases, epidemiological methods are now being used to test different approaches to disease control and to lay the groundwork for future field studies on vaccines (against leprosy and malaria, for example). □

Box 11.1 Highlights of activities in 1983-84

- Multidisciplinary, multidisease, population-based epidemiological studies in selected areas were completed and have yielded information on disease patterns in several endemic countries, which should prove useful for the planning and execution of disease control programmes.
- Faster, more efficient epidemiological methods have been applied to the study of tropical diseases, including case-control methods to determine special risk factors and to evaluate the effects of intervention procedures, and simple techniques for disease surveillance and diagnosis for use by primary health care workers.
- Facilities have been set up for the assessment and development of promising new diagnostic tests, particularly those simple enough for field use.
- Training in epidemiological research has been provided by postgraduate courses initiated by TDR in several institutions in endemic countries and by workshops on epidemiological research methods.
- A field manual on practical epidemiology has been prepared for health officers and is being evaluated.

Report of activities in 1983-84

Multidisease epidemiological studies

The Programme has supported multidisease, multidisciplinary, population-based epidemiological studies in a few selected localities. These are descriptive studies to quantify disease patterns and answer such questions as: Who has what infection or disease, where and when? The specific aims are, first, to provide details of direct value to ministries of health in reviewing their control programmes and strengthening their health services; second, to enable national field staff to be trained in survey techniques, primary health care delivery and basic laboratory procedures, as well as to ensure more advanced field research training for postdoctoral students; and third, to acquire the baseline data needed for analytical studies designed to test specific hypotheses and for intervention trials of new diagnostic, preventive or therapeutic tools.

Zambia

The Tropical Diseases Research Centre (TDRC) in Ndola, Zambia, has undertaken analytical studies of risk factors for selected diseases; population-based intervention studies; and descriptive, baseline epidemiological studies of populations in defined, ecologically differing geographical areas (Fig. 11.1).

The activities of the Ndola Centre have focused on methods of data collection, recording and analysis (including ways of identifying individuals), record-linkage techniques to keep track of multiple observations in individuals over time, and computer-assisted records to monitor consistency of laboratory results over time. The Centre has placed special emphasis on quality control, which includes, in addition to routine quality-control measures, indepen-

dent checks of answers to interviews, re-examination of physical findings in random samples of patients and duplicate runs of all laboratory tests.

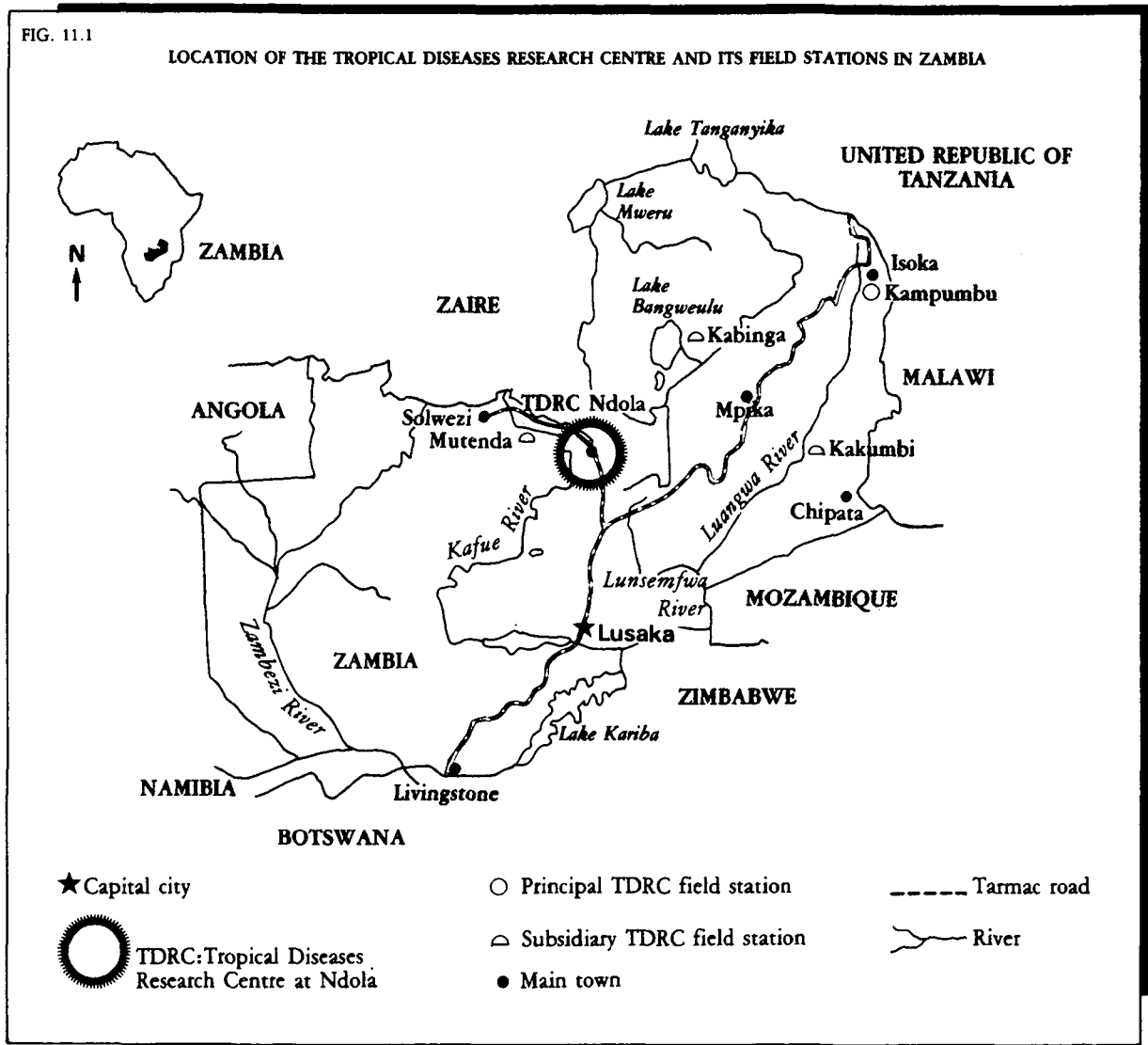
Descriptive baseline studies

Multidisciplinary, population-based, longitudinal studies have been started in the Northern Province of Zambia, an area endemic for several tropical diseases. The first study area is centred around the village of Kampumbu in Isoka District in the north-eastern corner of the country (Fig. 11.2) and has a total population of 10 000 living in 154 villages. The second study area, with a population of similar size, is located around the village of Kabinga in the Mpika District in the Northern Province of the country. Ecologically the two areas are very different: the Kampumbu area is predominantly savanna and scattered woodlands and is inhabited by subsistence farmers, whereas the Kabinga area is one of vast plains covered with short grass and scattered trees, the main livelihood of the people being fishing and small-scale subsistence farming.

The baseline studies were undertaken to assess the demographic, socioeconomic, clinical, parasitological, haematological and ecological factors underlying the multiple disease problems of the two populations. They have confirmed the high level of multiple disease in rural Zambia, but have demonstrated major variations in disease patterns related to different ecological settings and ways of life. In Kampumbu, more than half the children showed signs of malnutrition; malaria (*Plasmodium falciparum*, *P. malariae* and *P. ovale*) was widespread (hyper- to holoendemic), as was *Schistosoma mansoni* infection (found in 70% of adolescents tested, many of them with heavy infection); and African

FIG. 11.1

LOCATION OF THE TROPICAL DISEASES RESEARCH CENTRE AND ITS FIELD STATIONS IN ZAMBIA



trypanosomiasis was a major public health problem (*Trypanosoma brucei rhodesiense* parasitaemia was found in one per 200 individuals, equivalent to a lifetime risk of fatal disease of over 40%). In Kabinga, nutritional status was better, the level of malaria endemicity was lower, there was no transmission of *T. brucei rhodesiense* and schistosomiasis infection (predominantly *S. haematobium*) was very focal in distribution, with very high prevalence in some villages and virtually none in others.

Although descriptive, cross-sectional, baseline studies are carried out primarily to provide information for subsequent analytical or intervention studies, several findings from the Kampumbu studies are intriguing in themselves. For each age group, individuals with *P. falciparum* infection, for example, were found to be 20 times more likely to be infected

with *P. malariae* and twice as likely to be infected with *P. ovale* than those without *P. falciparum* infection; those infected with more than one malaria parasite had significantly larger spleens than those infected with only *P. falciparum*, suggesting a possible impairment in immunity to malaria. Follow-up studies are under way to verify these findings and determine possible underlying factors.

Post-baseline studies

The Kampumbu studies provided the necessary background for research on simple, rapid methods of diagnosing African trypanosomiasis and malaria that may be appropriate for use by primary health care workers. In order to better define diagnostic criteria, clinical signs and symptoms of the two



PHOTO: Courtesy of WHO/L. R. Rickman

An examination tent at the Kampumbu field station. The station, which includes a laboratory, supply and transport station and radio and communications centre, is the base for many field activities: field trials of the antischistosomiasis drug praziquantel; studies on rhodesiense trypanosomiasis; field studies on malaria surveillance through the primary health care system; operational research on distribution of praziquantel in and by the community; and clinical, entomological, ecological and case-control studies.

diseases were examined for sensitivity, specificity and predictive value. A simple combination of signs and symptoms easily elicited by medical assistants in primary health care centres was found to have a better predictive value for the diagnosis of African trypanosomiasis than currently available serological tests. Further testing and refinement of this approach is in progress.

Primary health care for malaria control

A pilot scheme for research on a primary health care approach to malaria control has been established in the Isoka District of Zambia with a view to: reducing malaria morbidity and mortality at an acceptable cost; providing adequate treatment, using trained community health workers employed by existing health services; monitoring malaria infection, parasitologically, serologically and clinically, through appropriate periodical sample surveys; determining changes in malaria morbidity patterns, through

village and health centre records of malaria infection episodes; obtaining mortality estimates by registering vital events through a system based on the active participation of the community; and maintaining adequate ongoing records of control costs so that *per capita* costs can be accurately monitored.

P. falciparum resistance to chloroquine

The current strategy of the malaria control programme in Zambia is largely based on the use of chloroquine. The recent emergence of resistance to this drug is therefore a major setback. In some areas of the country, such as the copper belt around Ndola, *P. falciparum* infection has been shown to be sensitive to a dose of chloroquine as low as 10 mg/kg but in 1983, RI and RII chloroquine resistance was demonstrated *in vivo* in up to 25% of Kampumbu schoolchildren, a finding since confirmed *in vitro*. This area of Zambia has had relatively little exposure to chloroquine, but it is fairly near the area where

chloroquine resistance was first reported in Africa.

Since the discovery of chloroquine resistance, doctors and laboratory technicians from the Zambian Ministry of Health are being trained to perform *in vivo* resistance tests in order to establish the pattern of chloroquine resistance in the country and provide baseline data for monitoring changes over time. Such studies may lead to a better understanding of the factors that contribute to the spread of resistance.

Sabah State, Malaysia

Epidemiological studies designed to provide information for improved disease and malnutrition control began in 1981 in Sabah State and on Banggi Island, East Malaysia. They include descriptive epidemiological studies on the distribution, frequency and severity of malaria, filariasis, leprosy, malnutrition and associated factors; analysis of environmental factors involved in the epidemiology of these infections; identification of risk factors; and the design and testing of control measures, based on epidemiological data.

Research coupled with on-site training of field staff has led to the development of an epidemiological research unit within the Sabah Medical Directorate's Laboratory Services and Disease Control Division.

These studies have shown that malaria transmission in much of Sabah is of a mesoendemic pattern. *P. falciparum* predominates (75%), with *P. vivax* and *P. malariae* accounting for the remaining cases. On Banggi Island, malaria is hyperendemic, with parasitaemia and splenomegaly observed in over 50% of children. The apparent ineffectiveness of malaria control could be due to a combination of incomplete application of control measures, outdoor resting of mosquitoes (perhaps enhanced by "excito-repellance" towards DDT), migration of people from uncontrolled areas resulting in incomplete detection and treatment of cases, and resistance of *P. falciparum* to antimalarial drugs. Detailed studies on these factors are being planned in collaboration with the national Malaria Control Programme.

Filariasis is endemic throughout the study area, with an overall microfilaraemia rate of 21% (ranging from 5 to 35% among the different villages). Nocturnally periodic *Brugia malayi* is the dominant parasite on Sabah itself, but *Wuchereria bancrofti* is also present and mixed infections are not uncommon. In contrast, *W. bancrofti* alone is endemic on the nearby island of Banggi. Clinical disease is uncommon in all communities investigated.

No important filariasis reservoir was found in wild and domestic animal populations closely associated

with man, although *B. malayi* was identified in the one leaf monkey (*Presbytis rubicunda*) examined. *Anopheles balabacensis* was incriminated as an important vector of malaria, and *Mansonia bonneae* and *M. dives* were found to be vectors of *B. malayi*.

The leprosy case-contact survey of the Kudat Residency (population ca. 100 000), revealed a detection rate of 26 new cases per 1000 household contacts examined (about four times that in peninsular Malaysia) and an estimated prevalence rate of 2.5 per 1000 persons (about twice that in peninsular Malaysia).

Development and testing of analytical epidemiological techniques

Analytical epidemiological techniques are used to examine the relationships between infection or disease and personal and environmental factors. They have proved particularly valuable in testing hypotheses on determinants of chronic noninfectious diseases, like cancer and arthritis, and they are proving invaluable in the study of the six diseases of concern to the Special Programme.

Case-control studies

Case-control studies are designed to identify risk factors that may be causally related to a disease. The frequency of suspected causal factors in patients having the disease is compared with that in matched control individuals not having the disease. Case-control studies have been little used in research on tropical diseases, but they may require much less time and expense than alternative approaches for the identification of disease risk factors. For example, a case-control study on African trypanosomiasis in Zambia was carried out to test the hypothesis that those born and raised in endemic areas acquire some degree of protection from the disease, an impression supported by anecdotal information. However, the study failed to show an association between risk of trypanosomiasis and birthplace or duration of residence in an endemic area. Together with the fairly frequent observation of individuals with a history of two or more distinct episodes of *T.b. rhodesiense* disease, this finding suggests that man does not acquire natural immunity to *rhodesiense* infection.

Studies being planned in India on filariasis will use the case-control method to investigate whether those who now have chronic disease with elephantiasis or hydrocoele have a history of more frequent acute attacks of lymphadenitis (or have received less treatment) than those who have not developed chronic disease. Although previous studies were in-

conclusive, some countries decided that the best way of reducing the effects of filariasis would be to treat patients with acute symptoms through their primary health care system. Case-control studies may provide data on the merits or shortcomings of this new control strategy.

A study being carried out in Peru is also using a case-control approach to investigate why some people develop the mutilating form of mucocutaneous leishmaniasis, whereas others, in the same area, infected with apparently the same organism, develop the more benign cutaneous form of disease. Risk factors being investigated include a previous history of partially or improperly treated cutaneous leishmaniasis (a variety of therapies have been used in the area, including traditional methods).

Microepidemiological studies

Detailed studies on the epidemiology of infection and disease may reveal substantial variations in disease patterns from one locale (village, neighbourhood or even household) to another — variations which may be obscured when an area is looked at as a whole. Examination of these “microvariations” may reveal useful clues about factors determining prevalence and distribution of infection and disease. In Papua New Guinea, studies being conducted in six closely situated villages with different levels of malaria endemicity should make it possible to relate entomological variables to parasitological findings, to determine the pattern of age-specific morbidity and to identify risk factors on a household basis. Baseline data are being collected before testing different intervention strategies.

Another study on local variations in the epidemiology of malaria has been carried out in Thailand with support from TDR's Research Strengthening Group and the SWGs on Applied Field Research in Malaria (FIELDMAL) and on Epidemiology. The study sought to identify factors contributing to persistent low-level transmission of *P. falciparum* and *P. vivax* malaria. Three neighbouring villages of similar size, with similar ecological settings and with well-run malaria control programmes, had markedly different epidemiological patterns of malaria transmission (Fig. 11.3). In one village (Hin Dard Tong), malaria attacks occurred at relatively low levels throughout the year; in the second (Pong-Chang), nearly all transmission occurred during the dry season from May to July and involved men, women and children; in the third (Lum-E-Zoo), where the total annual incidence was much greater than in the other two, men were predominantly infected in the early part of the rainy season but by October-November the

whole community was involved. These different epidemiological patterns were found to be due to a combination of interacting factors involving human and vector behaviour. The principal occupation in each village was different and exposed the inhabitants to different vectors at different times. In the first village, the main work was in an irrigated sugar plantation where year-round breeding of *An. minimus* occurs; in the second, the families would “forest-gather” in the dry season and be exposed to *An. dirus*; and in the third, the men would mine the forests in the rainy season and be exposed to *An. dirus* and, at the height of the *An. minimus* breeding season, bring infection back to the village. Knowledge of these transmission factors should help in the design of more effective control measures.

Intervention studies

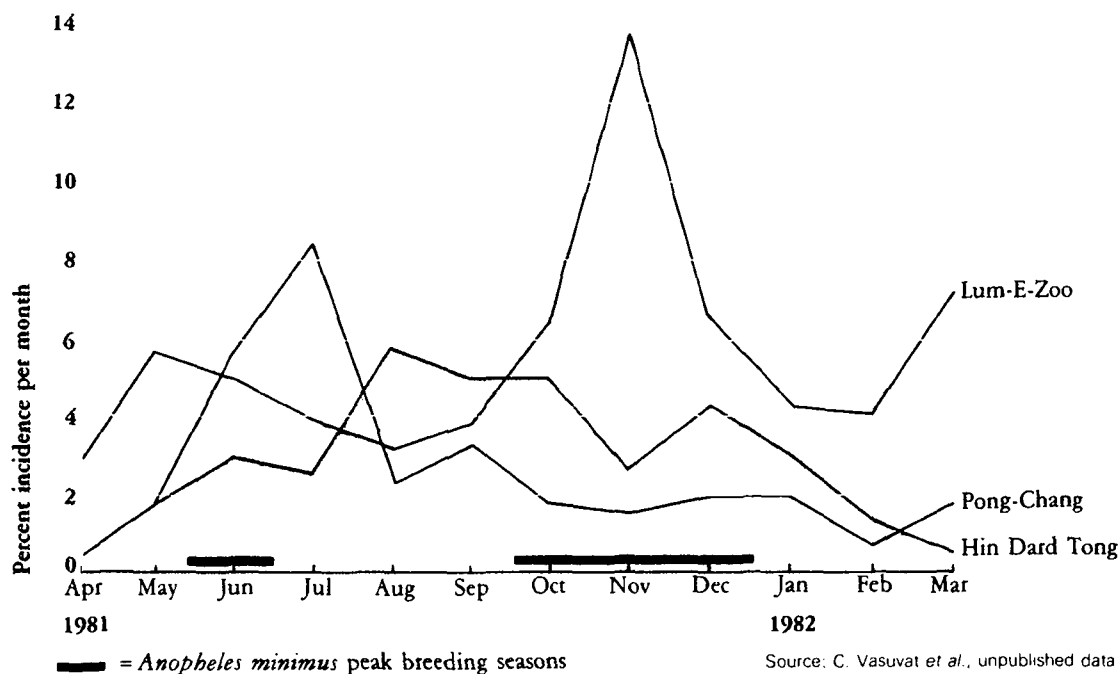
An understanding of the epidemiology of a disease is essential in designing experiments to test new disease control tools, such as those being developed by TDR. Special attention has been given to the design of trials of a leprosy vaccine, which, in view of the relatively low incidence and the long incubation period of the disease, will require repeated, meticulous examinations of hundreds of thousands of people for periods of 15 to 25 years and may well be the most challenging vaccine trials ever to be undertaken.

The impact of chemotherapy on malaria morbidity is being studied in a holoendemic area of malaria in Liberia. Suppressive monthly chloroquine treatment was compared with monthly doses of chlorproguanil and pyrimethamine, and also with chloroquine treatment of febrile episodes. With monthly doses of chloroquine, there was a marked reduction in parasite rates and in the frequency of fever, and a rise in haematocrit levels. Chlorproguanil had a substantial, though weaker, impact, whereas the effects of pyrimethamine, after the first few months, did not differ significantly from those of placebo. Despite the beneficial effects of chloroquine seen in these studies, the rapid spread of chloroquine resistance to other parts of Africa now precludes large-scale suppressive treatment with this drug. Proguanil and chlorproguanil are currently being evaluated as possible alternatives for selected high-risk groups.

In several countries, community-based trials using epidemiological principles are being conducted on praziquantel and other antischistosomal drugs and are providing information that can be used to develop the most effective strategy for controlling schistosomiasis morbidity under various conditions (see Chapter 3, under “Epidemiology and snail control”).

FIG. 11.3

MALARIA IN THREE VILLAGES IN KANCHANABURI PROVINCE, THAILAND



Diagnostic methods

A major constraint on epidemiological research is the inadequacy of many current diagnostic tests of infection. Parasitological tests are often specific for infection but can be difficult, laborious and often unsuitable for field use. Moreover, morphological examination is not always capable of distinguishing between pathogenic and nonpathogenic species (of *T.b. brucei*, for example) or between parasites causing different forms of diseases (*Leishmania*, for example). Several SWGs are now developing improved tests. The specificity of immunodiagnostic tests is being enhanced by the use of monoclonal antibodies and carefully selected antigens, and work is in progress on tests based on antigen detection. DNA probes are also being used in the identification of several parasite species.

New diagnostic tests need to be validated on an epidemiological basis and standardized, and training in their use must be provided. With support from the Programme, the WHO Immunology Research and Training Centre at the University of Geneva will assess and further develop promising tests, assist in standardizing simple techniques for use in the field,

assist in quality control in developing countries and serve as a teaching centre on simplified immuno-diagnostic methods for investigators from developing countries.

Training

The shortage of trained epidemiologists is widely recognized as a major constraint on the design and conduct of research in endemic countries. TDR is attempting to fill this need by supporting various training activities:

Postgraduate training in epidemiology

Postgraduate programmes in developing countries have been set up in collaboration with TDR's Research Strengthening Group. Support for more advanced-level epidemiological courses with emphasis on epidemiological methods, particularly as applicable to tropical diseases, has been provided to the Universidad del Valle in Cali, Colombia; the University of Nairobi, Kenya; the University of Singapore; and the Muhimbili Medical Centre in Dar es Salaam, United Republic of Tanzania.

Workshops on epidemiological research methods

A series of workshops have been held on epidemiological methods for research in tropical diseases, with emphasis on improved disease control and case-control methodology, in: Sichuan, China; Nazareth, Ethiopia; Varanasi, India; Nairobi, Kenya; and Bamako, Mali. Several of the studies described above were prompted by these workshops.

Field manual

Because of the dearth of suitable textbooks dealing with practical epidemiology in developing countries, a manual, *Epidemiology for the Health Officer: A Field Manual for the Tropics*, has been prepared and is now being evaluated.

Since 1984, TDR, together with WHO's Diarrhoeal Diseases Control Programme (CDD), has planned workshops in several countries on epidemiological methods for tropical and diarrhoeal diseases. In the future, close liaison will be maintained between the two programmes for epidemiological research training, and TDR will work with other WHO divisions that have specific interests in epidemiological research training.

TDR is also developing close links with other groups concerned with the training of epidemiologists and the stimulation of research in this field (such as the International Network for Clinical Epidemiology [INCLIN], the Rockefeller Foundation and the European Tropical Epidemiology Course).

Box 11.2 The future

The future work of the SWG on Epidemiology will cover several areas:

The development of epidemiological methods for field trials of new intervention tools:

- new diagnostic tests, particularly those suitable for simple and rapid use in the field;
- new therapeutic agents as they become available for community-based trials and new strategies for existing agents;
- new preventive techniques, especially vaccines.

The application of analytical epidemiological methods to three areas:

- identification of factors underlying the pathogenesis of tropical diseases;
- determination of the relative importance of different diseases in tropical countries and of the cost-effectiveness of control programmes, for the purpose of rationally allocating resources;
- evaluation of tropical disease control programmes as starting points for operational research.

The continuing promotion of epidemiological research training by several mechanisms:

- postgraduate epidemiological training programmes in selected institutions in developing countries;
- workshops on epidemiological research methods;
- short advanced-level courses on research methods for tropical diseases;
- promotion and development of teaching tools;
- coordination of epidemiological training activities with other agencies.

TDR scientific reports which became available in 1983-84

- C 1. TDR. Report of the Steering Committee of the Scientific Working Group on Epidemiology, July 1978—June 1983. Document TDR/EPI/SC-SWG(78-83)/83.3. (English only)

12 Social and economic research

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Note on lists of publications

Publications and other documents relating to the subject of this chapter are listed, at the end of the chapter, under two headings: Publications acknowledging TDR support (list A) and TDR scientific reports which became available in 1983-84 (list C). Since most of the work described in the Report is too recent to have appeared in the literature, only a small proportion of publications listed relate directly to the text. List A comprises publications acknowledging TDR support that have not appeared in any previous Programme Report. All the documents in list C can be obtained by writing to: The Office of the Director, TDR, World Health Organization, 1211 Geneva 27, Switzerland; requests should carry the full document title and reference number.

12 Social and economic research

The context

□ In the early stages of TDR's activities it was realized that social and economic factors must be taken into account if the control of tropical diseases were to be improved to any significant degree. The result of this realization was the establishment, in 1979, of the Scientific Working Group (SWG) on Social and Economic Research (SER).

Scientists from many disciplines are involved in the work of SER (Fig. 12.1): anthropologists, sociologists, psychologists and linguists probe people's attitudes, perceptions and behaviour in relation to disease and disease transmission; economists assess, from national and international perspectives, not only the cost-effectiveness of control programmes but also the economic rationale behind decision-making in the household and how it is influenced by and influences disease transmission.

In the past, these scientists have often worked in isolation. Effective social and economic research can only be achieved through the application of sound epidemiological knowledge combined with social science principles: in other words, through a collaborative effort on the part of community health specialists and social scientists. Too frequently, in the past, health specialists were well trained in epidemiology but lacked expertise in social sciences, whereas social scientists lacked training in epidemiology. This dichotomy may explain why the findings of earlier studies on the social and economic impact of tropical diseases were often erroneous or did not take into account the importance of underlying social and economic conditions. Scepticism about the value of social science studies on tropical diseases was the

result. Projects supported by SER are now demonstrating that well-conceived, well-executed studies can give results directly applicable to the improvement of disease control.

All projects supported by SER are based within institutions in tropical countries and carried out by investigators from these countries, who have a greater understanding of local situations and closer contact with communities and disease control authorities than foreign experts. Contacts have therefore been made with interested social scientists and with control programmes in tropical countries, and interdisciplinary teams have been set up to work on problems of high priority.

There are a great variety of problems to be solved and topics to be studied. SER first supports research on basic social and economic factors influencing disease transmission and control and then examines how control operations are organized and put into effect. On completion of a research project, recommendations for improving disease control are given directly to national control programmes and local communities.

Each study topic is usually determined by a research team that includes national control programme staff. SER supports projects which are likely to fill important gaps in knowledge and to gain support from national authorities. They cover many different population groups, diseases, social and economic issues, and methodologies (Fig. 12.1 and Table 12.1). SER has funded research projects, held state-of-the-art review meetings and begun to develop short- and long-term training activities to ensure that this research can be sustained locally. □

Box 12.1 Highlights of activities in 1983-84

Results directly applicable to the improvement of disease control that have emerged from SER projects during the reporting period include:

- a method of analysing the cost and performance of malaria control (in Thailand);
- evidence that local beliefs and behaviour influence the effectiveness of filariasis control (in Malaysia and the Philippines);
- new methods of identifying and solving leprosy control problems related to stigma and local attitudes (in Malaysia and the Philippines);
- new multidisciplinary methods, currently being developed, of assessing the social and economic impact of disease (in Brazil, Colombia, the Philippines, the Sudan and the United Republic of Tanzania);
- evidence of wide variability in the applicability of community participation and new methods of assessing locally its effectiveness in disease control (in Brazil, Kenya, Nigeria and Sri Lanka).

Report of activities in 1983-84

The emphasis of SER has so far been on research to identify social and economic factors impeding disease control (Table 12.1). As baseline studies are completed, increasing attention will be paid to research on ways of integrating the findings of social and economic research into disease control operations.

Fifty-four field research projects have now been supported by SER in 23 countries. They focus on such problems as: community resistance to mosquito control; noncompliance with treatment programmes; movement of populations into disease-endemic areas; continued use of water sources that are disease vector habitats, despite the availability of alternative sources; cultivation of needed crops attractive to disease vectors; community or individual non-compliance with control measures requiring sustained and/or repeated effort; lack of demonstrable public health benefits from disease control programmes, resulting in inadequate resources for disease control; and creation of new disease hazards by development projects, despite an awareness of the risks they incur.

Four of the 54 research projects have been completed and preliminary findings from these and others are described below. It would be premature at this stage to draw general conclusions from these studies, but initial results already throw light on some methodological issues. Optimal methods for project design, data collection and data analysis, for example, differ from project to project. In particular, a survey or questionnaire approach cannot be relied upon exclusively for data collection: anthropological approaches, involving work with communities and control programme staff, may be required to assess human factors at the level of the individual, household, community or control programme. Information from such assessments may then be used to prepare larger-scale studies.

Collection of such a variety of data poses problems of analysis. In some projects, the household is the most useful unit for analysis. In others, it is the individual, with the focus on conventional parameters, such as age, sex, income and infection, or on less traditional elements, such as beliefs about disease. Two meetings — one focusing on social science research and its importance in health education, the other on research on the economic aspects of tropical diseases — are now being planned to review study designs and methods.

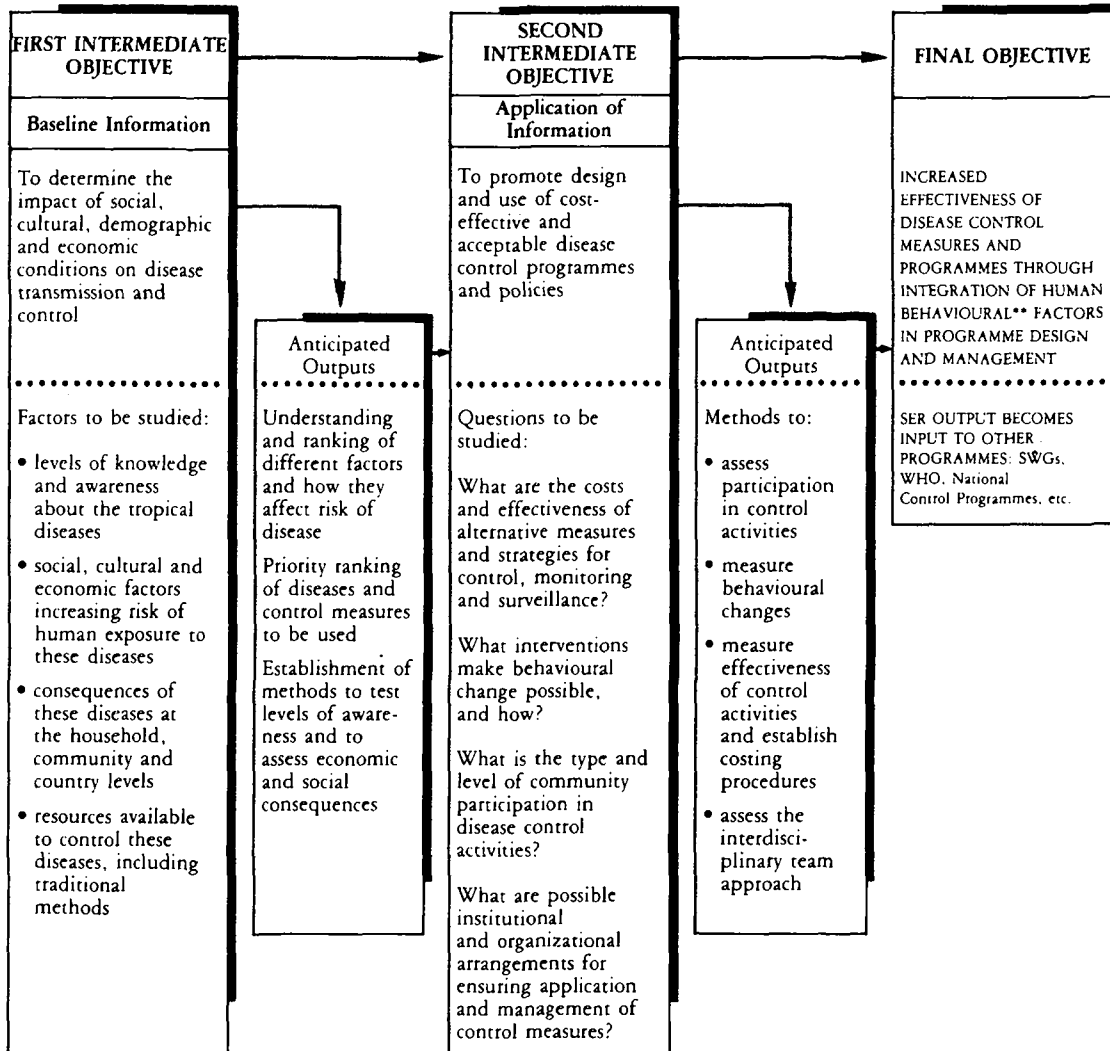
Malaria

Findings from SER studies are already influencing disease control programmes and demonstrating the importance of establishing at the outset close collaboration between research workers and the users of research products. In the Dominican Republic, for example, activities of the Malaria Control Programme are being modified in the light of an SER project involving the sociomedical analysis of living conditions and population movements. In some countries, ongoing SER research projects and meetings have prompted national agencies to support research. In Brazil, the national disease control programme is now supporting research on population movements and disease transmission, and has been encouraging investigators to submit projects to TDR. Behavioural science research is now in progress, both independently and in conjunction with TDR-supported studies, under the aegis of the Blue Nile Health Project in the Sudan. Among the topics covered are community participation, human-water contact in relation to disease transmission, health education and economic analysis of the consequences of disease.

Two projects illustrate the need for "situation-

FIG. 12.1

SER STRATEGIC PLAN OF RESEARCH*



* All studies are carried out in coordination with disease-specific and trans-disease Scientific Working Groups (SWGs) and, when applicable, with other WHO programmes.

** Behaviour is defined to include social, cultural and economic factors.

specific" approaches to the development of community-based malaria control. In Saradidi, in western Kenya, a church-based group is the basis for successful community participation in the distribution of antimalarial drugs. In Sri Lanka, on the other hand, involvement of local inhabitants in reducing mosquito breeding sites is proving more difficult, probably because of competing demands for time and effort.

Methods of evaluating costs and performance of

malaria control have been developed in an SER project and will soon be applied in Thailand (A9). The project was carried out by a team from Chulalongkorn University in Bangkok, Thailand, and the Malaria Division of the Thai Ministry of Public Health, with the cooperation of the community involved. A common problem in evaluating disease control priorities is the difficulty of identifying the different cost components of control activities. Expenditures are often listed under "wages, equipment

and supplies'', and it may be difficult to measure the precise outcome of any part of the control programme in relation to its cost. Yet, since available resources are limited, this information is essential. The Bangkok team developed a practical mathematical solution to the problem.

Although costs of operational services and units can be determined retrospectively (by apportioning expenditure under budget headings for the different activities), the new system, using an algorithm to determine costs for each activity, makes concurrent costing simpler and more reliable. In two major zones, differences were revealed in costs per case detected and costs per patient treated depending on whether malaria control clinics, village health collaborators or active case-detection services were used. Based on this research, a Thai manual for control programme staff has been prepared. The new procedures are now being implemented and some malaria staff have used them in their current budget requests. A follow-up study is being conducted jointly by the SER research team and the Thai malaria control programme to analyse in greater detail the siting of malaria clinics and the use of the primary health care village volunteer system.

Schistosomiasis

A study is in progress in a periurban community of Bahia State, in Brazil, on aspects of health education and community participation related to schistosomiasis control: 44% of adults interviewed were unaware that schistosomiasis was transmitted through exposure to water. Heavy work, heat, "evil eye" and malnutrition were mentioned as important causative factors. Religion and folklore were found to be the principal bases for organization in the community and lack of sanitation was a major concern of the population.

The community and the project team are jointly developing social action for disease prevention. Informal group discussions have been held on the relationships between social conditions, health and disease control. A youth group has produced a play about the health hazards of local living conditions, and the effectiveness of newsletters, radio and television in health education is being assessed. Material from the project is already being used in postgraduate community health training in Brazil.

Chagas' disease

At Cabeceira do Cansanção, in the State of Minas Gerais in rural Brazil, where it is estimated that 71% of the population is infected with *Trypanosoma cruzi*,

popular knowledge and new control strategies for Chagas' disease are being studied with the aim of developing disease control programmes through community participation. Few people have been found to associate triatomid bugs with disease transmission, although most are familiar with the bugs and their relationship to housing conditions, and with the symptoms of chronic Chagas' disease. The project is developing, with active community involvement, a programme to control Chagas' disease through insecticide spraying, house improvements and income-generating activities.

Filariasis

Two recently completed studies underline the need for a local approach to disease control. Both studies examined social and economic features of communities in which filariasis has been a persistent problem despite control measures. In the Magallanes municipality of Sorsogon Province, in the Philippines, social practices and attitudes to filariasis were analysed in relation to disease transmission, treatment and prevention (A10). The study's findings on local beliefs and patterns of treatment were communicated to the community and also to the filariasis control programme staff. One major finding was that the standard health education programme had not influenced beliefs and behaviour: people still believed, for example, that working in water and lifting heavy loads could cause disease. At the request of the local control programme, a follow-up study is being conducted on treatment compliance and diagnostic procedures. From the findings of these studies, guidelines will be developed for a health education programme.

In a study of health behaviour among rural households in Selangor State, Malaysia, perceptions of brugian filariasis were found to be based on an "ancient curse" and on guardian spirits (A14). Elephantiasis, considered incurable and therefore not to be medically treated, was thought to have originated from a spirit which lived in the elephant's jungle habitat and became angry at man's intrusion. The disease was also thought to be associated with water, which washed down the soil from the elephant's habitat. Without knowledge of such beliefs, control measures cannot be fully effective.

These studies show that widely differing beliefs about disease causation and prevention can be held in apparently similar rural communities. The Selangor study was conducted in a stable community, where tradition is the predominant force in daily life. In the Philippine study, concern with the more modern, economic aspects of living predominates,

TABLE 12.1

Distribution* of social and economic research projects by disease, topic, country and principal investigator**

<i>Topic/ disease</i>	<i>Malaria</i>	<i>Schistoso- miasis</i>	<i>Leprosy</i>	<i>Filariasis</i>	<i>African trypanoso- miasis</i>	<i>Chagas' disease</i>	<i>Leishmaniases</i>
FIRST INTERMEDIATE OBJECTIVE							
Social/ behavioural	Botswana (Mazonde) Colombia (Franco) (Sevilla-Casas) Costa Rica (Escobar) Egypt (Rakha) Peru*** (Mora) Philippines (Lariosa) Sri Lanka (Jayewardene) (Jayatileke) (Fernando) Thailand (Chitparop) (Hongvivatana)	Ghana (Boateng) Malawi (Kamwendo) Sierra Leone (Yumkella)	Kenya (Migot-Adholla) (Nyamwaya) Philippines (Valencia - 2) United Rep. of Tanzania (Muhondwa)	Malaysia (Mohd. Riji) Philippines (Lu) (Ventura)		Venezuela (Briceno-Leon)	Peru*** (Mora) Kenya (Kaendi)
Migration	Brazil (Sawyer) Dominican Rep. (Cury) Mali*** (Maiga - 2)	Mali*** (Maiga - 2)		Mexico (Ortega) (Quesada)	Ivory Coast (Hervouet)		
Economic/ social consequences	Colombia (Bonilla da Ramos) Sudan*** (Haridi)	Philippines (Paqueo - 2) Sudan*** (Haridi) United Rep. of Tanzania (Makembe)					
SECOND INTERMEDIATE OBJECTIVE							
Health education	Nigeria*** (Okafor)	Brazil (Loureiro) Nigeria*** (Okafor)	Malaysia (Chen) Nigeria*** (Okafor)	Nigeria*** (Okafor)	Nigeria*** (Okafor) Cameroon (Mafiamba)		
Community participation	Kenya (Kaseje) Nigeria*** (Adeniyi) Sri Lanka (Ruberu) Sudan*** (Gaddal)	Egypt (Khaity) Nigeria*** (Adeniyi) Sudan*** (Gaddal) Venezuela (Torrealba)		Nigeria*** (Adeniyi)		Brazil (Borges Dias)	
Economics/ management/ use of new tools	Thailand (Kaewsonthi - 2)	Egypt (EL Zeimity) (El Hak) (El Alamy)		Nigeria*** (Adeniyi)			

A hyphen followed by a number indicates the number of projects for which the principal investigator is or has been responsible.

* This distribution relates to the intermediate objectives identified in the Strategic Plan (Fig. 12.1). The table includes all field projects: feasibility studies, larger-scale projects and follow-up projects.

** Names of principal investigators are shown in parentheses below the country in which the project is/was based.

*** A single project under the responsibility of a single principal investigator but concerning more than one disease and/or topic.



PHOTO Courtesy of L. Valencia

Filipino leprosy patient being interviewed at her home, as part of an innovative study on stigma and local attitudes to leprosy: the data collection team included a psychologist, linguist, sociologist and public health nurse working together to construct a comprehensive cultural image of leprosy in the community.

and a direct relationship was seen between economic activities and disease transmission. The two villages have the same problem — the need to control filariasis — but the ways of solving it are likely to be quite different.

A successful guinea worm control project in Oyo State, Nigeria, based on piped water supplies, had been achieved with outside funding and with contributions from the community. Maintenance of the water system was not provided: it broke down, the community was forced to return to contaminated water and guinea worm again became a problem. The SER project is determining whether, by training and adult education programmes, and changes in the school health education curriculum, the community can be motivated to avoid contaminated water by the use of simple technology, like wells and water filters, which can be maintained by the community itself (A1).

Leprosy

Social, linguistic and psychological factors relating to the stigma associated with leprosy are being studied in the Ilocano communities in the Philippines (Fig. 12.2). Responses of people to short stories or “vignettes” and to illustrated cartoon stories about leprosy (Fig. 12.3) were used to obtain information about their attitudes and behaviour in relation to the disease (A19). Leprosy patients in these communities were found to refer to their condition in negative terms denoting, for example, shame and despair. Such attitudes had a profound effect on their readiness to seek and comply with treatment, which of necessity requires a long-term course of therapy.

Understanding people’s attitudes is essential for effective case-finding and case-holding. However, poor compliance and ineffective disease control were seen to be not necessarily nor entirely attributable to patients. Individuals not suffering from the disease

tended to refer to leprosy patients in terms indicating an imminent culmination of life's activities. Such a negative orientation, especially if held by members of control staff, may reinforce patients' own negative views and hamper control efforts. A study on this aspect of the leprosy problem has now begun.

Community participation

An SWG meeting was held in November 1983 to review research on community involvement in tropical disease control and to assess the progress of TDR projects involving community participation. More research is needed, it was agreed, particularly on ways of enhancing community participation and of assessing its overall cost and its cost-effectiveness at various stages of the disease control process. The meeting concluded that no single approach or standard protocol could be used to plan and conduct such

research, which, on the contrary, must be geared to the particular circumstances of the community involved.

Improvement of project design

Three workshops have been held to promote interest in social and economic research and to increase skills in preparing research proposals. All three were organized by local institutions and co-sponsored by the International Development Research Centre (IDRC) of Canada. Additional support was received from the Economic and Social Commission for Asia and the Pacific (ESCAP), the Ford Foundation, the United Nations Children's Fund (UNICEF) and the United States Agency for International Development (USAID).

One result of a workshop held in Kenya in 1982 was the creation of a network of social scientists in

FIG. 12.3

CARTOON CHARACTERS IN CHILDREN'S BOOK ON LEPROSY



One aim of TDR's social and economic research strategy is to provide culture-specific educational material on the six diseases of concern to the Programme. In Sarawak, Malaysia, the cultural backgrounds of population groups differing in age, ethnic origin and geographical setting, are the subject of one TDR-supported study. Information from this study was used to prepare a children's book on leprosy. The characters depicted in this prototype cartoon book (above) tell the story of a young leprosy patient, the reactions he encounters in his family and the community, and how he is made aware of the need for early treatment. The book is being field-tested for use by the Malaysian leprosy control programme and local community health workers.

Nairobi interested in SER-related research. M.Sc. students in sociology at the University of Nairobi are now conducting research on tropical diseases to meet thesis requirements. Similar networks are emerging from SER workshops held in Cameroon and Thailand in 1984. Workshops of this type have proved to be an effective mechanism for stimulating interest in social and economic research as applied to tropical diseases and for eliciting research proposals to SER.

Collaboration with other TDR Components and participation in other WHO activities

Collaboration has formed an integral part of a number of SER activities:

- TDR's epidemiology and disease-oriented SWGs collaborate with SER to ensure sound epidemiological input into SER projects and SER in turn reviews the social and economic aspects of projects supported by other SWGs.
- SER and the SWG on Applied Field Research in Malaria (FIELDMAL) support one project jointly and collaborate in the assessment of certain research proposals, and SER investigators are involved in a

number of FIELDMAL technical reviews.

- An SER study, suggested by the Steering Committee on Filariasis, is in progress on the social acceptance of guinea worm filters. The study draws on baseline data provided by an SER-supported field project (A1).
- WHO's Malaria Action Programme has provided technical support for SER research projects and an SER investigator is now a member of WHO's expert panel on malaria. SER-supported scientists have been invited to participate in activities of WHO's programmes on malaria, filariasis, schistosomiasis, leprosy, vector biology and control, and health education.
- A network of research workers studying migration is currently being promoted as a result of collaboration between SER, WHO's Regional Office for the Americas (AMRO) and the IDRC. Cooperation on the promotion and review of research projects is being developed with WHO's Regional Office for South-East Asia (SEARO), which has recruited a social scientist to work, among other things, on the development of research projects related to the social and economic aspects of tropical diseases.

Box 12.2 The future

- SER will give priority to research projects which seek to incorporate the findings of social and economic studies into disease control programmes. To this end, more extensive links will be established with WHO's operational disease control programmes and with ministries of health.
- In collaboration with other agencies, SER will seek to establish additional networks of scientists involved in social and economic research on tropical diseases.
- Training programmes will be developed in social sciences related to tropical diseases.
- A publication series is being started which will present case-studies in social and economic research on tropical diseases. The first in the series will be a "symposium by correspondence" on the "cost and performance of malaria control" project in Thailand.
- More research is required on the cost-effectiveness of intervention and delivery systems (including community participation) and resource allocation. Consultations will take place with other TDR Components, WHO's Expanded Programme on Immunization (EPI) and Diarrhoeal Diseases Programme (CDD), and other agencies, including The World Bank, to review research methods and to identify other topics in this area on which research is needed.
- SER will promote research on social and economic factors involved in the transfer of new technologies developed by TDR — new diagnostic techniques, new vaccines, new vector control tools (tsetse fly traps and guinea worm filters, for example) and new drug regimens.

PUBLICATIONS

Social and economic research

Publications acknowledging TDR support

- A1. ADENIYI, J.D. & BRIEGER, W.R. Guinea worm control in Idere. *World Health* (April-May): 8-11 (1983).
- A2. BANGUERO, H. Socioeconomic factors associated with malaria in Colombia. *Social Science and Medicine*, 19(10): 1099-1104 (1984).
- A3. BRIEGER, W.R., RAMAKRISHNA, J. & ADENIYI, J.D. Yoruba disease classifications for planning in health care. *International Quarterly of Community Health Education*, 4(2): 117-122 (1984).
- A4. BRIEGER, W.R., RAMAKRISHNA, J. & ADENIYI, J.D. A PHC approach to medical education. *Africa Health* (June-July): 22-23 (1984).
- A5. FERNANDO, M.A. (ed.) *Human Population Movements and Their Impact on Tropical Disease Transmission and Control. Proceedings of the workshop organized by the Faculty of Medicine, University of Peradeniya, Sri Lanka, in collaboration with the Social and Economic Section of the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases, 28 January - 1 February 1983*, 1984, 180 pp.
- A6. HONGVIVATANA, T. The application of social science research to tropical diseases. *Southeast Asian Journal of Tropical Medicine and Public Health*, 14(1): 47-53 (1983).
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TDR scientific reports which became available in 1983-84

- C1. TDR. Report of the second Scientific Working Group on Social and Economic Research: Guidelines to assess the social and economic consequences of the tropical diseases, Geneva, 22-27 October 1980. Document TDR/SER-SWG(2)/80.3. (English only)
- C2. TDR. Report on informal consultation on training in social sciences for tropical disease studies, Geneva, 28-29 April 1982. Document TDR/SER/SC(6)-TRN/82.3. (English only)

13 Strengthening of research capabilities in developing endemic countries

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Note on publications

All publications stemming from work supported by TDR's Research Strengthening Group (RSG) have been included in the publications lists of the different "disease-specific" chapters of the Report, as have publications from work outside the Programme that might have bearing on research capability strengthening. This chapter has, therefore, only one publications list, comprising TDR reports which became available in 1983-84 and which can be obtained from: The Office of the Director, TDR, World Health Organization, 1211 Geneva 27, Switzerland; requests should carry the full document title and reference number.

13 Strengthening of research capabilities in developing endemic countries

The context

□ TDR supports research throughout the world. A special effort is made, however, to involve scientists and institutions in developing countries in the planning and execution of research towards new methods of preventing, diagnosing and treating the endemic diseases, and of controlling the vectors that transmit them.

Scientists from developing countries are engaged in all aspects of research funded by the Programme, from basic biomedical sciences to clinical and field activities. Already, they are playing vital roles in the final development of research products — drugs, vaccines and diagnostic tests — and in the adaptation of these products to local use within national disease control programmes.

Much of this work is carried out under TDR's dif-

ferent Scientific Working Groups (SWGs). In addition, the Programme has a direct mechanism for strengthening the research capabilities of the developing countries: the Research Strengthening Group (RSG). The respective roles of the RSG and the SWGs have been clearly defined in relation to the developing countries, and mechanisms have been established to ensure that their activities are mutually supportive and complementary. Both RSG and SWG grants have provided opportunities for the training of young research scientists, in some cases enabling them to complete requirements for higher degrees (Table 13.1). RSG grants have made it possible for institutions in developing countries to make significant contributions to priority areas identified by the SWGs. To enhance local biomedical and health

TABLE 13.1

Numbers of theses reported to TDR resulting from work supported by the Special Programme

<i>Nature of degree</i>	<i>RSG</i>	<i>SWG*</i>	<i>Total 1983-84</i>	<i>Cumulative total 1975-84</i>
Ph. D.	13	21	34	54
M. Sc.	19	27	46	64
Other	8	15	23	28
Total 1983-84	40	63	103 **	
Cumulative total 1975-84	55	91		146

* Some theses were written by scientists from developed countries.

** Breakdown by disease, 1983-84:

Malaria	29	Chagas' disease	10
Filariasis	23	Trypanosomiasis	5
Leishmaniases	16	Leprosy	4
Schistosomiasis	10	Other	6

resources, the Programme strengthens the infrastructures of selected national institutions and trains key personnel, but always in the context of national health development plans and existing programmes of research and disease control.

Since the start of the Programme, the proportion of operational funding going to developing endemic countries has steadily increased, reaching a record 60% for 1983-84 (Table 13.2). □

TABLE 13.2

Percentage of total TDR funds obligated to developing endemic countries 1975-84

Year	% Funding
1975-78	44
1979-80	56
1981-82	58
1983-84	60

Report of activities in 1983-84

Institutional support

The first phase of the strategic plan established in 1979 by the Research Strengthening Group (RSG) (see the *Sixth Programme Report*, pp. 380-382) has now been completed. In this phase, emphasis was placed on strengthening relatively well-developed institutions. The selected institutions were supported according to their needs, taking advantage of existing strengths and local opportunities. Some institutions, for example, were given a one-time capital grant to purchase essential equipment. Others received long-term support grants normally stretching over a five-year period or more (Box 13.1 and Fig. 13.1). Some of these strengthened institutions are now supporting less developed institutions, training their scientists and collaborating with them in joint research

activities in a spirit of technical cooperation among developing countries. A number of institutions being strengthened have also established links with institutions in developed countries, thereby promoting collaboration within a global research network.

During 1983-84, fourteen institutions came to the end of their long-term support grants and of these, three were granted an extension (Table 13.3). Eleven new long-term awards were made to institutions in Africa, Latin America and Asia (Table 13.4), with particular emphasis on the development of field research activities. Since the beginning of the Programme, the RSG has made a total of 82 grants to institutions in developing countries:

Box 13.1 Types of grants awarded for strengthening research capabilities

Institutional grants

- *Long-term support grants*, normally awarded for a period of five years, are intended to help an institution carry out research on one or more of the six diseases of interest to TDR. The grants, initially made for one year but renewable annually depending on progress achieved, are given on a sliding scale, the institution progressively taking over recurrent costs — an essential condition for receipt of such a grant.
- *Capital grants* are awarded to fairly well-established institutions which require a single grant to improve

research and research training facilities or an initial capital outlay to purchase equipment and initiate research activities.

- *Short-term support grants* are awarded for initial pilot activities and the preparation of plans for long-term institution-strengthening activities. They are normally awarded for one or two years.

Training grants

- *Support for degree courses* is given towards the cost of formal postgraduate courses, usually in a university setting.

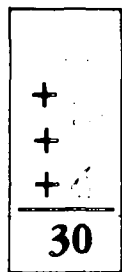
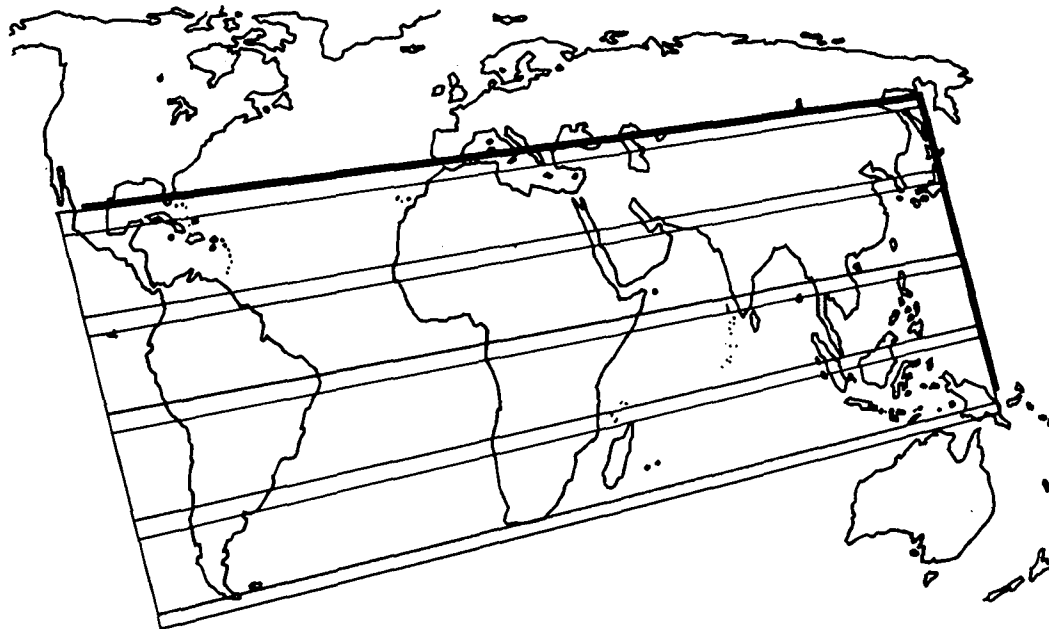
- *Re-entry grants* are awarded to researchers returning to work in their institution, to enable them to begin research after training.

- *Visiting scientist grants* are awarded to senior scientists and research managers already engaged in activities in their institutions, to help them visit other institutions and scientists engaged in related work.

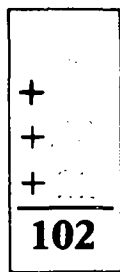
- *Research training grants* are awarded to staff holding regular career positions in a research or training institution, to enable them to participate in training programmes in institutions outside their own countries.

FIG. 13.1

INSTITUTIONAL GRANTS AWARDED BY TDR



Total 1983-84



Cumulative total 1975-1984

- Long-term support grants
- Capital grants
- Short-term support and small grants
- Support for degree courses

Training of research personnel

Individual training

The training of research scientists and other personnel is an important part of the Programme's institution-strengthening strategy (Fig. 13.3). During 1983-84, 68 scientists completed their training programmes and returned to their home institutions. The conditions a Ph.D. candidate has to fulfil to receive support from the Programme have been altered to encourage the candidate to carry out the research in the home institution: the first part of the training period is spent abroad on foundation courses and in planning the research project which will form the substance of a thesis. The candidate then returns home to carry out research with appropriate support, including visits from the supervisor from the train-

ing institution abroad, where the candidate spends the final stage of the training period writing the thesis and completing degree requirements.

Group training

Since the Programme began, it has supported 10 M.Sc. courses in medical entomology, epidemiology and public health (Box 13.3) and, in conjunction with the SWG on Biomedical Sciences, several workshops and short courses to facilitate the transfer of new technology to developing countries (Box 13.4).

Management and evaluation

Institutions being strengthened are encouraged to improve the management of their programmes and to develop appropriate mechanisms for evaluating progress, with emphasis on critical self-assessment.

Box 13.2

The Tropical Diseases Research Centre in Ndola, Zambia

The Tropical Diseases Research Centre (TDRC) in Ndola, Zambia, was established in 1975 as a multidisciplinary WHO centre for biomedical research and training in Africa. Its management was handed over to national authorities in 1981; there are now 26 Zambian scientists and technicians at the TDRC, including those being trained abroad (Fig. 13.2). The present staff includes nine Zambians who have returned to the Centre after completing training abroad under a staff development scheme.

The TDRC has taken a multi-disciplinary approach to the study of locally endemic diseases, closely integrating clinical, parasitological, epidemiological and socioeconomic studies. More specifically, it has conducted clinical and pharmacological evaluations of mefloquine; longitudinal, multidisease, population-based studies designed to produce descriptive epidemiological data; and case-control studies to test hypotheses on risk factors in trypanosomiasis. The Centre has also developed an official link with the two Schools of Tropical Medicine in Britain, in Liverpool and London, respectively.

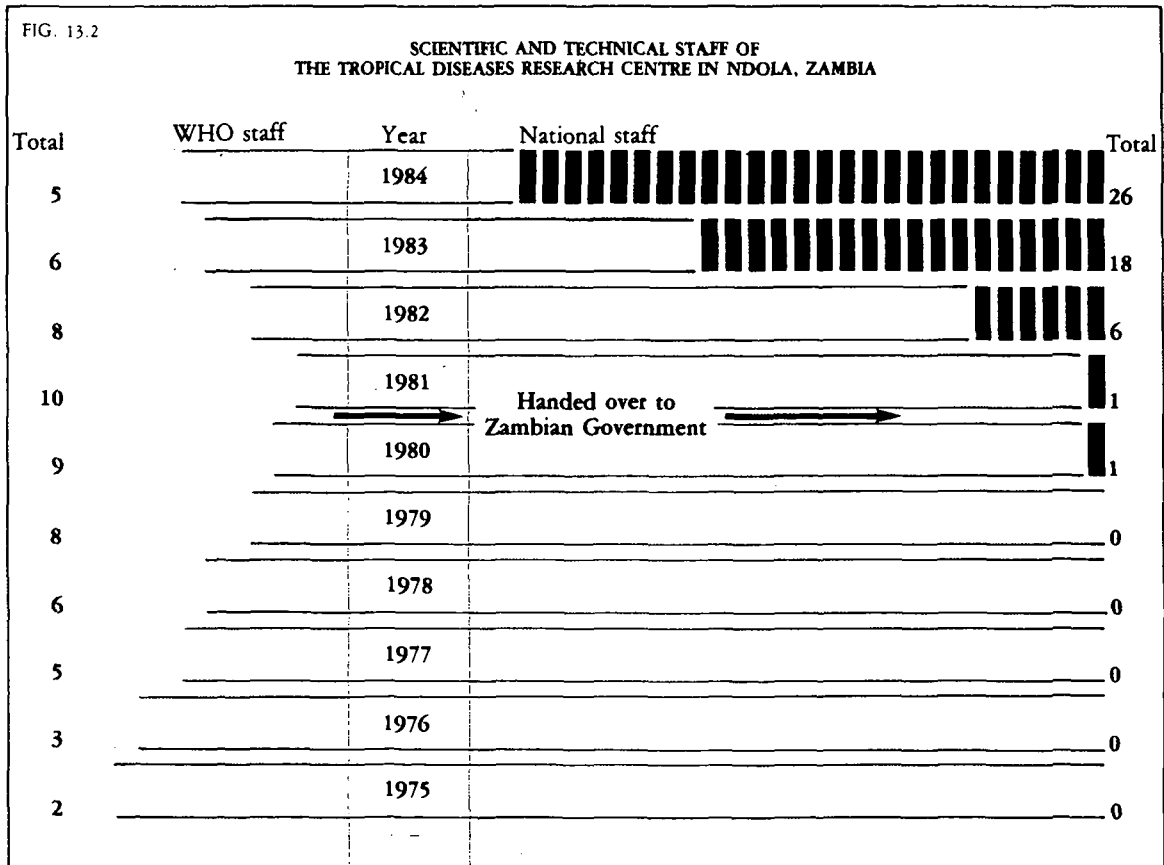


TABLE 13.3 Long-term support grants that reached completion before the end of 1984

<i>Institution</i>	<i>Research area</i>	<i>Years of support</i>
AFRICA		
• Service de Parasitologie Faculté de Médecine et de Pharmacie Université de Dakar Dakar, Senegal	Immunology of malaria	1977-81
• International Centre of Insect Physiology and Ecology (ICIPE) Nairobi, Kenya	Entomology of parasitic diseases, especially leish- maniases, malaria and trypanosomiasis	1979-81
• Département d'Immunologie et Médecine Cliniques Centre Universitaire des Sciences de la Santé Université de Yaoundé Yaoundé, Cameroon	Immunology of parasitic diseases	1980-84
• Clinical Research Centre Kenya Institute of Medical Research Nairobi, Kenya	Clinical, parasitological, immunological and epidemiological studies of parasitic diseases	1980-84
• Department of Pharmacology and Therapeutics College of Medicine University of Ibadan Ibadan, Nigeria	Clinical pharmacology	1980-84
ASIA		
◦ Institute of Public Health University of the Philippines System Manila, Philippines	Schistosomiasis	1978-83
◦ Department of Parasitology and General Pathology Faculty of Medicine University of Indonesia Jakarta, Indonesia	Epidemiology, immunology and chemotherapy of filariasis and malaria	1979-83
◦ Institute for Medical Research Jalan Pahang Kuala Lumpur, Malaysia	Epidemiology, biostatistics and immunology of tropical diseases	1978-84
◦ * Institute of Malariology, Parasitology and Entomology Hanoi, Socialist Republic of Viet Nam	Epidemiology, chemotherapy and entomology of malaria	1979-83
◦ Faculty of Tropical Medicine Mahidol University Bangkok, Thailand	Epidemiological and socioeconomic aspects of tropical diseases	1979-83
◦ Department of Biology Faculty of Science Mahidol University Bangkok, Thailand	Research and Training Centre in applied malacology	1979-83
THE AMERICAS		
◦ Centro de Educación Médica e Investigaciones Clínicas (CEMIC) Sección Immunología Buenos Aires, Argentina	Immunology of Chagas' disease	1979-83
◦ Fundação Oswaldo Cruz (FIOCRUZ) Rio de Janeiro, Brazil	Immunoparasitology of Chagas' disease	1979-84
◦ * Instituto de Investigaciones en Medicina Tropical "Pedro Kouri" La Habana, Cuba	Tropical disease research	1979-84
◦ National Institute of Dermatology Caracas, Venezuela	Epidemiology of tropical diseases, with emphasis on leprosy	1979-83
◦ * Instituto de Medicina Tropical "Alexander von Humboldt" Universidad Peruana Cayetano Heredia Lima, Peru	Tropical disease research	1979-84

* Completed but received one-year extension

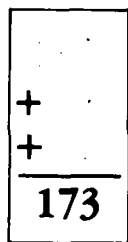
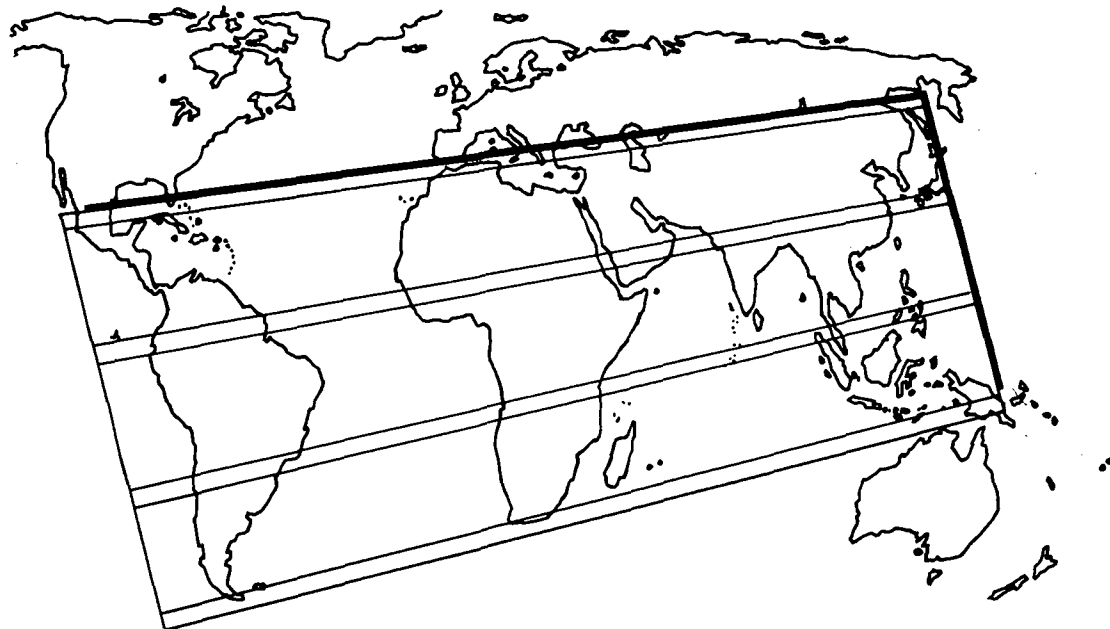
TABLE 13.4

New long-term support grants awarded in 1983-84

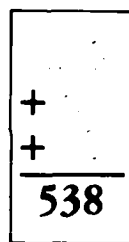
<i>Institution</i>	<i>Research discipline</i>	<i>Year initiated</i>
AFRICA		
• Institute of Primate Research National Museums of Kenya Nairobi, Kenya	Studies of tropical diseases using animal models	1983
• Centre Universitaire de Formation en Entomologie Médicale et Vétérinaire (CEMV) Faculté des Sciences Université d'Abidjan Bouaké, Ivory Coast	Entomology and epidemiology of trypanosomiasis	1984
• Institut de Recherches sur la Trypanosomiase et l'Onchocercose (IRTO-OCCGE) Bouaké, Ivory Coast	Entomology and epidemiology of trypanosomiasis	1984
• Institut Marchoux (OCCGE) Bamako, Mali	Drug resistance and epidemiology of leprosy	1984
• Tropical Medicine Research Institute and Immunology Training and Research Centre Khartoum, Sudan	Multidisciplinary research on leishmaniasis	1984
ASIA		
• Department of Parasitology Faculty of Medicine University of Colombo Colombo, Sri Lanka	Malaria research	1983
• Health Services Research and Development Centre Ministry of Health Surabaya, Indonesia	Field research in malaria and leprosy	1983
• National Drug Research Centre Universiti Sains Malaysia Penang, Malaysia	Clinical pharmacology	1984
THE AMERICAS		
• Chagas Research Group Faculty of Medicine Universidad de Chile Santiago, Chile	Clinical and basic biology of Chagas' disease	1983
• Department of Microbiology Universidad Nacional Autónoma de Honduras Tegucigalpa, Honduras	Epidemiology and immuno- parasitology of malaria and Chagas' disease	1984
• Instituto Nacional de Diagnóstico e Investigación de la Enfermedad de Chagas "Dr Mario Fátala Chabén" (INDIECH) Buenos Aires, Argentina	Integrated control of Chagas' disease and diagnosis of malaria and leishmaniasis	1984

FIG. 13.3

INDIVIDUAL TRAINING GRANTS AWARDED BY TDR



Total 1983-84



Cumulative total 1975-1984

- Research training grants
- Visiting scientist grants
- Re-entry grants

Improving management skills

The Programme holds management workshops to improve managerial abilities in institutions receiving long-term support grants. During 1981-82, two management workshops were held in, respectively, Flaine, France, and Penang, Malaysia. A further two workshops were held during 1983-84: one in Nazareth, Ethiopia, which was attended by directors of TDR-supported institutions in Africa; the other in Iquitos, Peru, to which directors of TDR-supported institutions in Latin America were invited. Each workshop, which employed management consultants as "facilitators", was attended by about 20 participants, who used the opportunity to plan and develop collaborative activities among their respective institutions.

Evaluation of progress

The Programme has devised mechanisms for internal and external evaluation of institutions being strengthened and for the formulation of proposals for improving efficiency. Several workshops on evaluation have been held since the Programme began: in Geneva, Switzerland (June 1979), with 7 participants from all parts of the world; in Nairobi, Kenya (March 1980), with 11 participants from Africa; in Cali, Colombia (July 1982), with 10 participants, all directors of M.Sc. courses, from different parts of the world; and in Islamabad, Pakistan (November 1982), with 10 participants from Asia. During 1983-84, a fifth evaluation workshop was held in Bogor, Indonesia, at which 12 internal evaluators were trained in conducting systematic reviews of their institu-

Box 13.3 M.Sc. courses supported by the Programme

Medical Entomology

- Medical Entomology Programme
Graduate Faculty
Bogor Agricultural University
Bogor, Indonesia
- Centre Universitaire de
Formation en Entomologie
médicale et vétérinaire
Bouaké, Ivory Coast
- Department of Zoology
University of Nairobi
Nairobi, Kenya
- Department of Zoology
University of Jos
Jos, Nigeria

- Faculty of Medicine
Universidad de Panama
Panama, Panama

- Faculty of Graduate Studies
Mahidol University
Bangkok, Thailand

Epidemiology and Community Health

- Division de Salud
Universidad del Valle
Cali, Colombia

- Department of Community
Health, Faculty of Medicine
University of Nairobi
Nairobi, Kenya

- Department of Social Medicine
and Public Health
Faculty of Medicine
National University of Singapore
Singapore

- Department of Community
Health, Muhimbili Medical Centre
Dar es Salaam, United Republic
of Tanzania

tions' activities, starting with the collection of basic data. Internal evaluation is completed by site visits by members of the Programme Secretariat and appropriate consultants.

Using criteria established during a consultation held in March 1984, the Programme is now evaluating progress made by the first 13 institutions to complete a period of long-term support. These criteria include changes in: physical resources; management practices; research output (as seen in scientific publications); competence of research staff and other personnel; interaction with national disease control programmes; and ability to attract research funds from government and other sources.

Progress in research capability strengthening

Strengthening research capabilities in developing countries is a long-term process. Even at this relatively early stage there are encouraging signs that significant progress has been made: the number of projects from scientists in developing countries has grown steadily, as has the number of scientific publications resulting from projects supported in developing countries.

Scientific contributions

Scientists and institutions in developing countries have now made significant contributions to the work of the Programme by participating in the work of

the different TDR Scientific Working Groups and in activities supported by the RSG.

- Institutions in Brazil, Thailand and Zambia have carried out 18 clinical trials of the antimalarial drug mefloquine; these trials provided the bulk of the clinical information required to obtain registration of the drug.

- In collaboration with the Liverpool School of Tropical Medicine in the United Kingdom, the Onchocerciasis Chemotherapy Research Centre in Tamale, Ghana, has made significant contributions to the development of new drugs for the treatment of onchocerciasis.

- The Clinical Research Centre in Nairobi, Kenya, has produced evidence that led to the revision of traditional drug regimens using pentavalent antimonials in the treatment of visceral leishmaniasis. The revised schedule was endorsed by a WHO Expert Committee on the Leishmaniasis.

- The Institute of Tropical Medicine at the University of Sao Paulo, Brazil, is the coordinating centre for a network of 14 institutions collaborating on the standardization of serodiagnosis in Chagas' disease. A high level of concordance has been achieved in results from the different laboratories.

- Scientists working at the Centro de Educación Médica e Investigaciones Clínicas (CEMIC) and the Instituto Latino-Americano de Investigaciones Médicas (ILAIMUS) in Argentina have produced lesions in the cebus monkey comparable to those of chronic Chagas' disease.

- A long-acting insecticidal paint has been developed at the Federal University of Rio de Janeiro, Brazil, and is being used by the Brazilian Chagas' Disease Control Programme (SUCAM) in a large-scale field trial in Goiás State.
- The Malaria Eradication Service in Manila, Philippines, in collaboration with the WHO Regional Office for the Western Pacific and TDR, has organized the production of "micro" field kits for testing the sensitivity of *P. falciparum* to 4-aminoquinolines and mefloquine.
- Work on the clinical pharmacology of chloroquine, conducted in Ibadan, Nigeria, in collaboration with scientists at the Karolinska Institute in Sweden, and at the National Drug Research Centre, Universiti Sains Malaysia, in Penang, Malaysia, has provided new information about the drug's pharmacokinetics.
- The Institute of Malaria, Parasitology and Entomology in Hanoi, Viet Nam, has carried out baseline research on the epidemiology of *P. falciparum* malaria, which should serve as a basis for improving the national control programme.
- In the Faculty of Tropical Medicine at Mahidol University in Bangkok, Thailand, a multidisciplinary group of epidemiologists, behavioural scientists, clinicians and parasitologists has contributed substantially towards the development of strategies for malaria control.

- At the Fundação Oswaldo Cruz, Rio de Janeiro, Brazil, molecular biologists are using DNA restriction enzyme analysis to classify *Trypanosoma cruzi*. The new classification is now being applied to epidemiological research on Chagas' disease.
- Scientists in Thailand have made important contributions to the understanding of the basic biology of malaria parasites, and the mechanisms of anti-malarial drug action and of *Plasmodium* resistance to drugs.

Regional role of strengthened institutions

Some strengthened institutions are now becoming regional reference and training centres in their respective disciplines.

The Centre for Malacological Research in the Department of Biology, Faculty of Sciences, Mahidol University, in Bangkok, Thailand, for example, has now become a regional reference and malacology training centre for South-East Asia. The Special Programme is making increasing use of training facilities available in endemic countries. Of 139 training grants awarded during 1983-84, 52 went to students who obtained part or all of their training in another developing country.

Box 13.4 Workshops on the transfer of technology

<ul style="list-style-type: none"> • November-December 1983 Fundação Oswaldo Cruz, Rio de Janeiro, Brazil (in collaboration with the SWG on Biomedical Sciences [BIOS]) 	<ul style="list-style-type: none"> 16 participants from Latin America 	<ul style="list-style-type: none"> Focus: Genes and antigens of parasites
<ul style="list-style-type: none"> • January 1984 Institute for Medical Research Kuala Lumpur, Malaysia 	<ul style="list-style-type: none"> 12 participants from Africa and Asia 	<ul style="list-style-type: none"> Focus: <i>In vitro</i> culture of filarial parasites
<ul style="list-style-type: none"> • September-October 1984 Madurai Kamaraj University, India (in collaboration with BIOS) 	<ul style="list-style-type: none"> 20 participants from Africa and Asia 	<ul style="list-style-type: none"> Focus: Molecular approaches to research in tropical diseases
<ul style="list-style-type: none"> • November 1984 National Institute of Health Islamabad, Pakistan 	<ul style="list-style-type: none"> 14 participants from Africa and Asia 	<ul style="list-style-type: none"> Focus: Isoenzyme characterization of old world leishmaniasis
<ul style="list-style-type: none"> • November 1984 Mahidol University Bangkok, Thailand (in collaboration with BIOS) 	<ul style="list-style-type: none"> 18 participants from Africa and Asia 	<ul style="list-style-type: none"> Focus: Genetic engineering techniques in tropical disease research

National impact of strengthened institutions

The national impact of TDR support is reflected in the increasing role of local institutions in national control programmes. Institutions are now generating epidemiological information for the planning and evaluation of control programmes for Chagas' disease, malaria, schistosomiasis and filariasis. Increasingly, national authorities are using the expertise of strengthened institutions to solve control problems and to support national health services.

Some institutions receiving long-term support — the National Institute of Health in Maputo, Mozambique; the Instituto Nacional de Diagnostico e Investigación de la Enfermedad de Chagas ("Dr Mario Fatale Chaben") (INDIECH) in Buenos Aires, Argentina; the Institute for Medical Research in Kuala Lumpur, Malaysia; the Health Services Research and Development Centre in Surabaya, Indonesia — are managed directly by ministries of health. Others, located in university departments or institutes, also work closely with national disease control programmes. These include the Vector Control

Research Centre in Pondicherry, India; the Clinical Research Centre in Nairobi, Kenya; the Institute of Public Health of the University of the Philippines, Manila; the Department of Parasitology of the Faculty of Medicine, Colombo Medical School, Sri Lanka; and the "Alexander von Humboldt" Institute of Tropical Medicine in Lima, Peru. Strategies, involving community participation, for the control of malaria, filariasis and other tropical diseases have been developed in the National Institute of Health in Maputo, Mozambique, and, in Pondicherry, India, the Vector Control Research Centre has devised and tested an integrated vector control approach for pest management.

An example of collaboration with local control authorities is seen in Thailand: in order to promote the rapid application of research findings, all investigators receiving TDR support met in January 1984 with representatives of the Office of the National Advisory Board for Disease Prevention and Control in the Ministry of Health to review ongoing and completed TDR-supported projects.

Box 13.5 The future

As the first phase of the RSG's strategic plan comes to an end, the second phase is being put into effect. In the immediate future, activities will focus on field research and basic biomedical research.

Field research

The steady stream of new products arising from TDR-supported activities requires careful clinical evaluation and field trials. There is therefore an urgent need to increase the capability of scientists and institutions in endemic areas to design and execute clinical trials and conduct other activities essential for the evaluation of these new products. The Programme will intensify its efforts to train appropriate personnel,

especially in epidemiology and medical entomology, where trained staff are in short supply. One of the most important constraints on the strengthening of field research is the lack of appropriate career structures in many developing countries. This has made it difficult to attract and retain the services of suitable staff to tackle the sometimes arduous field research. Although limited progress can be made in isolated cases by supporting special projects, a far-reaching solution would be for national health authorities (1) to make a long-term commitment to this high priority area as a prerequisite to promotion of research activities in their countries and (2) to design career structures, including appropriate incentives, to encourage

staff recruitment and maintain high staff morale.

Biomedical research

The RSG has been given the task of strengthening selected institutions in developing countries so that they are able to use modern biomedical techniques in immunology, molecular biology, biochemistry and genetics — techniques which are already proving their value in the development of vaccines, diagnostic tests and new drugs for the control of tropical diseases. The RSG will therefore be increasingly engaged in facilitating the transfer of these techniques to institutions being strengthened in developing countries.

TDR reports which became available in 1983-84

- C1. TDR. Report of the seventh meeting of the Research Strengthening Group (RSG), Geneva, 30 August - 3 September 1982. Document TDR/RSG(7)/82.3. (English and French)
- C2. TDR. Report of the eighth meeting of the Research Strengthening Group (RSG-8), Geneva, 22-26 August 1983. Document TDR/RSG(8)/83.3. (English and French)

14 Management and finance

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Pertinent TDR and WHO documents	14/10

Note:

TDR's scientific and technical reporting biennia — 1983-84, 1985-86, etc. — do not correspond to TDR/WHO's financial biennia — 1982-83, 1984-85, etc. The overlap between these two reporting periods enables the Joint Coordinating Board, TDR's top management body, to review both the scientific and technical progress of the Programme for the biennium immediately preceding its annual session and the Programme budget proposed for the biennium immediately following its annual session. Some sections of this chapter refer, therefore, to the financial biennial cycle, others to the scientific and technical biennial cycle.

14 Management and finance

The context

□ It took almost three years of negotiations to establish the *modus operandi* of the Special Programme. While the appropriateness of the Programme's objectives was never in doubt, the concept that TDR would operate through existing national institutions and their scientists raised many questions: Would institutions and scientists from many different countries be willing to work together? How would goals and priorities be established? How would the proposed worldwide scientific network be managed? How would related activities in many disparate institutions be coordinated? Would the creation of a number of international research centres not be more effective?

Two factors played a key role in the decision made in 1978 to base the Programme on a network of national institutions rather than on specially created new research centres. First, "on site" participation of tropical countries in the research and development process increases the chances of new technologies being locally appropriate and acceptable. Methods of controlling tropical diseases must be evaluated in the endemic countries themselves, and the participation of these countries as full partners in the research and development process greatly facilitates both the clinical testing and subsequent deployment of these methods by national health services. Second, drug and vaccine development require additional technologies and skills to those found in academic institutions. Only the pharmaceutical industry has the experience and the facilities to carry new products through to the stage of national registration.

So came into being the TDR network — a concept which makes effective use of both factors and whose worth and timeliness are borne out by the number of scientific and technical results now being produced.

Today the network pervades every aspect of TDR operations, from peer reviews of projects by individual scientists to policy decisions that are taken by the Joint Coordinating Board (JCB), TDR's top administrative body. From the start of TDR's scientific activities in 1977 up to 31 December 1984, TDR

has supported over 2000 projects in 100 WHO Member Countries, and over 4000 scientists and health administrators from 125 countries have taken part in the planning, research activities and evaluation of the Programme.

The enthusiastic participation of the world's scientific community is in itself a measure of the success of the TDR network. The diversity of involvement is illustrated not only by the geographical, cultural and political span of the 125 collaborating countries but also by the wide spectrum of scientific disciplines participating in the network, from social science field research in tropical villages to molecular biology in sophisticated research laboratories.

Some 50 "products", ranging from potential vaccines against malaria to biologically produced toxins for the control of disease vectors, are being developed through the TDR network, many with the active participation of the pharmaceutical industry.

Up to 31 December 1984, TDR's research and development efforts, which have brought several products to, or close to, the stage of field use, have cost less than US\$ 100 million — a fraction of the drug development costs reported by industry (US\$ 60-80 million per drug). On this basis alone, the TDR network would appear to be an effective mechanism for product development. Costs are kept low through the sharing of resources by the institutions, organizations and industrial firms participating in the network. Universities, government ministries and institutions, and public and private companies all contribute knowledge, manpower, facilities and operating costs to the collaborative efforts coordinated by TDR. The catalytic role of TDR increases the effectiveness of the collaboration and reduces the cost to each participant, as well as reducing the total cost for the development of a product.

Academic centres frequently provide the basic knowledge and the leads required for new technology. Industry develops the product to the stage of early trials in man, and academic centres and ministries of health in tropical countries then carry out the later phases of clinical and field testing.

TDR's unique role is to coordinate, through its worldwide network, the activities involved in every stage of this process. The development of new antimalarial drugs and potential vaccines against leprosy and malaria are typical examples of such collaboration and coordination.

The TDR network system of management has provided a demonstrably efficient mechanism whereby academic institutions, government ministries and agencies, and industrial concerns from many countries, as well as other international programmes (including other WHO programmes) work together

effectively towards well-defined common goals.

Unfortunately, the scientific and technical success of the Programme has not been matched by sustained, adequate financial support. Consequently, TDR's co-sponsoring agencies — the United Nations Development Programme (UNDP), The World Bank and the World Health Organization — together with the Joint Coordinating Board, have launched a major effort to publicize TDR's technical successes and obtain for the Programme the funds required to bring new disease control methods and capabilities to the millions of people who need them. □

Report of activities in 1983-84

The Scientific and Technical Advisory Committee (STAC)

STAC, which comprises 18 eminent members of the world's scientific community, acts as the Programme's scientific "watchdog" and adviser, and recommends to the JCB the main directions to be taken in TDR's scientific activities.

In 1983, the fifth STAC meeting analysed priority Programme activities for the period 1984 to 1987 and recommended a budget of US\$ 60.5 million for research and development and research capability strengthening for the 1984-85 financial biennium. STAC also called for the phasing out of the Programme's Biomedical Sciences Component (BIOS) by the end of 1985. At its sixth meeting, in 1984, STAC recommended that designated funds could be accepted for research on the immunology of malaria, leprosy vaccine development and research training, if such funds were contributed in accordance with the conditions established by the Sixth Session of the JCB held in 1983 (see below).

The Standing Committee

The Standing Committee, which is composed of senior representatives of the three co-sponsoring agencies — UNDP, The World Bank, WHO — continued to monitor the management of the Programme and its financial status. At its meeting in April 1983 the Committee recommended to the JCB a budget of US\$ 66.7 million for the 1984-85 financial biennium.

During 1983 and 1984, the Executive Heads of the three agencies made a personal appeal for additional funds for TDR. The Standing Committee also sponsored the production of a booklet, *Venture for Health*, to publicize the Programme.

The Joint Coordinating Board (JCB)

Government representatives of countries participating financially or technically in the Programme, together with representatives of the three co-sponsoring agencies, meet annually as the Joint Coordinating Board. The Sixth Session of the JCB, held in 1983, approved a maximum Programme budget of US\$ 66.7 million for the 1984-85 financial biennium and decided that designated funds would be accepted for a one-year trial period on condition that STAC identify activities suitable for this purpose, designated funds be truly additional contributions and such funds do not distort Programme priorities as established by STAC.

The Seventh Session of the JCB, held in 1984 in Bangkok, Thailand, was the first to take place outside of WHO headquarters, Geneva. It included a one-day visit to a site of malaria field research activities, which, together with technical presentations by Thai scientists, gave JCB members and observers a close-up look at health problems facing Thailand, and how the Special Programme was working with the Government of Thailand, its research institutions and scientists, to find solutions to these problems. In view of the rapid scientific and technical progress of the Programme, the Board discussed problems and mechanisms of transferring to national health services technology developed with TDR support.

Financial status

The audited financial report for the 1982-83 financial biennium was accepted by the JCB at its 1984 Session. Table 14.1 shows available funds and obligations between 1 January 1982 and 31 December 1983, and Fig. 14.1, the distribution among the four Programme Areas of obligations during the 1982-83

TABLE 14.1

Summary of 1982-83 income and obligations as at 31 December 1983 (US\$)

			Total
Balance as at 1 January 1982			6 564 559 ^a
	WORLD BANK*	WHO	
Income to 31 December 1983			
Contributions	31 470 079 ^b	8 807 385	40 277 464
Other income	1 593 441	3 249 955	4 843 396
Total funds available 1982-83			51 685 419
Obligations to 31 December 1983			49 372 631 ^c
Balance as at 1 January 1984			2 312 788

* The Tropical Diseases Research Trust Fund managed by The World Bank.

^a This figure includes a contribution of US\$ 2 973 573 for 1982 received from the Government of Denmark on 29 December 1981.

^b This figure includes a contribution of US\$ 1 126 199 for 1984 received from the Government of the Netherlands on 23 December 1983.

^c The JCB-approved budget for the biennium was US\$ 61.6 million, which was revised to US\$ 53 million by Director TDR and Chairman STAC.

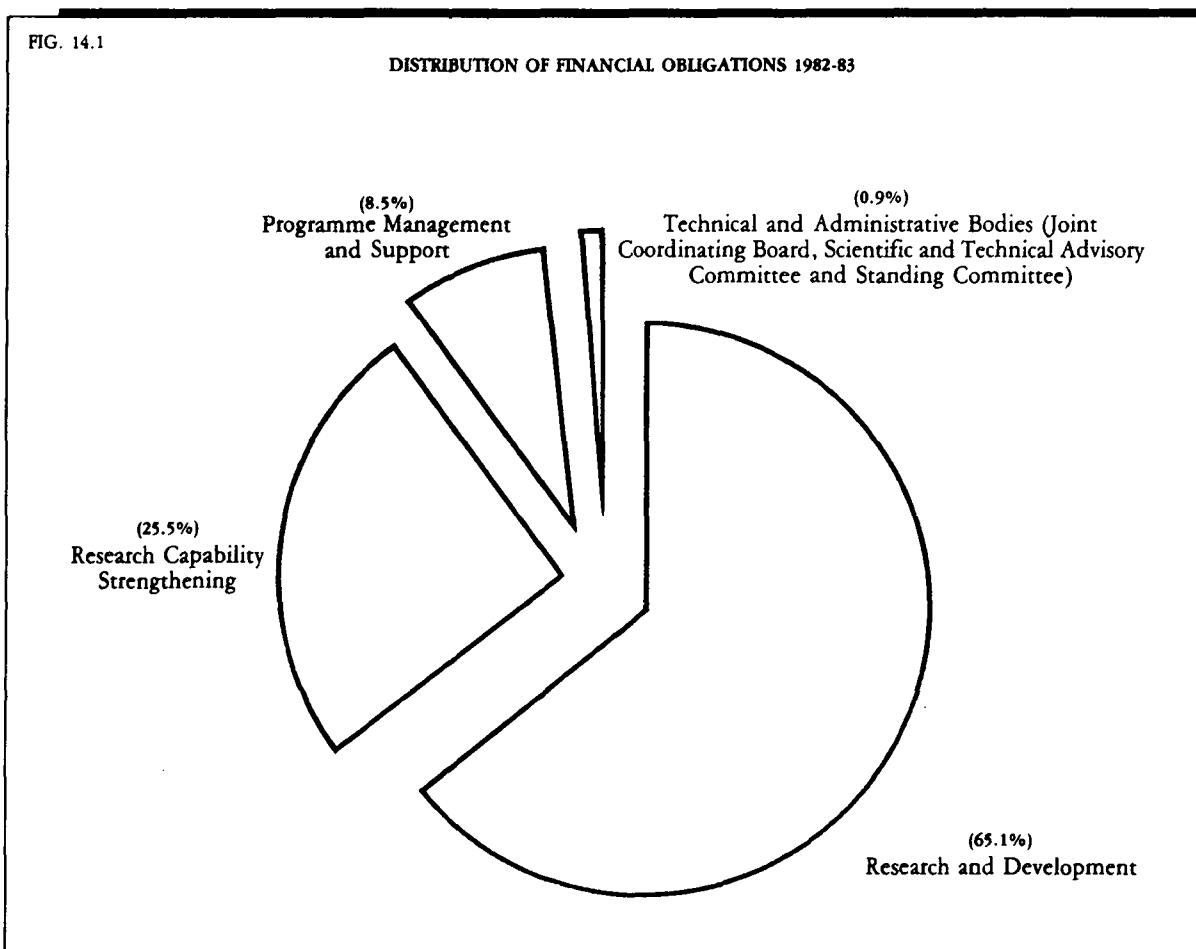


TABLE 14.2 Approved and revised budgets and obligations for 1980-81 and 1982-83 biennia (in thousands of US\$)

	1980-81					1982-83				
	1	2	3	4	5	6	7	8	9	10
	JCB- approved budget	Revised budget	Obliga- tions	% 3/2	% of total	JCB- approved budget	Revised budget	Obliga- tions	% 8/7	% of total
Technical and Administrative Bodies	595.0	708.0	515.0	72.7	1.0	685.0	540.0	422.0	78.1	0.9
Research and Development										
General activities	2 823.0	2 279.1	1 792.7	78.7	3.6	2 834.0	2 395.0	1 984.4	82.9	4.0
Malaria	8 864.0	7 744.7	7 372.2	95.2	14.7	9 920.0	8 767.0	8 149.5	93.0	16.5
Schistosomiasis	3 994.0	3 448.5	2 973.3	86.2	5.9	3 739.0	2 950.0	2 361.3	80.0	4.8
Filaria	3 989.0	3 727.0	3 725.3	100.0	7.4	4 250.0	3 452.0	3 233.0	93.7	6.5
African trypanosomiasis	3 992.0	3 860.0	3 820.9	99.0	7.6	3 961.0	3 183.0	3 140.6	98.7	6.4
Chagas' disease	1 905.0	1 798.0	1 752.1	97.4	3.5	2 000.0	1 785.0	1 653.5	92.6	3.3
Leishmaniasis	1 577.0	1 482.0	1 448.6	97.7	2.9	2 053.0	1 701.0	1 598.4	94.0	3.2
Leptosy	4 075.0	3 904.0	3 884.2	99.5	7.7	4 582.0	3 931.0	3 829.3	97.4	7.8
Biomedical sciences	1 699.0	1 572.0	1 311.0	83.4	2.6	1 563.0	1 196.0	1 116.9	93.4	2.3
Biological control of vectors	1 926.0	1 816.0	1 544.6	85.1	3.1	2 177.0	1 762.0	1 630.6	92.5	3.3
Epidemiology	1 965.0	1 827.0	1 816.2	99.4	3.6	2 399.0	2 111.0	2 101.5	99.5	4.3
Social and economic research	1 610.0	1 478.0	1 325.8	89.7	2.6	1 669.0	1 464.0	1 338.7	91.4	2.7
Total	38 419.0	34 936.3	32 766.9	93.8	65.3	41 147.0	34 697.0	32 137.7	92.6	65.1
Research Capability Strengthening										
General activities	1 343.0	1 033.5	1 028.3	99.5	2.1	1 983.0	1 462.0	1 457.9	99.7	3.0
Institution-strengthening activities	7 700.0	7 260.5	7 259.3	100.0	14.5	6 976.0	5 982.0	5 964.4	99.7	12.1
Training activities	3 750.0	4 513.0	4 510.7	99.9	9.0	5 378.0	5 159.0	5 191.3	100.6	10.5
Total	12 793.0	12 807.0	12 798.3	99.9	25.5	14 337.0	12 603.0	12 613.6	100.1	25.5
Programme Management										
Office of the Programme Director*	2 888.0	2 673.2	2 252.3	84.3	4.5	2 814.0	2 735.0	2 027.0	74.1	4.1
Information systems/services	210.0	190.0	174.5	91.8	0.3	196.0	196.0	164.1	83.7	0.3
Scientific and public information	410.0	481.0	411.0	85.4	0.8	145.0	145.0	205.8	141.9	0.4
Regional Offices	676.0	741.0	739.5	99.8	1.5	833.0	768.0	668.9	87.1	1.4
Administrative support costs	579.0	525.0	357.4	68.1	0.7	574.0	574.0	392.6	68.4	0.8
Common services and premises	137.0	137.0	134.4	98.1	0.3	912.0	748.0	740.6	99.0	1.5
Total	4 900.0	4 747.2	4 069.1	85.7	8.1	5 474.0	5 166.0	4 199.0	81.3	8.5
Grand Total	56 707.0	53 198.5	50 149.3**	94.3	100.0	61 643.0	53 006.0	49 372.3	93.1	100.0

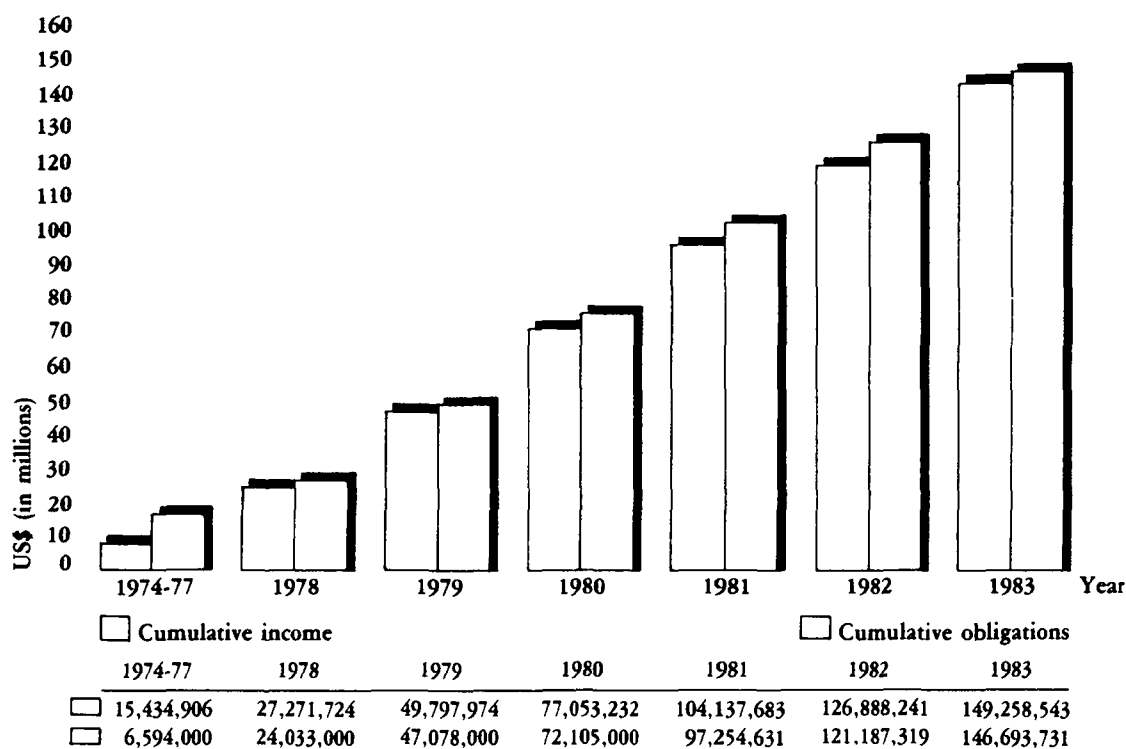
n. b. Entries showing .0 indicate less than one-tenth of one percent.

* This consists of personnel services, duty travel, and supplies and equipment.

** This does not include US\$ 26 700 for a project funded by TDR during the period.

FIG. 14.2

CUMULATIVE INCOME AND OBLIGATIONS 1974-1983



financial biennium. This distribution pattern reflects that stipulated by the budget approved by the JCB, although total obligations amounted to only 80.2% of the approved figure.

To enable the Programme to adjust the budget to the level of available funds, the JCB has maintained in operation the Plan of Financial Management first approved at its Third Session, held in 1980. The successful implementation of the Plan is illustrated in Fig. 14.2, which compares cumulative funds available to the Programme with actual obligations up to 31 December 1983. Details on budget revisions called for during the last two financial biennia are shown by Programme Area and Component in Table 14.2.

The shortfall in available funds with respect to the approved budget increased further during 1984, the first year of the current financial biennium. This was partly due to the strength of the US dollar in relation to the currencies in which most contributions are made. Contributions to the Programme up to 31 December 1984 are listed in Table 14.3 and the financial status at the mid-point of the 1984-85 bien-

nium is shown in Table 14.4.

The budget approved by JCB reflects the scientific and technical opportunities which TDR is ready and able to take advantage of. However, since 1980, due to the shortfall in funding, there has been a steady downward trend in the level of scientific and technical activities (obligations) that could be undertaken in proportion to that approved by the JCB: obligations amounted to 94% of JCB-approved funding in 1980, 84% in 1981, 82% in 1982, 80% in 1983 and 64% in 1984. This restriction comes at a particularly unfortunate time, since it slows the rate at which technologies can be developed and severely limits the Programme's capacity to pursue promising new leads. The slowing down, postponement or cancellation of high-priority activities is hampering TDR's ability to fulfil its role in the scientific community, as well as in developing countries, where the Programme has been looked upon as a leader in collaborative research and development, and a key instrument in solving the health problems caused by the six tropical diseases of concern to TDR.

TABLE 14.3

Financial contributions up to 31 December 1984 (US\$)

<i>Contributor</i>	<i>Total 1974-1980</i>	<i>1981</i>	<i>1982</i>	<i>1983</i>	<i>1984</i>	<i>Total 1974-1984</i>
African Development Bank, Ivory Coast	500 000	250 000	—	—	—	750 000
Australia	770 122	297 125	389 970	690 780	621 530	2 769 527
Austria	359 701	43 484	28 249	54 826	50 251	536 511
Bahamas	500	—	—	—	—	500
Bayer AG, Federal Republic of Germany	—	—	10 000	—	—	10 000
Belgium	2 397 100	388 249	321 839	587 648	240 828	3 935 664
Brazil	—	20 000	20 000	20 000	20 000	80 000
Cameroon	2 566	4 047	—	—	—	6 613
Canada	2 059 765	702 694	816 727	1 058 707	1 210 945	5 848 838
China	—	50 000	—	50 000	50 000	150 000
Cuba	4 102	1 987	2 500	2 000	—	10 589
Cyprus	239	—	—	—	—	239
Denmark	17 393 474	5 068 548	166 972	1 534 292	1 396 766	25 560 052
Finland	426 668	177 000	204 545	220 183	234 783	1 263 179
France	466 901	176 772	332 226	275 152	255 269	1 506 320
Germany, Federal Republic of	2 631 240	978 261	1 165 938	912 374	914 939	6 602 752
India	102 469	25 000	—	50 000	—	177 469
International Development Research Centre, Canada	806 973	186 892	177 972	—	120 192	1 292 029
International Federation of Anti-Leprosy Associations, UK	374 893	88 393	86 663	82 831	28 676	661 456
Iraq	5 000	—	—	—	—	5 000
Italy	—	—	—	—	623 551	623 551
Japan	—	—	100 000	100 000	100 000	300 000
Japan Shipbuilding Industry Foundation, Japan	2 730 916	400 000	259 851	250 000	200 000	3 840 767
Leprosy Trust Board, New Zealand	44 576	—	18 566	6 536	5 000	74 678
MacArthur Foundation, USA	—	—	—	—	1 000 000	1 000 000
Mexico	—	—	9 953	10 000	—	19 953
Netherlands	4 455 064	786 396	717 681	1 507 224	318 370	7 784 735
Niger	2 252	—	—	—	—	2 252
Nigeria	332 103	89 286	—	—	—	421 389
Norway	3 871 094	1 067 961	1 108 742	1 790 318	1 843 611	9 681 726

TABLE 14.3 (cont.)

Financial contributions up to 31 December 1984 (US\$)

Contributor	Total 1974-1980	1981	1982	1983	1984	Total 1974-1984
OPEC Fund for International Development, Austria	—	—	—	—	345 000	345 000
Pan American Health and Education Foundation, USA	—	—	—	5 000	—	5 000
Romania	1 995	—	—	—	—	1 995
SANOFI, France	—	—	—	39 216	—	39 216
Sweden	9 896 912	2 355 250	2 500 000	1 582 747	1 628 061	17 962 970
Switzerland	2 581 211	880 813	786 164	835 921	825 229	5 909 338
Thrasher Research Fund, USA	—	—	10 000	10 000	10 000	30 000
Turkey	—	—	—	—	5 000	5 000
United Kingdom	4 055 967	793 971	—	151 154	284 272	5 285 364
United States of America	6 372 912	4 001 000	5 030 000	3 000 000	2 000 000	20 403 912
Wellcome Trust, UK	25 000	—	—	—	—	25 000
Miscellaneous	6 761	2 750	3 500	4 287	4 940	22 238
Total	62 678 476	18 835 879	14 268 058	14 831 196	14 337 213	124 950 822
United Nations Development Programme (UNDP)	4 867 708	2 552 100	1 840 600	2 337 610	2 179 360	13 777 578
World Bank (IBRD)	—	2 480 000	2 400 000	2 500 000	2 580 000	9 960 000
World Health Organization (WHO)	5 551 500	1 050 000	1 050 000	1 050 000	1 282 500	9 984 000
Grand Total	73 097 684	24 917 979	19 558 658	20 718 806	20 379 073	158 672 200

TABLE 14.4

Summary of 1984 income and obligations as at 31 December 1984 (US\$)

			Total
Balance as at 1 January 1984			2 312 788
	<u>WORLD BANK</u>	<u>WHO</u>	
Income to 31 December 1984			
Contributions	14 259 512	6 119 561	20 379 073
Other income	829 669	991 916	1 821 585
Total funds available 31 December 1984			24 513 446
Obligations to 31 December 1984			21 441 609
Balance as at 1 January 1985			3 071 837

Box 14.1 The future

At a cost of less than US\$ 75 000 a day, TDR has catalysed academia, industry, and government ministries and institutions to collaborate and produce new methods for the control of tropical diseases. If this investment were increased by 25%, a steady stream of new therapeutic and diagnostic tools could emerge over the next five to ten years to help the tropical countries in their struggle against their endemic diseases. The investment is small, the potential pay-off for the entire world, enormous.

The success of the Programme has created new responsibilities for TDR and for WHO, the Programme's executing agency. TDR and WHO are now looked upon as coordinators and facilitators among the growing number of national and international tropical disease research programmes. These new responsibilities have increased the scope and magnitude of the activities of the Programme Secretariat and have created opportunities to increase even further the effectiveness of WHO and the TDR network.

It is hoped that governments and agencies will continue to endorse TDR as a unique international venture for health which provides a universally acceptable mechanism for international collaboration. Sufficient resources must be made available to ensure that today's scientific advances will, tomorrow, become the tools needed to bring under some measure of control the parasitic diseases that have deprived so many millions of the earth's inhabitants of their right to a healthy life.

Pertinent TDR and WHO documents

TDR. Collaboration with the industrial sector and the scope and management of patent issues. Document TDR/JCB(7)/84.6. (English and French)

TDR. Financial report for the 1982-1983 biennium. Document TDR/JCB(7)/84.8. (English and French)

TDR. Programme financing. Document TDR/JCB(6)/83.6. (English and French)

TDR. Report of the External Auditor of the World Health Organization on the statement showing the status of the trust fund for the Special Programme for Research and Training in Tropical Diseases as at 31 December 1983. Document TDR/JCB(7)/84.5. (English and French)

TDR. Report of the fifth meeting of the Scientific and Technical Advisory Committee (STAC-5). Document TDR/STAC-5/83.3. (English and French)

TDR. Report of the sixth meeting of the Scientific and Technical Advisory Committee (STAC-6). Document TDR/STAC-6/84.3. (English and French)

TDR. Report of the Standing Committee to the Sixth Session of the Joint Coordinating Board. Document TDR/JCB(6)/83.4. (English and French)

TDR. Report of the Standing Committee to the Seventh Session of the Joint Coordinating Board. Document TDR/JCB(7)/84.4. (English and French)

TDR. Seventh Session of the Joint Coordinating Board. Document TDR/JCB(7)84.3. (English, French and Spanish)

TDR. Sixth Programme Report: Proposed programme budget for the 1984-1985 biennium and estimates for 1986-1987, 10 May 1983. (English and French)

TDR. Sixth Session of the Joint Coordinating Board. Document TDR/JCB(6)/83.3. (English, French and Spanish)

TDR. The transfer to national health services of technology developed with TDR support. Document TDR/JCB(7)/84.7. (English and French)

TDR. *Venture for Health*. Geneva: UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases, 1984, 33 pp. (English, French and Spanish)

WHO. Resolution of the 71st Session of the Executive Board of the World Health Organization—Special Programme for Research and Training in Tropical Diseases. Document EB71.R10 (22 January 1983). (Arabic, Chinese, English, French, Russian and Spanish)

Annex: Other publications acknowledging TDR support

A number of publications stemming from work supported by TDR concern aspects of research which, although pertinent to the objectives of the Programme, do not relate directly to topics covered by the different chapters of this Report. They are therefore listed separately below.

CHAN, S.H. The application of monoclonal antibodies to studies of cell surface — a review. In: *Properties of the Monoclonal Antibodies Produced by Hybridoma Technology and Their Application to the Study of Diseases*. (V. Houba & S.H. Chan, eds.) Geneva: UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases, 1982, pp. 171-180.

GUERRA, H. Perspectivas en la prevención de las enfermedades tropicales. *Revista de la Sanidad de las Fuerzas Policiales*, 43(1): 78-81 (1982).

INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR, MALAYSIA. Tropical diseases research in Sabah. *Bulletin from the Institute for Medical Research (Kuala Lumpur)*, 20: 119 pp. (1983).

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OKIA, M. *Comparative studies of different formulations of a larval juvenile hormone mimic and conventional insecticides in distilled water and sewage against Culex quinquefasciatus*. Thesis, M.Sc., University of London, London, UK, 1984.

ORTEGA-GUTIERREZ, M. Control integrado de las endemias tropicales. *Salud Problema*, 9: 2-6 (1983).

PIMENTA, P.F.P. & DE SOUZA, W. Surface charge of eosinophils. Binding of cationic particles and measurement of cellular electrophoretic mobility. *Histochemistry*, 74: 569-576 (1982).

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YONG, H.S., BURTON, J.J.S., CHEONG, W.H., CHIANG, G.L. & DHALIWAL, S.S. Isocitrate dehydrogenase phenotypes in three species of *Toxorhynchites* mosquitoes. *Southeast Asian Journal of Tropical Medicine and Public Health*, 13(2): 291-292 (1982).