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ANNOUNCEMENT

WORLD HEALTH ORGANIZATION ONCHOCERCIASIS CHEMOTHERAPY PROJECT

Onchocerciasis is a parasitic disease of man in the tropics caused by the nematode worm *Onchocerca volvulus*. The adult worms inhabit subcutaneous nodules within which they reproduce and release mobile larvae, the microfilariae. Biting blackflies, of the genus *Simulium*, transmit the parasite by the accidental ingestion of microfilariae and the subsequent reintroduction of infective third-stage larvae. The pathology of onchocerciasis involves skin changes including nodule formation, dermatitis, pigmentation changes, papule formation, oedema, skin scaling and atrophy, lymphadenopathy and ocular pathologies leading to blindness. Endemic foci of onchocerciasis exist in tropical Africa, Yemen, Mexico, and Central and South America; conservative estimates indicate that 40 million people suffer from the disease and many more are at risk of infection. Whole villages can become blinded by infection with this parasite.

The Onchocerciasis Control Programme (OCP) was initiated by the World Health Organization in 1974 in the Volta River Basin area and it is concerned with vector control by the widespread application of insecticides. This long-term programme, although in part successful, cannot now be regarded as the sole approach to parasite control and new drugs are urgently needed to kill the worms in infected humans. As a consequence the Onchocerciasis Chemotherapy Project (OCT) has been launched, in the spring of 1983, by the OCP with the remit of supporting research into the development of new drugs that will kill the adult parasite. Killing of microfilariae is regarded as a secondary target because of the resultant and intense allergic response evidenced when the larvae are killed (the Mazzotti reaction).

In order to define objectives and review current developments the OCT recently held a working group meeting of parasite biochemists at WHO headquarters in Geneva. The outcome of this meeting is summarised below, the aim of which is to bring to the attention of biochemists in general the nature of the problem and the considerable need for basic biochemical information to be gathered on *Onchocerca* and related parasites. The OCT Steering Committee has an annual budget of $3.5 m to disburse for research directed towards the development of new drugs and it recognises the importance of fundamental parasite biochemistry to this research programme. None of the currently available drugs (i.e. diethylcarbamazine citrate, suramin and mebendazole) is suitable for large-scale use. Two new drugs, ivermectin and flubendazole, are currently undergoing clinical trials in onchocerciasis but it appears probable that neither will possess the requisite macrofilaricidal activity. Three derivatives of amoscanate are at the stage of preclinical toxicological trials but with so few candidate drugs under investigation it is vital to undertake a large programme of basic research involving biochemical studies on filarial parasites, synthetic chemistry and screening in animal models in order to find novel filaricidal compounds. To achieve these aims in a logical manner, much more knowledge of the biochemistry of filarial and allied parasites in general, and of *Onchocerca volvulus* in particular, is needed before differing metabolic pathways in parasite and host can be exploited for chemotherapy.

(i) *Energy metabolism of nematodes.* Carbohydrate is the sole energy source for parasitic nematodes and catabolism, via linear anaerobic pathways, leads to the formation of reduced organic end products. There is a normal glycolytic sequence as far as phosphoenol pyruvate but the fate of this intermediate reveals significant differences from that typified by mammals. The variation in the terminal pathways of carbohydrate breakdown in nematodes is reflected in their range of end products. All parasitic nematodes use oxygen when available and are thought capable of oxidative phosphorylation. However the relative contribution of oxidative processes to the energy budget of parasitic nematodes is unknown and probably variable. Branched-chain cytochrome systems occur in which one chain resembles that of mammals while a second branch involves a terminal O-type cytochrome. The control of electron flow through these branches remains unresolved.

The control of glycogen metabolism in nematodes is modulated by phosphorylation of glycogen synthase and glycogen phosphorylase and the control itself is modulated by hormonal activation of serotonin followed by elevation of cyclic AMP levels and by muscle contraction via neurotransmitters in a cyclic AMP-independent pathway. The cAMP-dependent pathway involves cAMP protein kinase while the cAMP-independent pathway involves the calcium ion effect on phosphorylase kinase. Glycogenolysis is directly coordinated with glycolytic flux via phosphorylation of phosphofructokinase; this phosphorylation is catalysed by a cAMP-dependent protein kinase which has been partially purified in a large gastrointestinal nematode. The probability of the development of successful chemotherapeutic agents based on the distinctness of the parasite and host hydrate-metabolising systems is increased as basic information accrues.

(ii) *Nutrient and drug uptake by filarial nematodes.* The cuticle of the general body surface of filarial worms is clearly implicated in the uptake of amino acids, monosaccharides, some nucleic acid precursors and certain drugs (i.e. levamisole and melarsonyl potassium). Suramin and mebendazole cannot cross the cuticle and enter the worm via the alimentary canal. The activity of novel candidate filaricides will be limited by their ability to enter the worm so that the physico-chemical properties which confer cuticular permeability on compounds must be defined and incorporated into new drugs wherever possible.

Since filarial worms maintained *in vitro* do not ingest substances orally efforts to improve culture techniques are of a high priority.

(iii) *Induction of folate deficiency.* Filarial nematodes cannot synthesise folate and depend upon preformed 5-methyltetrahydrofolate from the host. Adult worms can oxidise methyl FH_4 to 5, 10-methyl FH_4 which is then converted to other FH_4 cofactors, which serve as one-carbon donors in serine, purine and thymidylate synthesis. In their ability to oxidise methyl FH_4 and their inability to use methyl FH_4 in methionine synthesis, filarial worms differ qualitatively from their hosts.

A number of enzymes in the folate metabolic pathway are regarded as promising targets for chemotherapeutic exploitation (i.e. thymidylate synthase, dihydrofolate reductase and 5,10-methylene FH_4 reductase). Methotrexate and 5-fluorinated uracil, inhibitors of dihydrofolate reductase and thymidylate synthase, sterilise filariae *in vivo* but it is not certain whether novel antifolates can be developed which selectively inhibit parasite enzymes and yet prove acceptable for human application.

(iv) *Enzymic reactions and enzyme inhibitors.* Parasite energy metabolism and its regulation represents a major potential target for chemotherapeutic intervention. The key branchpoint at phosphoenolpyruvate and the regulatory roles of PEP-carboxykinase and pyruvate kinase are perhaps suitable targets for selective drug action. Inhibition of the reoxidation of NADH could lead to the blockade of glycolysis. NADP-linked malic enzyme may also provide a suitable target. Regulatory enzymes, such as cyclic AMP-phosphodiesterase, cyclic AMP-dependent protein kinase, phosphorylase kinase and glycogen synthase, must be examined for their target candidacy.

Enzyme inhibitors can provide the basis of successful chemotherapy against onchocerciasis, but to increase the chances of success selection of target enzymes or metabolic pathways should be on the basis of biochemical differences predictive of potentially lethal damage to the adult parasite. Irreversible, mechanism-based enzyme inhibitors, such as suicide substrates and transition state analogues, are preferable to reversible competitive inhibitors. As yet no efforts have been made to produce selective, species-specific toxicity for parasite enzymes with such inhibitors, which could then be tested against *in vivo* and *in vitro* models and subjected to appropriate QSAR examination and lead development. This approach demands a detailed biochemical background which at present is not available.

(v) *Reproductive and developmental biochemistry.* The reproductive biochemistry of most nematodes is poorly understood but is an essential prerequisite for potential chemosterilisation. The role of hormones in the moulting and development of bilarial nematodes needs to be elucidated if drugs are to be developed that inhibit normal development. The developmental similarities between nematodes and insects encourage a belief that hormonal control of growth and moulting might provide a suitable chemotherapeutic target.

(vi) *Research areas that must be developed and encouraged.* Because of the difficulties of obtaining adequate material of *O. volvulus*, much of the basic biochemical data will have to derive from related filarial nematodes that can be maintained in the laboratory. The use of wide ranging but appropiate model systems is therefore a necessity. WHO recognises the need to provide laboratories with *O. volvulus* from human nodules and is establishing one or more supply centres. At the same time *in vitro* culture techniques must be developed. The glaring deficiencies in the basic biochemical knowledge argue compellingly for considerable efforts to be made in all areas of parasite biochemistry. Identification of key pathways is a major priority and should lead to the logical development of new drugs with a selective mode of action.

Major chemotherapeutic targets will include (a) energy metabolism; (b) neuromuscular and neural transmission; (c) hormonal regulators of growth and development; (d) ion pumps; (e) the parasite cuticle; (f) parasite tubulin; (g) biosynthetic pathways; (h) drug and nutrient uptake; (i) chemosterilisation; (j) parasite motility. All of these are regarded as areas in need of basic research to provide leads for the development of novel anti-parasite drugs.

Further information on the Onchocerciasis Chemotherapy Project can be obtained from Dr. L. H. Chappell (Department of Zoology, University of Aberdeen, Tillydrone Avenue, Aberdeen, AB9 2TN; Tel. 0224-40241, ext. 6440).

It is hoped that the above outline will stimulate biochemists into turning their thoughts towards parasite biochemistry and that some will be encouraged to approach WHO with appropriate research proposals.