1. Introduction

This comprehensive review of the origins and progress to date of the Onchocerciasis Chemotherapy Project (OCT) has been prepared at the request of the Joint Programme Committee (JPC) of the Onchocerciasis Control Programme in the Volta River Basin area (OCP).

2. Origin of the OCT

2.1 Recommendations of the WHO Independent Commission on the Long-Term Prospects of OCP (1981)

The origin of the OCT stems from a recommendation (No. 26) in the Report of the WHO Independent Commission on the Long-Term Prospects of the OCP (1981). The Commission concluded that if a safe, effective, easily administered and acceptable macrofilaricide were available for Onchocerca volvulus it would profoundly alter the possible strategies for the future of OCP. It recognized that the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) was already supporting a research programme on the synthesis and screening of new compounds for filaricidal action, but concluded that further resources would be required for the development of candidate drugs. The Commission recommended:

(a) that funds should be provided to pharmaceutical companies for drug development and testing, under the supervision of a small expert committee; and

(b) that the JPC should provide the necessary resources from the Onchocerciasis Fund to undertake such a programme and should delegate responsibility for it to TDR, perhaps with some representative of the JPC on the monitoring committee.

2.2 Decisions made at the second session of the JPC (1981)

The JPC at its second session in December 1981 gave particular attention to the report of the Independent Commission with regard to chemotherapy, and it was provided with a detailed statement of ongoing chemotherapy research under TDR and of planned developments. The JPC endorsed the views of the Independent Commission concerning the priority to be given to chemotherapy research, and approved the request for US$ 1 150 000 for accelerated chemotherapy research in 1982. These funds were to be used, as proposed, under technical services agreements awarded by OCP with technical advice of the Steering Committee of the Scientific Working Group on Filariasis of TDR. The World Health Organization as executing agency was requested to prepare at the same time a proposal for the establishment of a chemotherapy project, bearing in mind that, while some reasonable long-term financing would be essential, every effort must be made to assure that support without holding large sums in reserve.
3. The OCT Working Group

As a result of these decisions, in December 1981, the Committee of Sponsoring Agencies (CSA) of OCP, in collaboration with the Standing Committee of TDR, set up an Onchocerciasis Chemotherapy Project Working Group of 5 members (3 outside experts and 2 WHO Secretariat) under the Chairmanship of Dr A. Morrison. Two specific tasks were assigned to this OCT Working Group:

(a) to make recommendations on expenditures and monitor the use of funds made available in 1982; and

(b) to recommend a permanent mechanism for managing and operating the project.

The Working Group met 3 times in 1982 and visited 10 pharmaceutical firms in Europe and USA which were known to be interested in producing drugs for tropical diseases, including onchocerciasis. Its report, which was presented to the JPC at its third session in December 1982, contained the following main recommendations.

A. The objective of OCT should be to accelerate the discovery and development of a safe, effective, low-cost and easily administered drug for onchocerciasis, which will be suitable for large-scale use and which will meet the following criteria:

(i) it must kill or permanently sterilize the adult female worms of *O. volvulus* without at the same time causing severe allergic reactions in recipients from microfilaricidal action;

(ii) if it has a microfilaricidal action this should be of long duration, and reactions in the host should be minimal.

B. Cooperation with the pharmaceutical industry, academic institutions and individuals is essential for the development of an onchocerciasis drug. The low profitability in this field and the relatively low level of current industrial interest requires that industrial support should be stimulated by providing financial assistance to supplement (not replace) industrial effort in chemical synthesis, biochemical investigations, screening programmes, toxicology and clinical trials.

C. The OCT should take full advantage of existing Research and Development programmes in its field, particularly that of the TDR Filariasis Programme (TDR-PIL).

D. To achieve the critical mass of scientists necessary to make real progress from basic research studies towards new chemotherapeutic agents, two interdisciplinary groups, each of at least 6 professionals (chemists, biochemists, biologists) and related technicians should be set up.

E. Expenditure of those funds available in 1982 should, in the main, support the following work:

(i) toxicological work on new Ciba-Geigy macrofilaricidal compounds;

(ii) clinical and formulation studies on mebendazole, levamisole and flubendazole in association with Janssen Pharmaceutica;

(iii) an integrated chemical, biochemical and biological screening programme at the Wellcome Research Laboratories.
F. The management structure for the OCT should include the following:

(i) the project should be financed by OCP, whose Director will act on the scientific and technical advice of Director, TDR;

(ii) a Steering Committee of OCT should be established by Directors, OCP and TDR, to direct all work financed in the field by OCT and coordinate the interrelated activities undertaken by OCT and TDR/FIL; and a full-time Secretary to the Steering Committee should be appointed, a person extremely knowledgeable in the field of industrial drug research and highly results-oriented;

(iii) the plan of action and budget prepared by the Steering Committee of OCT should be reviewed and research progress evaluated annually by the Scientific and Technical Advisory Committee (STAC) of TDR;

(iv) a Scientific and Technical Review Committee (STRC) of the STAC of TDR, including members of STAC/TDR and of the Expert Advisory Committee (EAC) of OCP, should be set up to review and evaluate progress, plans and budgets of the OCT, and prepare special reports on it for submission to STAC;

(v) the report of STAC on OCT, along with the Steering Committee's plan of action and budget, should be submitted to the CSA of OCP and the Standing Committee of TDR. These in turn will submit them, with comments as appropriate, to the JPC of OCP and the JCB of TDR;

(vi) the amount to be budgeted for each of the next 5 years (based on 1982 US dollars) should be US dollars 3.45 million p.a. with unspent funds being held in reserve for release to support promising clinical leads as may emerge;

(vii) one of the two interdisciplinary research groups (see D above) should be located at the Wellcome Foundation Research Laboratories,1 which should be given support for at least a 3-year period beginning in 1982. Every effort should be made to find and develop a second industrially-based group as quickly as possible.

4. Decisions made at the third session of the JPC (1982)

The JPC at its third session in December 1982 approved the management structure proposed for OCT. It recognized the key importance to OCP of developing drugs to supplement the Simulium larviciding campaign, and approved the implementation of the proposed chemotherapy programme during Phase II of the OCP, even though its full 5-year funding was not yet fully assured. It considered that the limited reserves available at that time were adequate to justify such risk-taking. The OCT research and development activities were to be shown as a separate section of the OCP budget.

1 The recommendation to establish one interdisciplinary group at the Wellcome Research Laboratories was made after the members of the Working Group had made site visits to 10 pharmaceutical companies including the Wellcome Foundation. Wellcome was at that time the only company interested in cooperating with WHO to establish a group working on filaricides, regarding this as a logical extension of its then filaricide screening programme which had been supported by TDR since 1977.

It may be noted that all profits arising from the trading operations of the Wellcome Foundation are paid to the Trustees of the Wellcome Trust which, under the terms of the will of the late Sir Henry Wellcome, uses them to support medical research, particularly in tropical diseases.
5. Establishment, constitution and functions of the OCT Steering Committee

Early in 1983 the Steering Committee of OCT was established by Directors, OCP and TDR, to operate in a manner similar to TDR Steering Committees. Initially it comprised 6 members under the Chairmanship of Dr H.R. Taylor, with WHO secretarial support from OCP, FIL and TDR. The first meeting was held in April 1983, and subsequently the Committee has met twice each year (in March/April and in September/October).

The Committee solicits and reviews research proposals, monitors research projects, evaluates their results and generally promotes research towards the OCT objective.

Recommendations for support of research are implemented by way of Technical Services Agreements signed by Director, TDR in respect of the scientific programme, and by Director, OCP in respect of funding.

Adequate interchange between the Steering Committees of OCT and of TDR/FIL is assured by having the Chairman of one Steering Committee appointed as an ex officio member of the other. Thus, during 1983 and 1984, Dr E.A. Ottesen, Chairman of SC TDR/FIL served on the SC/OCT, and Dr H.R. Taylor, Chairman of SC/OCT served on the SC TDR/FIL. Likewise the WHO Secretariat for the two Committees comprises the same officers.

Subsequently, in March 1984, two new members of the SC TDR/FIL, having appropriate chemotherapy expertise, were also appointed to SC/OCT; and in March 1985 a further two members were appointed to SC/OCT, bringing the total Committee strength up to 10 and permitting a gradual turnover of membership to begin after March 1985.

Recruitment of a suitable Secretary to the SC/OCT proved to be a long business. It was necessary to advertise in the scientific press as well as through the usual WHO network. Eventually, out of some 65 applicants, Dr C.D. Ginger took up the post early in January 1985. For the previous two years the duties of Secretary had been performed, in an acting capacity, by Dr B.O.L. Duke in addition to his usual duties as Chief of the Filarial Infections Unit.

The members of the Steering Committee are selected on the basis of a consensus among the members of the Secretariat concerned, taking into account:

(i) that there must be adequate disciplinary coverage - viz. pharmaceutical chemistry, biochemistry, toxicology, clinical pharmacology, parasitology, together with experience in drug development, clinical trials of onchocerciasis, ocular onchocerciasis, clinical immunology, pathology, and general experience of the epidemiology of onchocerciasis;

(ii) that the members are committed to promoting research towards the OCT objective;

(iii) that the members perform well in a Committee;

(iv) that they shall be neutral, in the sense of not being affiliated to any pharmaceutical company receiving support from OCT.

The terms of reference of the OCT Steering Committee do not include any of the training or research strengthening activities which form part of the TDR programme. The mission of OCT is solely to promote, fund and direct research designed to achieve its chemotherapy objective as soon as possible, and in the most effective manner.
Nor is it part of the OCT Steering Committee's remit to develop a system of drug delivery in the OCP countries. This task, which is recognized as being of fundamental importance, is one that must be undertaken by the OCP Participating Countries themselves in close association with OCP and in relation to the development of their Primary Health Care and Referral Health Systems. Furthermore the complexity of the task will depend on whether any drug eventually developed is suitable for mass treatment (perhaps excluding certain age- and sex-groups) or only for large-scale treatment of O. volvulus carriers (thus requiring pre-treatment diagnostic surveys); and again it will vary with the route of administration (oral or intramuscular) and with the number of days that are needed for a complete treatment.

Finally it must be remembered, as has been repeatedly pointed out in many WHO documents, including the Report of the Onchocerciasis Chemotherapy Project Working Group, there can be no guarantee that the OCT will be able to meet its objective in a limited period of time. However much directed research, patience and money goes into the search for new chemical leads, there has to be an element of luck if success is to be achieved, and the good fortune must continue if the promising finding at the research level is to become a successful drug delivered to the field.

6. Lines of research followed by OCT

6.1 General research policy

Up to March 1985 the OCT had funded a total of 39 projects with an overall budget of some 4 million dollars. A Scientific Working Group, ever expanding, but currently comprising 87 persons associated with the research programme, has been set up. Three Scientific Working Group Meetings have been held (dealing with biochemistry, in vitro culture, and drug screening), and the Steering Committee has met five times.

The main lines of research followed by OCT since its inception have been two.

First, is the development of promising new drugs through preclinical toxicity testing up to and including clinical trial in patients infected with onchocerciasis. This work has involved close cooperation and the establishment of legal agreements with the pharmaceutical companies which own the compounds concerned.

The drugs involved in this part of the programme are (a) ivermectin (Merck Sharp & Dohme, USA); (b) flubendazole and mebendazole (Janssen Pharmaceutica, Belgium); (c) CGP 6140 and CGP 20376 (Ciba-Geigy Ltd, Switzerland). Further details of progress made with each of these are given below (6.2.1) and in Annex I.

Second, lest none of the above drugs should prove safe and useful for the treatment of onchocerciasis (a possibility which has to be faced) it has been necessary to set up a programme of basic filaricidal research designed to generate new groups of filaricidal compounds. This part of the programme involves chemical synthesis, biochemical and metabolic studies on O. volvulus and allied parasites, and screening of compounds for action as filaricides. It is centred around the two large basic research groups that have been set up, the first at the Wellcome Research Laboratories in UK, and the second with the Upjohn Company/Michigan State University consortium in USA; and it is supported by ancillary investigations into (a) parasite metabolic pathways, (b) improving in vitro culture and animal models for work on O. volvulus, and (c) supply of nodules of O. volvulus and allied species. This basic part of the programme is essentially a long-term venture. It is closely linked with, and supplements, the TDR/FIL research programme in the same field. It involves both intelligent selection and screening of compounds with potential anthelmintic action that already exist in the compound libraries of pharmaceutical companies, etc, and the synthesis of new compounds designed to interfere with metabolic processes of the parasite that have been elucidated in biochemical studies. Fuller details of progress made in this basic part of the research programme are given below (6.2.2) and in Annex II.
6.2 Scientific progress

6.2.1 Clinical trials

In view of the increasing number of clinical trials of new drugs for onchocerciasis that are planned, a team, free to move from place to place as required and consisting of an ophthalmologist and a clinician/epidemiologist, has been established, based on the International Center for Epidemiologic and Preventive Ophthalmology, Johns Hopkins University, Baltimore, USA (Taylor) and on the Department of Medicine, Case Western University, Cleveland, Ohio, USA (Greene). This team has so far been actively engaged in executing clinical trials of ivermectin on over 240 onchocerciasis patients in Liberia.

Since the beginning of 1985 the OCT has also taken over the full support of the Onchocerciasis Chemotherapy Research Centre, Tamale, Ghana under the direction of Dr K. Awadzi who is currently employed as a WHO Consultant. This centre, which had previously been supported first by OCP and then by TDR, has a 24-bed clinical facility and the ability to undertake extensive clinical trials in onchocerciasis.

6.2.1.1 Ivermectin

Cooperation between WHO and Merck, Sharp and Dohme

The drug ivermectin (Merck Sharp and Dohme) continues to show promise in the treatment of onchocerciasis and its further development towards registration is now being pursued by the company. WHO/OCT is cooperating closely with Merck in clinical and other trials of ivermectin. A legal agreement is being drawn up between the two parties and the ivermectin programme has been accorded top priority by the Steering Committee.

Clinical trials completed or in progress

An open trial on 19 patients at the Onchocerciasis Chemotherapy Research Centre (OCRC) at Tamale, Ghana has now reached the 15-month follow-up stage. Four double-blind trials to compare the effects of ivermectin versus DEC versus placebo at Bamako, Dakar, Liberia and Tamale have reached the 12-month follow-up period. The first and the last two of these trials are supported by OCT, the others by Merck. The trials all followed the same protocol which was developed with OCT input. A total of 69 patients in these trials have received a single dose of 12 mg ivermectin (i.e., in the range of 160-240 micrograms/kg).

Further trials on larger numbers of patients are now being planned and these include a dose-finding element in the range of 50, 100, 150 and 200 mg/kg and safety studies. In Liberia 200 patients have been treated in such a trial, and similar trials are due to start very soon in Bamako, Mali, in Tamale, Ghana, in Ivory Coast, in Togo, and possibly elsewhere including Mexico and Guatemala. In the main these trials will be funded by Merck with OCT input into the design, execution and assessment.

These trials include groups infected with the West African forest and savanna forms of *O. volvulus*, which will be investigated separately. In the savanna zone patients from inside and outside the area under OCP control will be included. Until more toxicological data are available children under 12 and pregnant or lactating women will be excluded.
Effect on skin microfilariae

From these trials it appears that in adult males and non-pregnant females ivermectin is well tolerated at a single dose of up to 12 mg. At this dosage it is an effective microfilaricide capable of reducing skin concentrations as effectively as DEC over 1-2 weeks, while exciting very little or no Mazzotti reaction. In the open study and in the double-blind study at Tamale counts of microfilariae in the skin have remained at very low levels for 12 months after treatment. These patients had stable infections and came from well-controlled areas of the OCP. In the other three double-blind studies counts at 6 and 12 months after treatment with ivermectin were lower than DEC in each instance. These three trials were all done in areas where transmission continues.

Effects on microfilariae in the eye

Ivermectin has very little direct action on microfilariae in the cornea or anterior chamber and causes less adverse effects on the posterior segment of the eye than DEC. However, numbers of microfilariae in the cornea and anterior chamber fall gradually over the weeks after treatment, possibly by a process of emigration without replacement.

Effects on adult worms

Morphological examination of nodules excised up to 9 months after dosage with ivermectin indicate that the drug does not kill the adult worms or affect their embryogenesis. However, it appears that intrauterine microfilariae in worms from treated patients may be unable to make their usual active escape from the vulva of the female and, after about 2 months retention in utero, they begin to degenerate. This process may account for the delayed repopulation of skin microfilariae after ivermectin treatment, which compares favourably with DEC and leads to prolonged microfilarial suppression.

Effects on the transmission of O. volvulus by Simulium damnosum s.l.

OCT-sponsored trials are currently being carried out in both the forest and savanna zones of West Africa to assess the effects of ivermectin on the intake and development of microfilariae of O. volvulus by Simulium damnosum s.l. Preliminary results indicate that both microfilarial counts in the skin and the intake of microfilariae by feeding flies are very greatly reduced after a single dose of ivermectin, the effect being more marked than after DEC. However, those microfilariae that are ingested appear to be fully capable of completing their development to infective larvae (L3) in the fly.

This marked reduction in transmission from treated patients lasts for 2 months (the longest period so far tested) in S. sirbanum areas in savanna (Ranque, Bamako, Mali), and for 3 months with S. yahense in Liberia (Cupp, Cornell University, USA). Further observations will be made at 12 months to assess the effect of annual single dosage on transmission. In S. soubrense/S. sanctipauli areas in forest Prod'hon (Bouaké, Ivory Coast) has shown that the reduction in transmission lasts for up to 6 months (the longest period so far tested).

Effects on the early developmental stages (L3 and L4) in the vertebrate host (chemoprophylaxis)

An OCT-sponsored investigation into the possible chemoprophylactic action of ivermectin on the L3 and L4 stages of O. volvulus in chimpanzees has recently been started in Liberia (Trpis, Johns Hopkins, Baltimore, USA). This will take about 2 years to complete. Important ancillary observations will be made on the immune responses of the inoculated animals.
Potential toxic effects

Ivermectin is said not to pass the blood-brain barrier in adult mammals, although the barrier may be deficient in neonates. In veterinary medicine this passage into the central nervous system has been recorded only in one breed of dog (the collie), which has a congenital weakness of the blood-brain barrier. Treatment with ivermectin may then lead in dogs to an ascending and sometimes fatal paralysis. No similar accident has been seen in tens of millions of cows and horses which have been treated with ivermectin. Since meningo-encephalitic conditions, which may weaken the blood-brain barrier, occur in onchocerciasis areas (examples in West Africa are human trypanosomiasis and cerebrospinal meningitis) these represent a potential hazard which needs to be assessed when considering the large-scale use of ivermectin. This problem is currently being investigated in monkeys infected with Trypanosoma b. rhodesiense by Njogu (Muguga, Kenya) and in dogs infected with staphylococcal meningitis by Armengaud (Toulouse, France).

Conclusions

The limited clinical studies carried out to date indicate that ivermectin holds promise of being a useful stop-gap drug for the control of onchocerciasis which is projected by Merck to reach registration by mid-1987. It appears to be an effective, non-toxic, single-dose microfilaricidal, which does not produce a significant Mazzotti reaction or damage the eye. As such, it has potential for use on a large scale as a long-acting microfilarial suppressant, which might need to be given only once a year and which could greatly reduce microfilarial concentrations (a) in the eye, thereby preventing the onset of eye lesions and blindness, and (b) in the skin, thereby reducing the reservoir of microfilariae available for transmission.

Limitations and cautions

It must be remembered that ivermectin is not a macrofilaricide and the search for a non-toxic drug that kills or permanently sterilizes the adult worms must still be vigorously pursued if ever a definitive treatment for onchocerciasis is to be found.

Furthermore, only just over 300 onchocerciasis patients (mostly adult males) have so far been treated with ivermectin. The possibility must be borne in mind that hitherto unsuspected toxic manifestations may reveal themselves as larger numbers of patients (including women and children) are treated. It is therefore essential to suspend final judgement about the drug until more information is available. Meanwhile we must continue with the evaluation of this compound, proceeding cautiously as required for any agent being newly tested in man.

6.2.1.2 Mebendazole

A number of trials with this compound have been completed at the high and prolonged dosage (1.5 g daily for 2-3 weeks) that appears necessary to produce an embryostatic effect of 3-4 months duration in O. volvulus infection. In view of the facts that such a dosage schedule is impractical for large-scale treatment, that the drug is teratogenic in some animal species, occasionally induces a fatal neutropaenia, and that it produces a degree of Mazzotti reaction, it has been decided not to pursue its investigation any further.
6.2.1.3 Flubendazole

Cooperation continues with Vanden Bossche (Janssen Pharmaceutica, Belgium) for the development of a more acceptable formulation of flubendazole, a drug which has shown promising action against *O. volvulus* in a single small trial in Mexico. The formulation used in this trial caused too much local pain and inflammation at the injection site, due mainly to its insolubility at physiological pH, for it to be further used in man. Several other formulations have since been tested in animals without success. A new approach combining the drug with cyclodextrins is now being followed and the results should be available by mid-1985.

6.2.1.4 Ciba-Geigy Compound 6140

This compound, CGP 6140, which has shown macrofilaricidal action against *O. gibsoni* in cattle in the Copeman screen (Townsville, Australia), has now been put through the necessary preclinical toxicology preparatory to a Phase I/IIA clinical trial being undertaken by Awadzi at the OCRC, Tamale. The Ames' test and the V79 hamster lung cell mutagenicity test have been completed with satisfactory negative results. The preclinical dossier and protocols for the trial have been prepared and approved by WHO/SCIHS and the Ghanaian Ethical Committee, and all equipment needed for the trial has reached Tamale. Capsules of the drug have been sent to Ghana ready for a step-by-step trial up to a dose of 100 mg in uninfected and lightly infected volunteers, at which level pharmacokinetic studies will begin in man. The trials started in February 1985.

6.2.1.5 Suramin

Despite its toxicity, suramin is still the only macrofilaricide available for treatment of onchocerciasis. Thus all potential means of improving its use need to be examined.

A long-term (6-8-year) examination is being undertaken by Rougemont (Geneva, Switzerland) of patients treated with a "low-dose" suramin schedule (total dosage 3.6-4.4 grams) who have subsequently resided in (a) a zone of OCP where transmission has been interrupted and (b) a zone of continuing transmission in Mali. It is hoped that this will reveal the long-term effects of this treatment on the adult worms, on the concentrations of microfilariae and on eye lesions. Recent work by Breckenridge (Liverpool University, UK) supported by TDR/FIL has led to the development of a reliable HPLC method for suramin estimation. Pharmacokinetic studies using this method indicate that critical macrofilaricidal levels of the drug might be achieved by a schedule of doses more widely spaced than the current 7-day intervals, and this perhaps with less risk of toxicity. Further trials of suramin on these lines are now being considered.

6.2.1.6 Other drugs

**Arsenicals**

It has been decided to stop further work on melaminylthioarsenites, a series of macrofilaricides prepared by Dr E.A.H. Friedheim, since there now appears to be no likelihood that new drugs in this series will be taken through to man for sleeping sickness treatment. In view of the risk of arsenical encephalopathy, it is not considered ethical to take these compounds initially to man for treatment of a non-fatal disease such as onchocerciasis.
Lodoxamide

Protocols have been developed for a trial of lodoxamide in the control of the Mazzotti reaction to DEC; and a supply of the drug has been donated by the Upjohn Company. However, in view of the recent promise shown by ivermectin, the need to control reactions to DEC has become less pressing and, since lodoxamide has shown no action in controlling the reaction to DEC in the guinea-pig eye model (developed recently by Donnelly (Scheie Eye Institute, University of Pennsylvania, USA) with OCT support), it has been decided for the time being not to block up our relatively limited clinical trial facilities with investigation of this ancillary drug.

6.2.2 Basic Research on Filaricides

Work continues at the large basic filaricide group set up in the Wellcome Foundation Research Laboratories in the UK. A number of promising chemical lines are being followed in the search for new filaricides but so far only one compound has emerged that is sufficiently promising to pass to the tertiary cattle screen, which requires that test compounds be resynthesized in multigramme quantities. This is only feasible for highly active drugs, or those which are under development for use in the veterinary or human market.

Five proposals to form a second large basic group on filaricide research have been reviewed by the Steering Committee. None was found immediately suitable, but a consortium of the Upjohn Company and various American Universities (notably Michigan State University) has since been recommended for funding following a site visit and further negotiation. A legal contract between WHO and the company has been signed, and work started on 1 January 1985. The Upjohn Company has a number of groups of novel compounds with anthelmintic activity, and the associated Michigan State University team has an onchocerciasis project in the Sudan.

As a result of the guidelines laid down by an OCT-sponsored Working Group on Biochemistry of Filarial Parasites held in 1983 a considerable number of projects on various aspects of filarial biochemistry and metabolism have been funded. These projects aim at finding metabolic pathways in the parasite which differ from those of man and which can thus be exploited for novel drug action.

A continuing supply of frozen nodules, both of Onchocerca volvulus and of O. gibsoni, is also necessary in support of the biochemical programme. Contracts have been entered into with the onchocerciasis control authorities in Mexico (Jaimes, Chiapas) and in Guatemala (Zea-Flores) in an attempt to provide adequate quantities of O. volvulus to the biochemists. The first nodules from Guatemala came on line early in 1985. Other potential sources of supply in Africa are also being explored; and regular and large quantities of O. gibsoni from cattle are being shipped from Australia by Copeman to biochemical laboratories in Europe.

The importance of in vitro culture and maintenance of Onchocerca worms is also recognized as a back-up to biochemical work. Work on this difficult subject is being supported, and a small working group meeting on the subject was held in September 1984. The report of this meeting has stimulated innovative approaches to the problem, and attracted additional research proposals, some of which were recommended for funding by the Steering Committee in March 1985.

In view of the great importance and unique relevance to OCT of the Onchocerca gibsoni and O. gutturosa screen maintained in cattle by Dr B. Copeman in Australia, this screen has now been incorporated into the OCT activities.
6.2.2.1 Organization of the basic research programmes for the biochemistry and chemotherapy of onchocerciasis

As noted by a recent STAC Subcommittee (TDR/STAC-6/SUBG/84.1) there is a paucity of basic information relating to the biochemistry and biology of *O. volvulus*, and studies in these areas should be given high priority in any programme for the rational development of novel filaricides. Thus OCT recognized that such fundamental research into comparative biochemistry of the parasite and its host should form part of the integrated research programmes of the two industrially-based groups, and also by the independent, academically-based research workers. While all OCT-funded research must have as its aim the discovery of inhibitors of essential metabolic processes of the parasitic worm, which do not significantly affect similar processes in man, today only pharmaceutical companies with a background in tropical medicine and parasitology have the knowledge and resources to take such discoveries from the laboratory, to carry out the necessary chemical synthesis for lead optimization, and then proceed with the drug metabolism, toxicology and preclinical testing, which is required before a novel drug can go forward for clinical trials in man.

The STAC subcommittee also noted that when OCT invests in a project within a pharmaceutical company, it results in technical and administrative services being provided to WHO whose value may exceed by 100-200% the funds actually expended. Thus access is provided to chemical series of novel structures whose physicochemical properties and biological test profiles are available in the form of computer data bases. Physical techniques such as electron spin resonance (ESR), nuclear magnetic resonance (NMR), and mass spectrometry are available for both chemical and biological applications, and computer-based technologies such as quantitative structure-activity relationships and Hansch analysis techniques, computer graphics, etc. can all be used to allow rapid lead optimization once initial activity is found. Usually, many biological test systems, some created for the study of gastrointestinal nematodes of veterinary importance, are available and are used to provide additional information on the mode of action of compounds synthesized initially for antifilarial purposes. In both companies selected for OCT support, fermentation techniques have been used to prepare chemical materials for use in the onchocerciasis research programmes.

Individual academic workers are encouraged to collaborate with the industrial groups to develop a functional network which is able to make best use of available resources, and to further the development of any inhibitors which seem promising for the chemotherapy of onchocerciasis. Such collaboration is occurring informally between workers in the UK and the Wellcome Group, and as a more formal consortium between university groups and the Upjohn Company in the USA.

By providing continuing support for basic biochemical studies on *Onchocerca volvulus* and related filarial parasites, it is hoped that important aspects of filarial metabolism will be revealed which will provide suitable targets for chemotherapeutic attack. The use of available inhibitors of known mode of action will provide a steady accumulation of useful information which will provide biochemical clues for the synthesis of candidate anti-*Onchocerca* compounds.
ANNEX I

The rationale for the clinical research programme and budget on new drugs for treatment of onchocerciasis, supported by OCT

1. General considerations

This annex describes the rationale for the selection of the three promising new filaricidal drugs which are currently at an early stage of clinical development by OCT in collaboration with the pharmaceutical companies which own the patents of the compounds concerned. It describes plans for the priority development of these drugs for use in the treatment and control of onchocerciasis, points out the relative contributions made by OCT and by the companies, and thereby provides also the rationale for the allocations of the OCT budget.

Since the beginning of OCT its ultimate goal has been to find and develop a drug that will kill or permanently sterilize the adult female worms of *Onchocerca volvulus* (i.e. a macrofilaricide or an embryostatic). Only a drug with this action can bring about a definitive cure of the infection. However, it has been agreed all along that a drug which kills only microfilariae (a microfilaricide) could provide a useful stop-gap measure for treatment and control provided that it does not cause severe reactions to the death of these parasites and that it has a reasonably long residual action.

The three drugs currently at various stages of their early clinical trials are, in order of advancement of their present development:

(a) ivermectin (Merck Sharp & Dohme) - a "non-reactive" microfilaricide;

(b) flubendazole (Janssen Pharmaceutica) - a long-acting embryostatic and possible macrofilaricide;

(c) CGP 6140 (Ciba-Geigy Ltd) - a potential macrofilaricide.

Further details of the plans and budgetary aspects of the development of each of these compounds are given below.

2. Ivermectin

Ivermectin (a macrocyclic lactone produced by a fermentation process) is the property of Merck Sharp & Dohme (MSD), USA, who market the drug widely for veterinary use as a gastrointestinal anthelmintic and ectoparasitocidal agent in stock animals.

Clinical trials in man have so far been confined to onchocerciasis, in which disease the drug appears to be a microfilaricide only, but one which is unexpectedly unique among all other microfilaricides in the following respects.

It is

(i) effective in a single dose;

(ii) produces little or no Mazzotti reaction;

(iii) has a prolonged (6-12 month) suppressive action on microfilariae.
During 1983 and 1984 WHO/OCT cooperated with MSD in clinical and other trials of ivermectin in onchocerciasis, and was the sole or major financial contributor, to the following investigations, some of which are still in progress:

(i) Open range-finding study of single-dose ivermectin on 19 heavily infected onchocerciasis patients at OCRC, Tamale.

(ii) Two double-blind trials, one in Liberia and one in Ghana, to compare ivermectin versus DEC versus placebo in 30 patients each. (Two other similar trials were conducted exclusively by MSD in conjunction with other investigators in Mali and Senegal.)

(iii) Three studies, in Ivory Coast, in Mali and in Liberia, to assess the effect of ivermectin on the intake and development of microfilariae of O. volvulus by Simulium damnosum, i.e. an assessment of the drug's potential for controlling transmission of onchocerciasis.

(iv) Trial of ivermectin as a chemoprophylactic for Onchocerca volvulus in chimpanzees.

(v) Trials in dogs with purulent meningitis, and in monkeys with Trypanosoma rhodesiense infection, to determine the risk of ivermectin crossing the blood-brain barrier and causing paralysis or death in such conditions.

These activities have accounted for about $453 000 of OCT-obligated funds over the period 1983 to April 1985.

In September 1984, having assessed the results of the trials mentioned in (ii) above, MSD made a policy decision to pursue the further development of ivermectin for use in the treatment of human onchocerciasis up to registration, with a target date of 1987.

Early in 1985 MSD expressed its desire and intention to assume full financial responsibility for all future Phase IIb or III clinical trials of ivermectin in onchocerciasis. As of April 1985 seven such trials, each involving 150 ivermectin-treated patients and 50 placebo-treated controls, are under way or are planned to start soon in Liberia, Ghana, Ivory Coast, Mali, Togo, Guatemala and Israel (the latter in Ethiopian refugees). These trials, which include dose-refining, safety and efficacy aspects, will give a total of some 750 ivermectin-treated patients.

Since MSD have now decided to fund the whole or the greater part of all future clinical trials, the financial cost to OCT of the further development of this drug will be relatively low, although it is likely to go ahead as rapidly as ever.

WHO/OCT involvement with MSD in the present stage of the development of ivermectin is thus limited to (i) providing scientific advice in designing trial protocols, (ii) undertaking ancillary tests of potential toxicity, (iii) the input of the OCT mobile clinical team to the Phase III trial in Liberia, (iv) the input of Dr Awadzi's expertise and the OCRC facilities to the Phase III trial in Ghana and (v) continuing the transmission and chemoprophylactic studies which are already under way.

It appears therefore that OCT has succeeded very well in catalysing the desired effort and commitment of MSD to develop its own product, as rapidly as possible, for use in the treatment of onchocerciasis.
Annex I

It should be noted that the OCT Steering Committee has considered that it
would not be wise to undertake trials of repeated doses of ivermectin until the
results of single-dose treatment have been thoroughly assessed and more information
is available on the toxicology of the compound in repeated doses and on its
half-life in the tissues. Thus the suggestion made by the EAC in 1984, that the
effect of repeated doses given at intervals of 6 months or a year should be
investigated as soon as possible, has not yet been acted upon. However, the
effects of repeated doses, given at intervals of 2-6 weeks, are being investigated
in Onchocerca gibsoni in cattle in Australia; and it is proposed later in 1985 to
test the effect of a second single dose of 200 mcg/kg given after one year to
volunteers in Mali, who have been taking part in experiments to assess the effects
of ivermectin on the intake and development of microfilariae of O. volvulus
by Simulium sirbanum, and thus to assess the effect of the drug in controlling
transmission.

The terms of a formal agreement between MSD and WHO for the further
development of ivermectin are under discussion. This agreement will be designed to
protect the public interest and ensure that the product will be available on the
most favorable terms to the developing countries where onchocerciasis is endemic.

3. Flubendazole

Flubendazole (a benzimidazole derivative) is the property of Janssen
Pharmaceutica, Belgium. The drug is a very promising embryostatic and
macrofilaricidal in many animal filarial screens.

Two small trials of the drug have been carried out in onchocerciasis. One in
Ghana, using an oral preparation, produced no effect on O. volvulus, due to lack of
absorption. A second trial of an intramuscular formulation was carried out on 8
onchocerciasis patients in Mexico. The net result of treatment was a slow decline
in microfilarial concentrations to near zero over the course of a year. The exact
mode of action of the drug in this small trial could not be clearly determined. It
was certainly a relatively long-acting embryostatic agent; it may have had a
macrofilaricidal action on some of the male worms; and a very slow residual
macrofilaricidal action due to deposits of drug in the tissues cannot be excluded.

The drawbacks of the intramuscular flubendazole formulation used in this trial
were the great pain and inflammation produced at the injection sites - so much so
that the clinicians involved considered that no further trials could be carried out
until a new and less painful formulation was devised.

OCT persuaded Janssen Pharmaceutica that the potential of this drug for the
treatment of onchocerciasis justified undertaking a programme of research to
develop a new, painless and well-absorbed formulation. A legal agreement was drawn
up with Janssen Pharmaceutica and a research project, with a relatively modest
budget ($97 190 over 1983-85) was funded to investigate and develop a suitable new
formulation.

To date the search for a satisfactory formulation, which is pain- and
inflammation-free, while yet being well absorbed and filaricidal in animals, has
not been successful. Although there is some hope that a recent new development
could provide a breakthrough, it is probable that, if this fails, the project for
an injectable benzimidazole derivative may have to be abandoned.
Nevertheless, in terms of OCT expenditure, no greater sums can profitably be laid out on flubendazole development until the formulation problem is solved. If it were to be solved, then research activity and expenditure on this drug, involving preclinical toxicity testing and further clinical trials, could suddenly rise sharply.

4. **Ciba Geigy Compound 6140 (and its back-up compound, CGP 20376)**

CGP 6140 and CGP 20376 (benzthiazole derivatives of dithiocarbamic acid) are the property of Ciba-Geigy Ltd, Switzerland. Both these drugs have a macrofilaricidal action against *Onchocerca gibsoni* in cattle as well as being microfilaricidal. It is an account of their macrofilaricidal potential that they have been developed to the stage of clinical trial.

Under the terms of a legal agreement between WHO and Ciba-Geigy Ltd, designed to accelerate the latter's programme to develop drugs for onchocerciasis, OCT expended some $304,400 in 1982 and 1983 in funding some of the preclinical toxicity studies which were needed to bring forward three CGP compounds (CGP 6140, CGP 20376, and CGP 24914) for possible Phase I clinical trial. Within this series of compounds, some indications of CNS toxicity, and possible mutagenicity, have been indicated in laboratory models, and these parameters will need careful monitoring during clinical development.

On the basis of the preclinical studies it was decided that CGP 6140 was both the least toxic and the most promising compound for *O. volvulus*; and it has gone into man in a Phase I/IIA trial at the OCRC, Tamale.

Further expenditure on CGP 6140 in 1984 was less than envisaged owing to the decision of Ciba-Geigy to pay for the ADM trials which had been foreseen both for this drug and for CGP 20376. Also in 1984 there was some delay in obtaining ethical clearance for the clinical trial of CGP 6140 and this in turn upset the timing of its planned trial at Tamale, where the OCTC capacity had by then been saturated with its various ivermectin trials. The trial of CGP 6140 could not therefore begin until early in 1985, during which year expenditure is likely to increase again as the dosage level in the clinical trial reaches the threshold where human pharmacokinetic studies can be done; and, provided the drug continues to prove safe, as dosage is raised in further trials towards the anticipated therapeutic effect level.

5. **General conclusions on the new drugs currently under clinical trial for onchocerciasis**

From the above it can be seen that the relatively low expenditure by OCT on drugs at the clinical trial stage stems from three reasons.

First there is no drug which is yet ready to be used in large-scale field trials. The drugs presently used for individual treatment, namely diethylcarbamazine citrate and suramin, are unsuitable for large-scale use, and the newer drugs have not yet advanced to this stage.

Second, the pharmaceutical companies concerned have all assumed a greater share of the costs of preclinical development and clinical trials than had been originally envisaged. This has resulted in a significant reduction in the anticipated costs of the OCT programme, while at the same time maintaining rapid progress.

Third, with both flubendazole and CGP 6140, technical difficulties have caused delays in the anticipated schedule of development and have thus led to temporary reductions in expenditure while these are being overcome.
Of the drugs currently under clinical development, CGP 6140 is a macrofilaricide and flubendazole, even if not macrofilaricidal, is certainly a long-acting embryostatic drug sterilizing the adult female worms. It is only ivermectin, which is primarily microfilaricidal in action; and the further development of this drug is being pursued solely on account of its unique properties, which appear to accord with the second criterion of the OCT objective, namely that the action of a microfilaricide that is to be useful in practice must be "of long duration, and reactions in the host should be minimal".

There are thus, at present, three new compounds, all belonging to different chemical groups, undergoing early clinical trials for use in onchocerciasis. It is greatly to be hoped that one or more of them can be developed into a useful drug for large-scale treatment of onchocerciasis. On the other hand it could very well be that all three candidate drugs will fail, for one reason or another, in the course of further trials.

Ivermectin has been tested so far in only a small number of patients (just over 300 as at April 1985). Further evaluation may show some hitherto unsuspected toxicity; it may be necessary to restrict its use in special groups, such as young children or pregnant and nursing women; it may have to be withheld during meningitis epidemics; or it may have to be administered repeatedly, at intervals of 6-12 months, if a continued suppression of microfilariae is to be achieved.

Flubendazole may have to be abandoned if the formulation problem cannot be solved.

CGP 6140 is currently only at the very beginning of Phase I/IIA trials using extremely small doses, and it may prove to be unacceptably toxic before the anticipated chemotherapeutic level is reached.

Within the pharmaceutical industry only about 1 in 10 of such active products are finally marketed successfully. Thus if the worst happens and all these leads should fail for one reason or another, then the OCT will be entirely dependent on the generation of new filaricide lead compounds from the results of its basic research programme. It is for this reason that the two large basic filaricide research groups (the Wellcome Foundation and the Upjohn Company/Michigan State University Consortium), along with supporting biochemical, in vitro culture and screening programmes, have been set up - all of which currently consume about $1 600 000 per annum, or about one half of the OCT annual budget for research projects. These basic research activities are described in greater detail in Annex II.
Details of basic research on filaricides supported by OCT

1. Individual research topics

As no laboratory host has yet been found for O. volvulus, and continuous and plentiful supplies of viable worms are not yet available from human nodulectomies, most research must be present, particularly in the early stages, be carried out either on equine or bovine species of Onchocerca, or on other more easily obtained filariae such as Brugia pahangi, Dipetalonema viteae or Dirofilaria immitis. One small study (Hardman, Aberdeen, UK) is using modern techniques of molecular biochemistry to study the degree of genetic relatedness shown between the DNA and RNA of Onchocerca volvulus, and other filarial and nematode worms. Initial work has been concerned with the direct nucleotide sequencing of ribosomal RNA using a reverse transcriptase method, and although only bovine Onchocerca species have been used to date, RNA from O. volvulus should soon become available. Results from this study will help to determine which model filariae are most similar to O. volvulus in biochemical terms, and thus predictive of metabolic processes in the human parasite.

It is of interest that both major industrial research groups have independently concluded that two major areas of biochemistry and physiology are exploitable in terms of filarial chemotherapy. These two areas are energy generation (glycolysis and associated metabolic pathways, together with electron transport systems), and neuromuscular function and neural transmission; both of which were defined by WHO Scientific Working Group (SWG) on the Biochemistry of Filarial Parasites (OCP/OCT/83.1).

1.1 Glycolysis and energy generation

When the Biochemistry SWG met in 1983 it was generally thought that, with the exception of L. carinii, glucose breakdown was mainly by glycolysis to form pyruvate, followed by the anaerobic production of lactic acid, in almost quantitative amounts. Thus initial work at the Wellcome Research Laboratories was aimed at trying to find inhibitors of glycolysis or lactate production which could be used to substantiate the hypothesis that inhibition of this fundamental pathway would be a lethal event for filarial worms. However, none of the available glycolytic inhibitors would kill the worms, and specific synthesis of novel inhibitors of lactic dehydrogenase failed to produce inhibitors better than the original lead compound.

Since that time OCT has given support to two academic workers to identify the regulatory enzymes in the glycolytic pathway, and to study the levels of intermediary metabolites found in model filarial worms (Barrett, University of Aberystwyth, UK and Harris, Texas College of Osteopathic Medicine, USA). Barrett has shown that all enzymes of the glycolytic sequence, and of the tricarboxylic acid cycle, are present in Brugia pahangi, Onchocerca gutturosa and Onchocerca lienalis, and that glycogen phosphorylase, hexokinase, phosphofructokinase and pyruvate kinase are the regulatory enzymes of the glycolytic sequence. Using Dirofilaria immitis, Harris has obtained very similar data for glycogen phosphorylase, hexokinase and phosphofructokinase, and has begun purification of the phosphofructokinase. Thus future work with inhibitors can be carried out on the known regulatory enzymes of the filarial glycolytic pathway, now that these rate limiting enzymes have been defined.
Annex II

Barrett and Harris have also obtained very similar results on the levels of adenylate nucleotides and other metabolic intermediates, in the four filarial species and, in conjunction with the Wellcome Group, Barrett has compared these intermediates in normal and drug-treated adult B. pahangi. Such studies will help to indicate the site of action of any inhibitor, prior to further detailed studies on individual enzymes.

NMR techniques can be used to assay metabolic intermediates in the intact, living worm, and Powell (London School of Pharmacy, UK) is applying this technique to B. pahangi with worms and active antifilarial compounds, supplied by Denham (London School of Hygiene and Tropical Medicine, UK - supported by TDR/FIL).

Regulatory enzymes are often controlled by a reversible phosphorylation/dephosphorylation process, sometimes dependent upon cyclic AMP (cAMP), and Harris hopes to investigate such control mechanisms in D. immitis with glycogen phosphorylase and synthase, and with phosphofructokinase and hexokinase. In other nematodes these enzymes have proved to be target sites of inhibition.

Kommuniecki (University of Toledo, Ohio, USA), a biochemist associated with the Upjohn Consortium, will also use these same named regulatory enzymes of the glycolytic pathway (in B. pahangi and D. viteae) to evaluate novel inhibitors available from Upjohn, and will also pay special attention to the cAMP-dependent phosphorylations of these enzymes. Like Barrett, Kommuniecki will also make use of the analysis of metabolic intermediates to locate sites of action of inhibitors, and both the Wellcome and Upjohn groups use glucose uptake and lactate production as an assay for inhibition of the glycolytic pathway. It is hoped that from its contacts in the Sudan the Upjohn Group will carry out some of these experiments on adult O. volvulus, whereas the Wellcome Group at present rely on bovine Onchocerca spp, together with other laboratory-reared filariae for their studies.

One other worker whose research is concerned with regulation of enzyme activity by reversible phosphorylation is Walter (Bernhardt-Nocht-Institut, Hamburg, Germany). Although his work on O. volvulus in the field of glycolysis and energy generation and general protein kinase studies is funded by TDR/FIL, OCT are currently funding his work on three other enzymes, which in mammalian systems, are regulated by reversible phosphorylation. These are acetyl CoA-decarboxylase, aminoacyl-RNA synthetases, and ornithine decarboxylase which are the rate-limiting enzymes in the biosynthesis of fatty acids, proteins and polyamines respectively; metabolic pathways essential to the adult worm with respect to reproduction, growth and turnover of macromolecules. An antifilarial analogue of Amoscanate (Ciba-Geigy) has already been shown to act on aminoacyl-RNA synthetases, while irreversible inhibitors of ornithine decarboxylase, such as difluoromethylornithine, are available for experimental use against the Onchocerca enzyme.

1.2 Polyisoprenoid biosynthesis

The isoprenoid biosynthetic pathway, in mammalian tissues, synthetizes cholesterol and other steroids as end products, but in nematode worms, like arthropods, sterols are taken up from the environment, and only the early part of the biosynthetic pathway remains in filariae. This however is still of great importance as products of this part of the pathway include ubiquinone and rhodoquinones (important in electron transport) and dolichols and other polyisoprenoids, probably involved in glycoprotein biosynthesis. Although not yet identified in filarial worms, the pathway may also provide juvenile hormones (as found in arthropods), and isopentenyl adenine which is involved in regulation of cell division processes in other systems. All aspects mentioned above are under study within the biochemical group at the Wellcome Research Laboratories, but special attention is being paid to the rate limiting, regulatory enzyme of the pathway, hydroxymethylglutarate-Coenzyme A reductase (HMG-CoA reductase), and to the interconversion of ubiquinones to rhodoquinones.
The natural products mevinolin and compactin inhibit HMG-CoA reductase from filarial worms (including O. gibsoni), block the incorporation of radiolabelled precursors into isoprenoid alcohols, dolichols and quinones, and in the B. pahangi L3 developing larvae assay they inhibit moulting and cause death. Mevinolin shows marginal activity in in vivo assays against B. pahangi. With UCT funding, analogues of these natural inhibitors are being synthesized by Robinson (University of Southampton, UK) to be tested in the Wellcome systems, in the hope of achieving specific inhibition of filarial HMG-CoA reductase and hence of the isoprenoid biosynthetic pathway.

The conversion by filarial worms of ubiquinone to rhodoquinone demonstrated by the Wellcome Group provides a metabolic pathway present in the nematode, but not in the host, as rhodoquinone is found only in helminths, bacteria and few protozoa. Thus the possibility of the synthesis of an inhibition with selective toxicity is high, particularly as quinones analogues and mimetics are an area of expertise of the Wellcome Group. The Wellcome quinone inhibitor parvaquone (marketed as an anti-Theileria agent) has been sent for evaluation in the O. gibsoni cattle screen.

1.3 Respiratory electron transport chains

Although metabolic roles for ubiquinone and rhodoquinone in filarial worms have not been demonstrated, in other systems they are involved in electron transport processes as a link between flavoproteins and cytochromes, and this is another metabolic area being studied in detail by the Wellcome Group. Although previous work seemed to indicate that glucose catabolism gave total conversion to lactate as an end product, it was known that filarial worms did take up oxygen, and it has now been shown that, like other nematodes, filariae possess two respiratory pathways which utilize oxygen. They possess one electron transport pathway similar to the classical mammalian cytochrome chain, and another, the so-called "alternative oxidase" which is resistant to cyanide. The quantitative importance of these aerobic pathways for maintenance of redox balance and generation of ATP remains to be established. Additionally, an anaerobic NADH-linked fumarate reductase is present as in other nematodes studied, which results in succinate as an end product. In mammalian tissues, this reaction usually runs in reverse, as part of the tricarboxylic acid cycle, and is then termed succinate dehydrogenase. Fumarate reductase in nematodes thus provides a relatively unique chemotherapeutic target, which has previously been exploited in some anthelmintic compounds. One problem is whether, with such a multiplicity of electron transport chains, inhibition of one of them will result in worm death or must all be inhibited? Wellcome chemists are currently synthesizing Antimycin A analogues (more than 70 made to date) which already are showing selective toxicity towards nematode systems, and have produced death of filarial worms in vitro.

1.4 Neurophysiology

Biochemists associated with the Upjohn Group (Pax, Bennett and Sulaiman) feel that neuromuscular function in filariae provides a valid assay system for inhibitors both of energy generation, and of neuro-transmission to the body musculature. Their research will focus on two different but interrelated objectives. The first will involve the evaluation and development of an in vitro assay system for quantitatively monitoring the effects of drugs on the muscle activity of B. pahangi and O. volvulus. The second objective will involve the gathering of relevant neurophysiological data on newly synthesized antifilarial compounds. They have demonstrated that filarial segments, or intact worms (B. pahangi and D. immitis mfs) can be used in neurophysiological experiments of the increasing complexity. Initially spontaneous movements of the worm can be assayed using a "micromotility meter", and the effects of inhibitors on this system can be quantitated. If positive effects are shown by novel compounds, they can be further studied by electrophysiological methods such as direct recording of muscle mechanical activity, and the response of the muscle to both direct and
Annex II

Synaptically-produced membrane potentials. Baseline studies of the filarial neuromuscular system will involve measurement of the membrane resting potential, its conductance and excitability. The use of suction electrodes and intramuscular microelectrodes will enable such electrophysiological work to be carried out, and allow characterization of receptor sites and their pharmacological properties. Ivermectin has already been used to validate these systems in preparations of B. pahangi, and other classes of anthelmintic inhibitors are currently under investigation. It is hoped that most of the experiments described can also be carried out on intact worms or segments of O. volvulus, obtained locally by nodulectomy and examined initially in a Khartoum laboratory. This will provide data on fresh worms which can be used later in comparisons with worms transported to the USA.

Following a lead in the patent literature that 3-carboxamidolevamisole possessed macrofilaricidal activity, the Wellcome Group considered that other analogues were worthy of further exploitation, since previous synthetic efforts in this area were probably directed towards gastrointestinal nematode activity. To support this work, Wellcome-funded ancillary studies are investigating the effects of novel analogues in both electron transport systems, and in electrophysiological studies of cholinergic receptors in filarial and other nematode systems. Such studies have revealed clear differences between the newly synthesized 3-carboxamidolevamisole analogues and levamisole itself, and indicate that the newer derivatives may well have a different mode of action.

1.5 Tubulin-polymerization inhibitors

Howells (Liverpool, UK) has developed a simple in vitro assay to demonstrate mitotic arrest in the reproductive tissues of filarial worms by colchicine, or those antifilarial agents such as benzimidazole carbamates, which are inhibitors of tubulin polymerization. This technique is potentially applicable to O. volvulus adult male worms obtained by nodulectomy, and would be a way of rapidly screening the many benzimidazoles and related compounds available to the OCT.

1.6 Transcuticular uptake of nutrients and drugs

In filarial worms generally, and particularly in Onchocerca, there has been much discussion regarding the relative importance of oral and transcuticular uptake of nutrients and drugs. Howells has made detailed studies on the uptake of amino-acids, sterols and sugars and their analogues, and the inhibition of such transport in filarial worms, and has also studied the uptake of drugs such as suramin. Synthetic chemistry by Jefford (University of Geneva, Switzerland, supported by TDR/FIL) has been directed towards analogues of phlorizin which may block the filarial sugar transport systems defined by Howells. Similar transport work on bovine Onchocerca spp. is included in the OCT-funded project of Muller (Commonwealth Institute of Parasitology, UK). Court (Wellcome Group) is also attempting to define those physicochemical properties of a drug molecule which are needed for transcuticular passage of molecules in adult filaria, and to date has made a study of non-electrolytes of varying lipophilicity, molecular weight, charge, etc. Such studies will hopefully enable predictions to be made of those substituents essential for entry of drugs into the worm. It is encouraging that one overall finding by all workers is that the cuticle of adult filarial worms is much more permeable to drugs than that of gastrointestinal nematodes.
1.7 Chitin metabolism

One speculative study being supported by OCT (Gooday, University of Aberdeen, UK) is concerned with the structure of the cuticle. There is evidence that at certain life-cycle stages, e.g., the membranes surrounding the egg, nematodes synthesize chitin, which occurs mainly in the exoskeleton of arthropods and in other invertebrates. If chitin and enzymes associated with it can be shown to occur in filarial worms, at any stage of their life-cycle, then damage by known specific inhibitors such as polyoxins and nikkomycins should occur. The biochemical aspects of this work are being carried out in Aberdeen, while the in vivo and in vitro testing against filarial worms will be done by the Wellcome Group.

2. In vitro and in vivo screening facilities and provision of filarial material for biochemical work

Both the major industrially-based research groups carry out the biological evaluation of novel compounds produced "in house" by means of in vitro and in vivo biological assays, using filarial species other than Onchocerca. In vitro test systems have the advantage of using only small amounts of the compound, are of shorter duration than in vivo tests, and, when these are available, can utilize larval and adult stages of various Onchocerca spp. Muller is being funded to establish bovine Onchocerca life-cycles, and to maintain, cryopreserve and cultivate both adult and larval stages in vitro (particularly O. lienalis) with a view to drug screening, and intercontinental transport of viable Onchocerca spp. This work is also being carried out in collaboration with the Wellcome Group, where much of the development of in vitro drug screening systems was done. Combinations of in vitro drug exposures, with reimplantations of treated worms (B. pahangi) into recipient host animals, is also proving of value to the Wellcome Group.

Following a recent Scientific Working Group meeting (OCP/OCT/84/3) on the in vitro culture of filarial parasites, especially Onchocerca, interest has been aroused in improvements in culture techniques for filaria, and research proposals have been put forward for consideration by the OCT Steering Committee. Good in vitro systems will allow biochemical experiments to be carried out, drug evaluation to occur over several life-cycle stages, and the transport of, and experimentation upon, adult O. volvulus worms obtained from nodulectomy. All these are essential to obtain the basic metabolic information, and chemotherapeutic responses, of the target species.

The establishment of filarial life-cycles for screening purposes (B. pahangi, D. viteae, D. immitis, L. carinii) can usually be expanded to provide material for biochemical studies, and the in vitro maintenance techniques developed for drug screening purposes are generally adequate for radiolabelled incorporation experiments, etc. However, to date very few biochemical workers have been able to use O. volvulus itself (Walter being a notable exception), and most workers exploit equine and bovine Onchocerca species (O. lienalis, O. gutturosa, O. gibsoni and O. cervicalis) but, as yet, not knowing whether the information gained is truly relevant to O. volvulus. Because of the way nodule formation occurs, the bovine species O. gibsoni has been used as the only biological in vivo screen for Onchocerca (Copeman, James Cook University, Townsville, Australia) and this screen is now being supported by OCT. Copeman has also been funded by OCT to supply O. gibsoni nodules to any laboratory carrying out OCT-funded research. Wellcome at present "banks" any nodules in excess of its own requirements, and supplies them on demand to European workers. At present the USA will not allow the importation of O. gibsoni nodules for veterinary health reasons.
Annex II

A major target for the OCT is to provide *O. volvulus* nodules, or collagenase freed adult worms, to workers throughout the world for biochemical and chemotherapeutic purposes, but this supply has not yet been established in the same regular way as for cattle species, although low temperature equipment is being made available in both Africa and the New World.
LIST OF OCT/SC MEMBERS

<table>
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<tr>
<th>Nationality</th>
<th>Name</th>
<th>First and final meeting attendance</th>
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<tbody>
<tr>
<td>British</td>
<td>Dr R. Branch, Clinical Pharmacologist Vanderbilt University, Nashville, USA</td>
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<td>Dr C.C. Wang, Biochemist University of California, San Francisco, USA</td>
<td>March 1985 - September 1987</td>
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