REPORT OF THE SCIENTIFIC WORKING GROUP
ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF SCHISTOSOMIASIS

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This report contains the collective views of an international group of experts convened to advise on the UNDP/WORLD BANK/WHO SPECIAL PROGRAMME FOR RESEARCH AND TRAINING IN TROPICAL DISEASES.
INTRODUCTION

Even before the introduction of the currently available safe and effective oral antischistosomal drugs, specific chemotherapy always played an important role in schistosomiasis control. Christopherson's treatment of 70 cases of schistosomiasis with intravenous potassium antimony tartrate in 1917-1918 marked the beginning of the use of trivalent antimonial compounds for schistosomiasis. Other preparations such as antimony sodium tartrate, sodium antimony dimercaptosuccinate, antimony bispyrocatechol-3,5-disulfonate and lithium antimony thiomalate have all been in frequent use in the past, both intravenously and intramuscularly.

Since 1960, orally administered antischistosomal drugs have appeared progressively. Three are now included in the WHO List of Essential Drugs: oxamniquine for *S. mansoni* infection, metrifonate for *S. haematobium* infection and praziquantel, which is effective against all human schistosomes. Niridazole and hycanthone are occasionally used clinically under strict medical supervision and, in the laboratory, serve as models for pharmacological studies of metabolism and mode of action. Oltipraz and amoscanate are in the preclinical development stages and much is still unknown about these compounds.

In spite of the apparent plethora of effective antischistosomal drugs, complacency towards further research is neither rational nor historically sound. Despite well-documented evidence of parasite resistance to oxamniquine, the large-scale use of the drug continues. This may be an early-warning signal which cannot be ignored by the scientific community. New drugs must be developed and to this end research on the biochemistry and physiology of the parasites must be pursued; the pharmaceutical industry, the source of compounds which reach the market place and are used in the endemic countries, should be encouraged to support the development of new drugs.

The Scientific Working Group on Schistosomiasis of the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases sponsored a three-day meeting in Geneva between 30 January and 1 February 1984. Its purpose was to identify areas of research likely to facilitate the discovery and development of future antischistosomal drugs. Participants were drawn from universities, research laboratories, the pharmaceutical industry and governmental agencies in several tropical countries.

Presentations made during the meeting were grouped into five major categories:

- **Specific compounds**: praziquantel, metrifonate, oxamniquine, oltipraz and amoscanate. The presentation on niridazole focused on the metabolic basis for this drug's differential toxicity in host and parasite. Three of the above drugs (i.e. oxamniquine, amoscanate and niridazole) have nitro groups as ring substituents; thus the structure-activity relationships and mode of action of nitroheterocyclic compounds were also discussed.

- **Metabolism**: the presentations involved glucose and energy metabolism as well as the pathways of pyrimidine and purine metabolism.
Physiology: glucose and amino acid transport into and between schistosomes as well as the effects of acetylcholine, dopamine and serotonin on longitudinal and circular muscle were considered.

Pharmacokinetics: the pharmacokinetic studies of niridazole and its metabolites in Filipinos as well as of oltipraz and oxamniquine in Sudanese were presented.

Resistance: available information on resistance to current antischistosomal compounds and experimental approaches to this question were discussed.

The summaries presented in this report are based on the working papers and discussions at the meeting.

1. PHARMACOKINETICS, METABOLISM AND MODE OF ACTION OF CURRENT ANTI SCHISTOSOMAL DRUGS

1.1 Praziquantel

The anthelmintic activity of praziquantel, 2-cyclohexylcarbonyl-1,2,3,6,7,11b-hexahydropyrazino[2,1-a]isoquinolin-4-one, was discovered in 1972. Extensive clinical trials have since established that it is effective against most cestode and trematode infections of man and of animals. Praziquantel is only sparingly soluble in physiological media but is readily soluble in ethanol. It is stable in aqueous solution.

Rapidly absorbed from the intestinal tract, it is subject to first-pass metabolism. Its metabolites are excreted in the urine, mainly as glucuronide and sulfate conjugates. Praziquantel is a rapidly acting drug. In vitro, praziquantel is quickly taken up by all susceptible parasites but not metabolized. This proves that praziquantel itself is active. The metabolites formed in man and animals, i.e. the 4-hydroxycyclohexyl derivative in serum, and sulfates and glucuronides of di-hydroxylated analogues in urine, are less potent or inactive as anthelmintics.

Although praziquantel is a newly developed drug, much progress has been made towards understanding its mode of action. At therapeutic serum levels (0.3 μg/ml), praziquantel causes contraction of the musculature of the parasites and vacuolization of their body surface, the tegument. Contraction is very rapid: half the maximal effect is obtained in 10 seconds. Surface alterations are equally rapid and can be noticed in 30 seconds and they are fully expressed in less than 5 minutes. Identical effects are observed in vitro and in vivo. The mechanism underlying contraction and tegumental vacuolization appears to be a direct effect of praziquantel on the permeability of membranes. It increases the permeability of the cell membrane to mono- and divalent cations, especially calcium, without being an ionophor. Besides these very rapid and therefore primary effects, praziquantel also causes a variety of secondary effects in tapeworms and schistosomes: a slow depolarization of the tegumental resting potential, inhibition of glucose uptake, a decrease in glycogen content and a stimulation of the release of lactic acid. Many other metabolic processes, such as protein and RNA synthesis, and the activities of many enzymes are not affected by praziquantel.

In the case of intestinal tapeworms, their inability to hold their position in the intestine, a consequence of the paralytic effect of praziquantel, may be sufficient to explain the elimination of these parasites. Other mechanisms must, however, be involved in the killing of parasites, such as lung or liver flukes and the blood-dwelling schistosomes, which cannot be evacuated easily from the body. In these instances, tegumental damage appears to be especially important. Partial erosion of the body surface causes
schistosomes to lose their immunological disguise: thus affected, they can be recognized as alien and are invaded by phagocytic cells within four hours of treatment of the mouse host. This then results in complete lysis of parasitic tissues.

Praziquantel has been reported to be more effective against late rather than early developmental stages of both *S. mansoni* and *S. japonicum*. In contrast to these *in vivo* findings, no difference in the susceptibility of different developmental stages of *S. mansoni* to praziquantel was observed *in vitro*. Two possibilities were explored to elucidate the apparent stage-specific resistance of *S. mansoni* to praziquantel in *in vivo* but not *in vitro*.

The first possibility was that young forms of *S. mansoni* might be intrinsically more resistant to praziquantel. In order to explore this hypothesis, an *in vitro* assay was developed to determine the pharmacological effects of praziquantel at different developmental stages of *S. mansoni*. Then, in order to compare the pharmacological effects at the different stages, the minimum effective concentration for each effect for each stage was determined. The stages studied were at Day 0, Day 3, Day 7, Day 14, Day 28, Day 35 and Day 42.

Four pharmacological effects were characterized *in vitro*: stimulation of motor activity, muscular contraction, formation of tegumental vesicles and ability of the parasite to recover from drug-induced damage. When stimulation of motor activity and muscular contraction were used as criteria, no stage-specificity was noted. When the formation of tegumental vesicles or the ability of the parasite to recover from drug-induced damage were used as criteria, Day 3 and Day 7 lung stages of *S. mansoni* were more resistant to the pharmacological effects of praziquantel. In order to determine whether the same pattern of resistance could be reproduced *in vivo*, schistosome-infected CF-1 female mice were treated with praziquantel either orally (382 mg/kg, 1/2 ED$_{50}$) or intramuscularly (202 mg/kg, 1/2 ED$_{50}$) on the same day as that corresponding to the different developmental stages tested *in vitro*. When quantitative worm recoveries were performed, more worms were recovered from infected mice treated on Day 3 (36% worm reduction) than mice treated on Day 42 (80% worm reduction). These findings suggest that Day 3 forms may be intrinsically more resistant to the action of praziquantel.

The second possibility was that the apparent resistance to praziquantel of early stages of *S. mansoni* in *in vivo* might result from their exposure to lower concentrations of unchanged praziquantel in the peripheral circulation. On the other hand, more mature stages, located in the liver and mesenteric veins, would be exposed to higher concentrations of the drug. In order to test this hypothesis, a high-pressure liquid chromatographic method was developed to measure praziquantel pharmacokinetics after oral (382 mg/kg) and intramuscular (202 mg/kg) doses of praziquantel. When levels of the drug obtained *in vivo* were compared to drug concentration and time of exposure required to damage parasites irreversibly *in vitro*, only the more mature forms of the parasite were irreversibly affected. Moreover, the same result was obtained whether the drug was given orally or intramuscularly. These results suggest that the route of administration and difference in serum level of the drug were not important in explaining the apparent *in vivo* resistance of young forms to praziquantel.

The combined results of these *in vitro* and *in vivo* studies suggest that the greater resistance of immature lung forms of *S. mansoni* to praziquantel may be an intrinsic property of the parasite relating to its tegumental membrane structure.

1.2 Metrifonate

Metrifonate, 0,0-dimethyl (-2,2,2-trichloro-1-hydroxyethyl) phosphate, was originally synthesized as an insecticide in 1952. Its potential use as an
anthelmintic was investigated in the 1960s and clinical studies with metrifonate as a drug against *Schistosoma haematobium* infections have been undertaken since 1965.

It has always been assumed that the inhibition of acetylcholinesterase is somehow related to the therapeutic effect of metrifonate. Acetylcholine functions as an inhibitory neurotransmitter in schistosomes. However, it has been difficult to reconcile the lack of major differences in the susceptibility to metrifonate of the cholinesterase preparations from *Schistosoma haematobium* and *Schistosoma mansoni* with the therapeutic refractoriness of the latter. Concentrations of 0.2 to 0.8 μg/ml that paralyse schistosomes are well below the maximal levels obtained in man (8 μg/ml). Measurements of the inhibitory effect of metrifonate on schistosome cholinesterases have yielded inconsistent *IC₅₀* values ranging from 20 000 μg/ml to 1.6 μg/ml. A recent analysis indicated that metrifonate is unlikely to react with schistosome cholinesterases while dichlorovos, 0,0-dimethyl-0-(2,2-dichlorovinyl), DDVP, which is produced from metrifonate by chemical rearrangement, is a more potent inhibitor of the schistosome enzyme (*IC₅₀* value 40 μg/ml). The concentration of DDVP in human plasma has only been observed to rise to between 0.02 and 0.04 μg/ml because of a very rapid destruction of DDVP (half-life 1–2 min). Thus, metrifonate seems to function as an intrinsic sustained-release formulation of DDVP.

However, the paradox remains unexplained, that metrifonate (or DDVP) is almost equally effective in vitro and in animal experiments against *S. mansoni* and *S. haematobium*, but therapeutically effective in man only against *S. haematobium*.

1.3 Oxamniquine

Oxamniquine, 6-hydroxymethyl-2-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline, was reported to have antischistosomal activity in 1969. It is effective against *S. mansoni* only.

The metabolism of oxamniquine was studied in the mouse, rat, hamster, rabbit, rhesus monkey and dog, and in man. The drug is well absorbed after oral administration and is extensively metabolized to two acidic metabolites which are largely excreted in the urine. The major metabolite in all species studied arises from oxidation of the 6-hydroxymethyl group to a carboxyl group. The second metabolite results from oxidation of the side-chain to give the 2-carboxylic acid and this is present in mice, hamsters, rabbits and dogs. The 6-acid is a major component circulating in plasma. Both metabolites are inactive as schistosomicides.

Pharmacokinetic parameters were estimated for oxamniquine from studies in human subjects. Oral bioavailability appears to be about 50% with reference to intramuscular data. A study in the dog suggested that there is first-pass metabolism in the gut wall. Mean value for the half-life of elimination in man is approximately 1.5 hours.

Studies have also been carried out to determine whether the systematic bioavailability of the drug is different in different ethnic groups and could account for the variations in dosage required in the African continent. Comparison of serum concentrations from patients in Brazil, South Africa, East Africa and Sudan shows that in the main there are no differences and that the variation in dose-response might be due to strain differences in the parasite.

* 50% inhibition of enzyme activity
There is some correlation between serum concentrations of oxamniquine in individual patients and therapeutic effect, which suggests that the active principle is oxamniquine itself. Oxamniquine is taken up by *S. mansoni* in vitro and has been shown to be present in parasites from infected mice given the drug either orally or intramuscularly.

In contrast to hycanthone and metrifonate, neither oxamniquine nor the 6-acid metabolite is an inhibitor of the cholinesterase of *S. mansoni*. Oxamniquine stimulates worm motility and inhibits the onset of carbachol-induced paralysis, indicating that oxamniquine possesses some anticholinergic properties, as has been reported for hycanthone. More recent studies on the possible mode of action of oxamniquine reported by other investigators were discussed.

1.4 Oltipraz

Oltipraz, or 4-methyl -5(2-pyrazinyl)-1,2-dithiole-3-thione, is a new antischistosomal agent with established experimental and clinical efficacy against *S. mansoni* and *S. haematobium*.

The pharmacokinetics of oltipraz were studied in the mouse, rat and monkey and in man. The protocol was designed to take into account, on the one hand, the physicochemical properties of the drug, including its high liposolubility and hydrophobicity, and on the other, the fact that it is active after short-term oral treatment.

The absorption of oltipraz is characterized as follows:

- the rate of absorption is relatively slow and does not vary with dosage form, sex or nutritional state;
- the absorption of 14c-labelled oltipraz in animals accounts for at least 50% of the orally administered dose;
- food intake markedly modifies plasma levels, which are 3 to 4 times higher in man when oltipraz is administered after a meal;
- the bioavailability of the drug is dose-dependent;
- uptake by the parasite is extremely efficient, rapid and independent of the plasma kinetics;
- no drug accumulation has been observed during therapeutic administration.

The excretion of oltipraz varies according to the species. Approximately half of the dose is excreted in the urine mostly as metabolites and half in the faeces predominately as unchanged compound.

In the three animal species studied and in man, the metabolism is characterized by rearrangement of the dithiole-thione ring with recyclization into a pyrrolo pyrazine. The compounds formed undergo oxidation of the sulfur and/or carbon atoms, which gives sulfoxides, sulfones and pyrrolo pyrazinones. Oxidation of the dithiole-thione into dithiolone is a secondary mechanism of metabolism. N-glucuronides have also been detected.

Finally, the experimental findings led to hypotheses concerning the mechanism of action of oltipraz. Administration of oltipraz causes a decrease in the glutathione level in the schistosomes. This effect is limited to the parasites and the glutathione level actually increases in various host tissues. Of the chemical analogues of oltipraz, only those with antischistosomal activity also induce a reduction of the glutathione level.
The mechanism underlying the activity of oltipraz may therefore imply the inhibition of enzyme systems linked to glutathione.

1.5 Amoscanate

This new isothiocyanate anthelmintic exhibits marked activity under experimental conditions against hookworms and all three major human schistosomes.

Amoscanate and its metabolites have been assayed *in vivo* by a variety of radioactive and non-radioactive techniques. Development of a non-radioactive method, for eventual use in man, has been complicated by the unusual chemical and kinetic properties of amoscanate, especially since the parent drug has a short half-life (t1/2) and since it is chemically quite reactive and thus binds covalently to serum proteins, forming adducts with t1/2's measurable in weeks. Most methods, including spectrophotometric, differential pulse polarographic, radioimmunological, and several gas-liquid chromatographic techniques, depend ultimately on the presence of a para-nitrophenyl moiety. However, reduction of the nitro group is thought to occur to some extent *in vivo*, thus resulting in "silent" metabolites for such assays.

A $^{14}$C-ring-labelled suspension (particle size approximately 2 to 3 μm) was administered orally at a dose level of 30mg/kg to mice and rats to determine the basic pharmacokinetics (absorption, distribution and excretion) of the drug. The results indicated that in the rat the absorption is both slow and incomplete. The systematic bioavailability of "apparent" amoscanate after the application of two small-particle-size suspensions to rats and dogs is dose-dependent. The extent of absorption is lower in dogs than in rats. Absorption seems to be increased following the application of oily suspension to rats in the lower dose range and at high and low doses in dogs. In 10 human volunteers given a single oral dose of 30mg/kg micronized amoscanate (approx. 5μm) in agar suspension, the mean peak plasma level (at 36h) was $0.37 \pm 0.18$ μg/ml, and the t1/2 was 18 - 23 days. Renal excretion in the first 72 hours accounted for 1.04% or less (mean 0.52%, range 0.24 to 1.04%) of the administered dose. Five human volunteers were treated orally with a single dose of 3.5mg/kg of a small-particle-size (2μm) suspension and renal elimination was monitored by differential pulse polarography. The mean excretion within 72 hours amounted to 9.6% (individual values: 4%, 5%, 9%, 11% and 19%) of the administered dose.

Amoscanate binds irreversibly to amino groups of amino acids and proteins, *in vitro* and *in vivo*, by reaction of the isothiocyanate moiety, to form thiourea derivatives. Some 80% of the drug in plasma is precipitated by HClO₄, is not extractable by acetone, and is thus presumed to be covalently linked to protein. The adducts have not been characterized.

The primary mode of action of amoscanate is unknown. When given to schistosome-infected rodents it induces a rapid hepatic shift which is prevented by a single i.p. dose of 40mg/kg of 5-HT (serotonin). This indicates that amoscanate might interfere with 5-HT in the schistosomes.

Under *in vivo* conditions the drug decreased succinate, lactate and acetate recoveries from the cestode *H. diminuta*, while ATP levels dropped. In addition, reduced glycogen levels were observed in drug-treated worms which were homogenized immediately upon isolation. Glycogen synthase I was inhibited 16-61% upon isolation from the host, but returned to normal levels after incubation for 90 minutes. In contrast to glycogen synthase I activity, the effects on phosphorylase A were not readily reversible. Attempts to demonstrate these effects *in vitro* have so far been unsuccessful. Effects of a single subcurative dose of amoscanate on the surface of adult schistosomes were studied by scanning and transmission electron microscopy. Surface alterations included pronounced swelling and ballooning of the tegument,
wrinkling and constriction of folds and channels, disruption and collapse of sensory bulbs, exfoliation of surface layers, erosion of large areas of the surface, and attachment of host cells. In addition, vitelline cells of female worms were also affected.

1.6 Niridazole

Niridazole, 1-(5-nitro-2-thiazolyl)-2-imidazolidinone, is effective against the three major species of schistosomes which infect man, and against a number of other facultative anaerobic organisms. Although the requirement of multiple oral doses and the occurrence of side-effects (central nervous system toxicity, immunosuppression and mutagenicity/putative carcinogenicity) make niridazole less than ideal for mass chemotherapy, it has been used as the prototype for antischistosomal nitro compounds. The side-effects of niridazole in the mammalian host and its toxicity to schistosomes have been found to relate to its metabolism in the two organisms, but its metabolic fate is fundamentally different in host and parasite.

Studies on the proximal metabolism of niridazole in adult *S. mansoni* support the hypothesis that reductive metabolic activation of the drug within the parasite is required for antischistosomal activity. When adult worm pairs were incubated in the presence of 14C-niridazole, the radiolabel became covalently bound to trichloroacetic acid-precipitable material in a dose- and time-dependent manner. Experiments with cell-free schistosome preparations revealed that the initial step of niridazole metabolism in vitro involves the NAD(P)H-dependent enzymatic reduction of the essential nitro group. Covalent binding of 14C-niridazole to parasite macromolecules was also demonstrated with the cell-free system. The requirements for covalent drug binding were identical to those required for proximal enzymatic nitro reduction. Other experiments with 4'-methylniridazole, a close structural analogue which has a nitro group but no antiparasitic activity, further substantiate the hypothesis.

In contrast to its reductive metabolic fate in the parasite, niridazole is oxidatively metabolized in mammalian liver by the cytochrome P450 mixed-function oxidase system. Based on the identification of certain niridazole metabolites, it is postulated that oxidative metabolism leads to the formation of a reactive epoxide intermediate which may be related to one of niridazole’s putative side-effects in the host, i.e. carcinogenicity. In support of this hypothesis, oxidative microsomal metabolism of 14C-niridazole also leads to macromolecular covalent drug binding. Covalent binding did not occur in the presence of carbon monoxide, a specific inhibitor of cytochrome P450. Precursor/product studies with 14C-labelled oxidative niridazole metabolites were also consistent with the formation of the putative epoxide.

Taken together, these results suggest that it may be possible to synthesize new derivatives of niridazole which retain full antischistosomal activity but which are less toxic to the host.

2. NITROHETEROCYCLES: MODE OF ACTION AND STRUCTURE-ACTIVITY RELATIONSHIPS

The nitroheterocyclic drugs with useful chemotherapeutic properties are the nitrofurans, used principally in the treatment of urinary tract infections, and the nitromidazoles, which have wide applications in the therapy of bacterial and protozoal infections and show potential as antitumour drugs. Emphasis was placed almost entirely on members of the nitromidazole group which, despite their wide antimicrobial spectrum, are effective only against anaerobes possessing low redox potential reactions. The nitro group of these drugs acts as a preferential electron sink in low redox systems and a short-lived reduction product generated in the process appears to be responsible for subsequent damage to DNA and for cell death.
Details of the interaction between reduced nitroimidazoles and DNA were described with brief reference to the methods used to measure and characterize DNA damage. Such interactions have possible predictive value \textit{vis-à-vis} the relative effectiveness of nitroimidazoles towards anaerobic microbial pathogens.

The nature of the short-lived reduction product has attracted much attention and recent evidence suggests it is the one-electron radical anion, a species which may also be responsible for the irreversible toxicity of the nitrobenzyl derivative chloramphenicol.

Finally, the major characteristic of nitroimidazoles and their pre-requisite for reductive activation of the nitro group enables quantitative structure-activity relationships to be established between the redox potential and any useful biological response. Examples were given and pitfalls in interpretation pointed out to illustrate the difficulties in this field.

3. **SCHISTOSOME BIOCHEMISTRY**

3.1 **Metabolism in the Schistosome**

Free-living stages of schistosomes (miracidia and cercariae) generate energy by oxidative phosphorylation from reserves of glycogen. However, the loss of the cercarial tail and the change in permeability of the body appear to trigger the change from oxidative phosphorylation to excretion of lactic acid under aerobic conditions. Some of the glucose which is taken up through the adult tegument by a Na\textsuperscript{+}-coupled mechanism is transferred from males to females. The glucose is metabolized by glycolytic enzymes, with properties not dissimilar to those of the mammal, and lactic acid is excreted. Inhibition of one of the enzymes of glycolysis, phosphofructokinase, has been suggested as the mechanism of action of antimonials. Certain sugar phosphates can inhibit glucose isomerase in worm homogenates, but these are not specific for the schistosome enzyme.

Although schistosomules and adult worms continue to use oxygen, \textit{in vitro} studies have failed to show a Pasteur effect or decreased amounts of ATP during anaerobiosis, which suggests that all schistosome energy is obtained by glycolysis. Oxygen is required for egg production, but its role in this process and in immature worms needs to be elucidated. Only small amounts of the key enzymes of the citric acid cycle are present in \textit{S. japonicum}, but it has been claimed that the administration of fluoroacetamide to infected mice resulted in the migration of worms, the loss of worm glycogen and an increase in citric acid. This would suggest a minor but not insignificant role for oxidative phosphorylation, as has been claimed for adult \textit{S. mansoni}. How far isolation procedures and culture conditions affect the results is not known; in fact, different strains of the same species could vary in energy metabolism. Further studies on possible strain and species differences of oxygen use and its relationship to energy metabolism are required. A relatively large amount of malate dehydrogenase, which occurs in multiple forms, is present, but its exact role has not been determined.

The problems of producing sufficient schistosome mitochondria have so far precluded a study of their metabolism. Such a study might help to elucidate their role in energy and intermediate metabolism in the parasitic stages of the life cycle.

3.2 **Energy Metabolism as a Target for Drugs**

The emphasis of this presentation was not on direct experimental results. Ideas, in a biochemical framework, as to possible targets for
antischistosomal drugs in the energy metabolism of the parasite were reviewed.

Since schistosomes, like other parasitic helminths, are completely dependent on glucose as a source of energy, three sites for the perturbation of energy metabolism by drugs seem to be present:

- the uptake of glucose,
- the metabolism of glucose,
- the excretion of products.

Inhibition of the parasite's glucose uptake would inevitably result in its death. The chances of lowering the glucose concentration in its habitat are poor. Therefore, attention should be focused on blocking the actual process of glucose uptake into the schistosome by inhibiting the glucose binding sites in the schistosomal membranes. Some aspects of this approach were discussed.

Since Schistosoma parasites were considered to be homolactic fermenters, previous attempts to interfere with their energy metabolism were directed towards designing drugs which would inhibit glycolysis. However, experimental evidence was discussed which cast serious doubts on the suggestion that the energy metabolism of the schistosome is completely anaerobic. If it can be demonstrated that schistosomes indeed derive in vivo a considerable part of their energy from mitochondrial oxidative phosphorylation, as preliminary in vitro data seem to indicate, this would have important implications for future drug development.

A specific inhibition of the excretion of end-products of energy metabolism might result in smothering the parasite in its own waste. This very attractive speculation has recently become more plausible due to the discovery of a biomembrane-bound lactate-carrier protein.

3.3 Hexokinase

Hexokinase appears to be an important regulatory enzyme in the glycolysis of fortified cell-free homogenates of S. mansoni worm pairs. Three lines of evidence support the view that hexokinase is the rate-limiting factor in the glycolysis of worm homogenates. First, hexokinase is the component enzyme of lowest specific activity. Furthermore, the rate of glucose consumption to form lactate by homogenates is the same as the rate of formation of glucose-6-phosphate by hexokinase in these homogenates. Second, when purified glycolytic enzymes are added individually to glycolyzing worm homogenates, only hexokinase stimulates the overall reaction rate. Third, lactate production is nearly tripled when glucose-6-phosphate, rather than glucose, serves as the initial substrate for glycolysis.

In worm homogenates, no measurable contribution to ATP-dependent glucose-6-phosphorylation is made by a high $K_m$ glucokinase-like enzyme. Although worms synthesize glycogen and live in the mesenteric and portal veins where glucose concentrations fluctuate, they do not possess a glucokinase, which in hepatocytes appears to provide a mechanism for coping with the same conditions.

In rapidly glycolyzing tumour cells, nearly 50% of the hexokinase is bound to mitochondria. Unlike its soluble counterpart, this hexokinase is relatively resistant to glucose-6-phosphate feedback inhibition and it phosphorylates glucose at the expense of readily available mitochondrial ATP. These characteristics are thought to contribute to the very high rates of glucose consumption by tumour cells. However, the hexokinase in worm homogenates is not particulate, eliminating the possibility that hexokinase bound to mitochondria or incorporated into glycosomes is responsible for the rapid glycolytic rate.
Hexokinase appears to be an important target for the inhibition of glycolysis in *S. mansoni*, and potentially a target of chemotherapeutic importance. Hexokinase activity of *S. mansoni* has been purified over 300-fold from a worm homogenate. Studies are under way to purify further and characterize the hexokinase of *S. mansoni*, with particular emphasis on its susceptibility to substrate analogue inhibitors.

3.4 Pathways of Pyrimidine Metabolism

The occurrence of all six enzymes of de novo uridine monophosphate (UMP) biosynthesis has been demonstrated, for the first time, in *S. mansoni*, indicating that this parasite is capable of de novo pyrimidine biosynthesis in contrast to its inability to synthesize purines de novo.

Significant differences in pyrimidine metabolism exist between *S. mansoni* and its host. In *S. mansoni*, the major product of orotate metabolism was found to be orotidine 5'-monophosphate (OMP), whereas in mouse liver it was UMP. These results suggest that, in contrast to mammalian cells, OMP in *S. mansoni* is not "channellled" from orotate phosphoribosyltransferase (OPRTase) to OMP decarboxylase, resulting in a significant degradation of OMP to orotidine. Marked differences in the inhibition of OPRTase by several orotate analogues between the *S. mansoni* and mouse liver were also observed. Another striking difference between the parasite and the host is the occurrence of two distinct OPRTases in *S. mansoni*. These enzymes differ from one another in their molecular weights and their substrate and inhibitor specificities. The higher molecular weight OPRTase is non-specific for the substrates tested, as it catalyses the conversion of orotate, 5-fluorouracil and uracil to the respective nucleoside 5'-monophosphate. The lower molecular-weight OPRTase is specific for orotate. Both enzymes are inhibited by 5-azaorotate, but only the orotate-specific enzyme is inhibited by 4,6-dihydroxypyrimidine.

Enzymes of pyrimidine salvage pathways were also studied. Activities of thymidine and deoxycytidine kinases are present; however, neither uridine kinase nor uridine nucleoside phosphotransferase activities were detected. *S. mansoni* has no thymidine phosphorylase but does possess a non-specific uridine phosphorylase and 5'-nucleotidase activities towards OMP, UMP, CMP, dTMP, dUMP and dCMP. The absence of uridine kinase, a major enzyme in the salvage of uridine and uracil in mammals, is another conspicuous difference in pyrimidine metabolism between schistosomes and their mammalian host. Despite the absence of uridine kinase, schistosomes efficiently incorporate uridine into their nucleic acids. Therefore, the absence of uridine kinase and the ability of OPRTase to utilize uracil in schistosomes indicates that OPRTase may play a central role in synthesizing UMP via both de novo and salvage pathways. This is in contrast to mammalian systems, in which uridine and uracil are salvaged by uridine kinase. It follows that inhibitors of OPRTase in schistosomes may be selectively toxic against the parasite by blocking the synthesis of UMP by both the salvage and the de novo pathways.

3.5 Purine Salvage Pathways

Purine metabolism in developing *Schistosoma mansoni* schistosomulae was investigated in erythrocyte-free and serum-free media to eliminate possible contamination from host metabolites or enzymes. A previously reported absence of de novo purine nucleotide synthesis in the adult parasites was confirmed by the lack of incorporation of radiolabelled glycine or formate into the schistosomula nucleotide pool. Adenosine and adenine were equally incorporated into adenine nucleotides. The incorporation was not affected by hadacidin, an inhibitor of succinyl AMP synthetase. Adenosine and adenine therefore appear to be converted to AMP without forming IMP as an intermediate. Guanosine was first converted to guanine which was then incorporated into guanine nucleotides. Hypoxanthine was incorporated into all purine
nucleotides, but a large percentage (90%) was found in the adenine nucleotides. The equilibrium however, was shifted by hadacidin in favour of guanine nucleotides, an indication that hypoxanthine was converted first to IMP and then to AMP or GMP. These findings, together with the previous observation that *S. mansoni* lacks functional purine nucleoside kinases, lead to the conclusion that all purine nucleosides are primarily converted to the corresponding purine bases. The latter are then incorporated into the nucleotide pool via individual purine phosphoribosyl transferases. The three enzymic activities for salvaging adenine, guanine, and hypoxanthine thus constitute the major network for purine salvage in *S. mansoni* schistosomules.

4. **SCHISTOSOME PHYSIOLOGY**

4.1 **Intertegumental Transport Mechanisms**

Integumental incorporation of a variety of metabolites is seen to be dissimilar when comparative studies of mated and unmated, male and female schistosomes are made. For example, the rate of glucose assimilation by male and female *Schistosoma mansoni* was observed to be significantly greater in copulating than separated worm pairs, and the magnitude of this increase was proportionally greater in female than in male schistosomes. Thus it is apparent that the surface nutrient-gathering capacities of the male and female schistosome are influenced by tactile and/or chemical communications.

Using pulse-labelling methods, a transfer of $^{14}$C-glucose from the male to the female partner was demonstrated over a period of minutes. Other hexoses are also transferred between the copulating partners and, to date, the transport of four compounds from the male to the female has been defined. It has been postulated that a considerable portion of the total glucose utilized by the female in vivo may be supplied by the male. This phenomenon was first demonstrated in *S. mansoni*, and extended to *S. japonicum* and *S. haematobium*, which suggests that it may indeed be a characteristic of all species of this genus. Further confirmation of this phenomenon was derived with different techniques; glycogen and protein assays using individual worms of all three schistosome species indicated that glycogen content was greater in males that were unpaired in vivo than in paired males. Thus it has been suggested that in copula the female is able to deplete glycogen stored in the male.

Integumental permeability of amino acids in male and female schistosomes has also been measured. In unpaired (separated) schistosomes, a saturable, carrier-mediated selective transport system for acidic amino acids was demonstrated. The ascaricide drug kainic acid shared an affinity for acidic amino acid binding sites in the mammalian central nervous system, but not for the binding sites on the schistosome surface. The most dramatic observation, however, was that in copula the mated (male or female) schistosomes do not incorporate acidic amino acids, but may actually exclude these compounds i.e. this facilitated diffusion mechanism is induced or operational only when the worms are unmated. Thus these studies suggest that data obtained by the in vitro analysis of separated male and female schistosomes should be interpreted with caution, as conditions may not resemble those in vivo or in copula. This observation should perhaps be considered in all studies (immunological, physiological or pharmacological) of surface phenomena where data are obtained in vitro from unmated male and female schistosomes.

4.2 **Acetylcholine, Dopamine and Serotonin**

The somatic musculature of the male schistosome is composed, for the most part, of longitudinal and circular muscles. The longitudinal muscle is important in locomotor activity, while the circular muscle is essential for formation of the gynecophoral canal and thus maintenance of male-female pairing. An experimental preparation was recently developed which permitted
examination of some of the physiological and pharmacological properties of these two sets of muscles. The cholinergic agonist carbachol has comparable tension-relaxing effects on both circular and longitudinal muscles. When the muscle is bathed in a high Mg²⁺ medium, carbachol no longer has an effect on circular muscle but still causes relaxation of longitudinal muscle. Thus it appears that longitudinal muscle membrane possesses cholinergic receptors, whereas the circular muscle does not.

Serotonin(5-HT) induces rhythmic contractile activity in both sets of muscles and a high Mg²⁺ medium does not block this effect. Consequently, it appears that the 5-HT receptor sites are associated with both circular and longitudinal muscle membranes. The 5-HT antagonist metergoline is equally effective in blocking 5-HT effects on both sets of muscles. It also interferes with the stimulus responsiveness of both sets of muscles.

Dopamine has mixed effects on circular muscle but decreases longitudinal muscle tone. Its effects on circular muscle are still present after exposure to a high Mg²⁺ medium, but its effects on longitudinal muscle are significantly reduced. It is concluded that dopaminergic sites are probably associated with circular muscle but not with longitudinal muscle. The dopamine antagonist spiroperidal blocks the stimulus responsiveness of circular muscle but not that of longitudinal muscle.

From these studies it appears that all three of the major compounds previously implicated as possible neurotransmitters in schistosomes act to modulate activity in both circular and longitudinal muscles, but the manner in which they exert their effects is not identical. Only longitudinal muscle membrane appears to possess cholinergic receptors, only circular muscle membrane appears to possess dopaminergic receptors, while serotonergic receptors appear to be associated with both longitudinal and circular muscle membranes.

5. PHARMACOKINETIC STUDIES OF ANTISCHISTOSOMAL DRUGS IN ENDEMIC COUNTRIES

5.1 Philippines/Niridazole

Although niridazole has been used extensively for the chemotherapy of schistosomiasis, the pharmacokinetics of known metabolites of this drug have not been studied in man. Niridazole and six of its metabolites have now been quantitated by high-pressure liquid chromatography in sera of four male Filipino patients with mild S. japonicum infections who were 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, reaching peak concentrations of 0.9 ± 0.3 µg/ml of serum (mean ± S.D., n = 4) and 0.7 ± 0.1 µg/ml of serum within 1-4 hours. 4-ketoniridazole achieved peak serum levels one hour after the other oxidative metabolites in three of the four patients and was the predominant metabolite in the sera of all patients 6 to 10 hours after dosing. By 24 hours, both 4-ketoniridazole and 4-hydroxyniridazole had largely disappeared from the serum. Niridazole and three other oxidative metabolites, 4,5-dihydroxyniridazole, 5-hydroxyniridazole, and 4,5-dehydroniridazole, appeared within an hour in serum but failed to exceed 0.4 µg/ml; none of these compounds was detected in the 24-hour serum samples. The pharmacokinetic pattern of niridazole and the oxidative metabolites showed marked interindividual variation but was quite reproducible in the same individual studied 10 days later.

1-thiocarbamoyl-2-imidazolidinone (TCI) was analysed in serum samples by a different high-pressure liquid chromatographic procedure. This reductive metabolite attained maximal levels of 50 to 150 ng/ml of serum 6 to 12 hours after drug dosing and remained at 40% or more of its peak concentration even after 24 hours. The persistence of TCI in serum after a single oral dose of
niridazole is consistent with the proposed role for this metabolite in mediating the prolonged immunosuppressive side-effects of niridazole.

5.2 Sudan/Oxamniquine

Oxamniquine is a drug with a high first-pass metabolism which is demonstrated to be effective against Schistosoma mansoni infection and surprisingly well tolerated by patients with periportal fibrosis. Drugs with similar characteristics have high plasma levels in patients with liver disease.

Nine patients with active schistosomiasis infection, who also had confirmed periportal fibrosis, were studied together with five healthy volunteers. Similar doses of oxamniquine were administered to both groups in the fasting state. The kinetics of oxamniquine was studied from the plasma concentration of oxamniquine. The drug half-life and area under the plasma-concentration curve in the disease state were not statistically different from those of healthy volunteers. It is suggested that an increase in the gut metabolism of the drug compensated for the increase in the plasma levels expected in liver disease.

5.3 Sudan/Oltipraz

Oltipraz is a lipophilic schistosomicidal agent which was recently shown to be effective in field trials in the Sudan when given orally in a single dose to patients infected with *S. mansoni*. However, in some children it produced finger-tip pain. Careful analysis of the conditions of the trial revealed that the children who had taken the drug had a high-fat diet. A formal investigation was then carried out in Khartoum, using seven healthy volunteers. Oltipraz, 25mg/kg body weight, was administered to each volunteer under three different experimental conditions: fasting; a low-fat diet (less than 5%); and a high-fat diet (20% fat). The concentration of oltipraz in the blood was about 4 to 5 times greater when oltipraz was given with food compared with concentrations found in the fasting state. The fatty diet gave the highest concentrations. The area under the plasma-concentration curve, the plasma peak concentration, time to peak and drug half-life were also significantly higher in the drug-with-food experiment. Further studies to assess the effect of this increase in plasma concentration of oltipraz with food on the efficacy of the drug were performed: 14 patients with *S. mansoni* (mean egg output 509 ± 345 per gram of faeces) took 1.5 mg of oltipraz with food and 11 patients (mean egg output 510 ± 199 per gram of faeces) took similar doses without food. There was a significant difference (p < .05) in the antischistosomal efficacy of oltipraz when it was administered with food. There were no differences in the side-effects of the drug in the two groups. It was concluded that there was a considerable increase in the plasma concentration of oltipraz when the drug was administered with food, and that this increase was reflected in an increase in the antischistosomal activities of the drug.

6. RESISTANCE TO ANTISCHISTOSOMAL DRUGS

In recent years, a number of patients in Brazil infected with *S. mansoni* were not cured after treatment with oxamniquine or hycanthone and subsequent laboratory investigations revealed that the remaining parasites were resistant to the drugs used. Furthermore, a number of authors have reported that drug "pressure" applied in vivo to laboratory-maintained *S. mansoni* can significantly reduce susceptibility to the same and related drugs in the progeny.

Our current knowledge of drug resistance in schistosomes was summarized and the problem of defining the term drug resistance was discussed. In view of the increasing emphasis currently being placed on large-scale chemotherapy
for the control of schistosomiasis, it is essential that field and laboratory workers collaborate in further investigating and monitoring this situation.

In the laboratory, rodent/parasite models can be used to investigate a number of aspects of this area of chemotherapy. Experiments must include species of schistosomes other than *S. mansoni*, and particular attention must be given to the appropriate use of praziquantel, metrifonate, oxamniquine and oltipraz.

The approaches for evaluation include:

- the exposure of successive generations of schistosomes to subcurative therapy, in order to examine the capacity of the parasites to reduce their susceptibility to drugs;
- determination of the magnitude and stability of such changes;
- the phenomenon of side- and cross-resistance;
- the controlled combination of drug-resistant and susceptible strains of parasite of similar geographical origin, and subsequent passage: susceptibility to the drug in question and comparison with other antischistosomal drugs can be assessed after passage through several generations to determine whether any biological advantage occurs in drug-resistant strains.

A small number of laboratories could be equipped to monitor regularly the drug susceptibility of different species of schistosomes obtained in the field from endemic areas where large-scale treatment is undertaken. Such an approach will complement the basic laboratory studies and serve as an early indicator of changes which may occur in the response to antischistosomal drugs.

7. SUMMARY

This SWG successfully brought together research forces drawn from academic, public health and pharmaceutical backgrounds. In assessing from the meeting, there was a general consensus on the following points:

7.1 A review of the current drugs available for treatment of schistosomiasis revealed a general lack of knowledge concerning the molecular basis of the modes of action of antischistosomal compounds.

7.2 Patterns of metabolism of antischistosomal drugs in the host have been described and much more is known, particularly about recently introduced compounds. However, the pharmacology, antischistosomal efficacy and toxicity of the individual drug metabolites requires further research.

7.3 The acquisition, mechanisms of glucose transport and metabolism of hexoses by the schistosome are poorly understood. In particular, the amount of energy the organism derives in vivo from glycolysis, versus other energy-producing pathways, has yet to be defined.

7.4 Significant differences in host and parasite purine and pyrimidine metabolism were described. Such differences seem to present an opportunity for the targeted blockade of worm nucleotide metabolism. However, de novo or salvage pathways are not defined with certainty and additional studies are clearly needed.

7.5 Differences between circular and longitudinal schistosome muscle were analyzed in response to serotonin, dopamine, and carbachol. These and other reactions to transmitter agents suggest that the worm neuromuscular system is substantially different from that in the mammalian host.
7.6 There is a paucity of clinical metabolic studies and pharmacokinetic data on known antischistosomal compounds given to patients in different endemic regions. Most of the available data have been obtained from apparently healthy volunteers living in developed countries; it was suggested that infected persons might handle drugs differently.

7.7 In regard to resistance to drugs, evidence from field studies or laboratory strains is still rather sparse. High-level resistance to hycanthone has been shown, while oxamniquine resistance appears to be of a lower order. The problem of resistance has not been broadly studied, and the mechanisms are not defined.

8. COMMENTS AND RECOMMENDATIONS

This SWG did not make formal recommendations for research directions for the future. However, the following comments can be made:

8.1 The SWG on Schistosomiasis has encouraged and supported fundamental research in promising areas of the biochemistry and physiology of these parasites. The study of drug action and pharmacokinetics is still very much within the strategic plan. These efforts should continue to have high priority for research funding.

8.2 Although this SWG dealt with important aspects of schistosome physiology and biochemistry, many equally important areas were not included. For instance, a central issue in schistosomiasis is egg production. The meeting did not concern itself with such themes as ovigenesis or spermatogenesis, vitelline function, or the nutritional apparatus which supports protein synthesis in these reproductive functions.

8.3 A number of intriguing possibilities for the design of chemotherapeutic intervention were discussed. Examples are: mechanisms of purine or pyrimidine biosynthesis or salvage; the transport and metabolism of glucose; the possibility that the molecular basis of drug action might soon be discerned. New opportunities arising from scientific breakthroughs in other fields should be followed closely by the SWG on Schistosomiasis.

8.4 Comments on the possible modes of action of nitro compounds were highly pertinent, especially since several antischistosomal molecules have a nitro group. The collaboration of outside experts who are not engaged in research on schistosomiasis may provide important insights into fruitful areas of research.

9. LIST OF WORKING PAPERS*

ANDREWS, P. Metrifonate: Metabolism, Pharmacokinetics and Mode of Action.

ANDREWS, P. Praziquantel: Metabolism, Pharmacokinetics and Mode of Action.

CATTO, B. Effects of Praziquantel on Different Developmental Stages of Schistosoma mansoni.

COLES, G. Schistosoma Energy Metabolism.

*copies of the working papers are not available
CORNFORD, E. Intertegumental Transport Mechanisms Between Schistosomes.


EL KOUNI, M. Pathways of Pyrimidine Metabolism in Schistosoma mansoni.


JOLLES, G. Oltipraz: Pharmacokinetics, Metabolism and Mechanism of Action.

KAYE, B. Oxamniquine: Metabolism, Pharmacokinetics, Mode of Action.

MARSHALL, I. In vivo Models for Evaluation of Schistosome Resistance to Antischistosomal Drugs.

PAX, R. Acetylcholine, Dopamine and Serotonin – A Comparison of Their Physiological Roles in Control of Circular and Longitudinal Muscles in Schistosoma mansoni.

SHAPIRO, T. The Hexokinase of Schistosoma mansoni.

STRIEBEL, H. Amoscanate: Metabolism, Pharmacokinetics and Mode of Action.

TRACY, J. The Metabolic Basis for Differential Nitidazole Toxicity in Host and Schistosome.

VALENCIA, C. The Necessity of Pharmacological Studies to Understand the Anthelmintic Properties of Old and New Antischistosomal Drugs.

VAN DEN BERGH, S. Pathways of Energy Metabolism as Targets for Drugs.

WANG, C.C. Purine Metabolism in Schistosoma mansoni.

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