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COMMENTARY

PECULIAR TARGETS IN ANTHELMINTIC CHEMOTHERAPY

HUGO VAN DEN BOSSCHE
Laboratory of Comparative Biochemistry, Janssen Pharmaceutica, B-2340 Beerse, Belgium

Worm infections are one of the major problems in countries where sanitary conditions, environment and a low standard of living are conducive to the preservation and spreading of parasites.

Schistosomiasis is a parasitic disease caused by trematodes living in the blood. Calcified eggs of schistosomes [1] were identified in the kidneys of two mummies of the twentieth dynasty (1250–1000 B.C.). To-day it is one of the major tropical diseases that affect 200,000,000–300,000,000 people in Africa, Asia, South America and the Caribbean Islands [2]. This geographical spreading is best explained by Chernin's recipe: "warmth, water and poverty are the basic ingredients, add a few snails and a dash of faeces or urine and you have schistosomiasis" [3].

At least 20 million people living in tropical Africa, Yemen, Mexico and Central and South America are infected by *Onchocerca volvulus* [4]. *O. volvulus* is transmitted by *Simulium damnosum*, the blackfly. This blackfly breeds in fast-flowing water, for example around the sluice-gates of dams. Dams create also breeding-grounds for mosquitoes that transmit *Wuchereria bancrofti* and *Brugia malayi*. These filariae infect about 250,000,000 people in West, Central and East Africa, Egypt, Madagascar, South-East Asia, China, the Philippines, Central and South America [2, 5, 6] with resulting lymphangitis and in more severe forms elephantiasis [7].

Hookworm disease (ancylostomiasis), an infection by *Ancylostoma duodenale* or *Necator americanus*, occurs in 20–25 per cent of the world population [8]. This disease primarily occurs in agricultural areas where soil temperatures between 23 and 33° and a high humidity create ideal living conditions for the development of infectious larvae.

In tropical countries 70–90 per cent of the population should be infected with *Ascaris lumbricoides*, but *Ascaris* also regularly occurs in countries with a moderate climate [2, 9].

Just like *Ascaris* and the hookworm, *Trichuris trichiura* is a soil-transmitted nematode. *Trichuris* is a cosmopolite. Infections by *T. trichiura* are transmitted especially by coprophagy, which explains why trichuriasis so often occurs in institutions for the mentally insane.

Human behavioural patterns are often significantly related to man's risk of acquiring parasitic diseases. The nomadic Turkana people living in the desert of north-western Kenya is one of the population groups in the world infected most heavily with *Echinococcus granulosus* [10]. The adult *Echinococcus* lives in the intestine of dogs and other carnivores; sheep, camels, goats act as intermediate hosts by ingesting grass or water contaminated with eggs. The cycle is completed when dogs eat livestock offal containing one of the resultant hydatid cysts. Man becomes infected by ingesting eggs present in water, soil or on his hands after stroking a diseased dog. The Turkana people are fond of their dogs which are left to look after young infants whom they lick and care for as they would their own puppies. The people rarely eat meat themselves; however, the dogs devour even the most diseased animals. This close interaction between man, dog and livestock, plus lack of water for washing, provides ideal conditions for *E. granulosus* [11].

Hydatidosis is not limited to the north-western Kenya. This zoonosis constitutes a public health problem of nearly global dimensions. For example, it is a well-known phenomenon in Europe [12].

Cattle infected with *Cysticercus bovis* cysts, the intermediate stage of the human tapeworm, *Taenia saginata*, represent another example of an important human health and economic problem.

A parasitic disease common in countries where raw fish is considered a delicacy or where fish is often the only source of animal protein is clonorchiasis. This disease is due to infestation with the Chinese liver fluke, *Clonorchis sinensis*. Clonorchiasis is prevalent in China, Vietnam, Korea and Taiwan [9]. Complete protection is achieved simply by cooking the freshwater fish. However, as pointed out by Schmidt and Roberts [13] "it would be a futile exercise to try to get millions of people to change centuries-old eating habits".

People who eat undercooked or 'underfrozen' bear, walrus, wild or domestic pig meat are at risk of becoming infected with *Trichinella spiralis*.

These are only a few examples of zoonosis, vector- or soil-transmitted worm infestations.

Therapy

Fortunately, several anthelmintics (chemical structures are presented in Table 1) are available to reduce the incidence of a great part of the worm diseases. A few examples to illustrate this follow. Trimestrial administration of levamisole to heavily infected African school children has almost led to a complete eradication of *Ascaris*, whereas the output of hookworm eggs and *Strongyloides stercoralis* larvae was reduced by 77 and 87 per cent, respec-

Table 1. Chemical structures

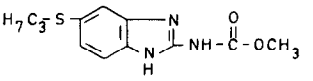
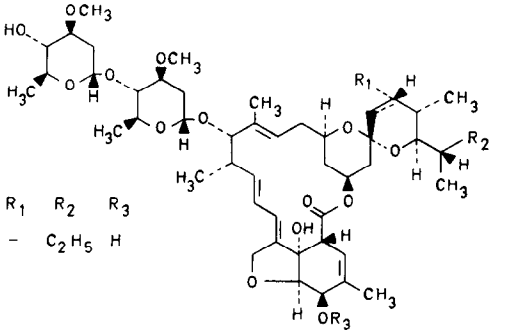
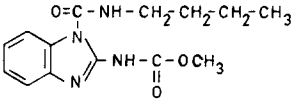
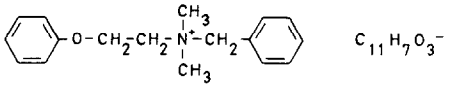
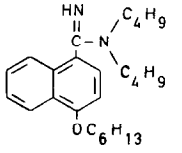
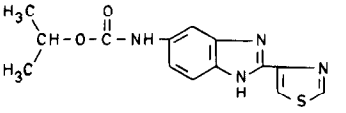
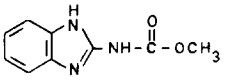
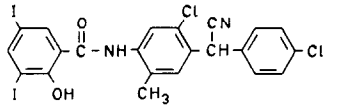
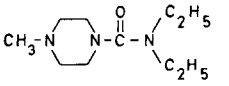
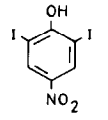
NAME	STRUCTURE
ALBENDAZOLE	
AVERMECTINE B1a	
BENOMYL	
BEPHENIUM hydroxynaftoate	
BUNAMIDINE	
CAMBENDAZOLE	
CARBENDAZIN	
CLOSANTEL	
DIETHYLCARBAMAZINE	
DISOPHENOL	

Table 1—*Continued*

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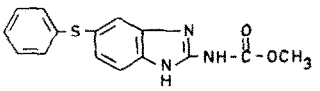
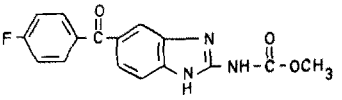
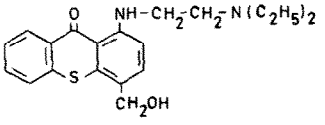
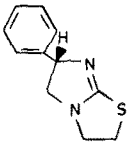
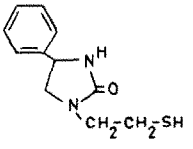
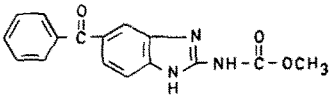
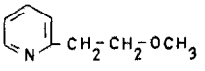
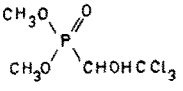
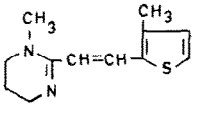
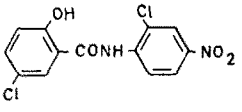
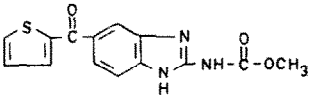
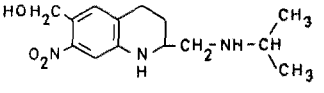
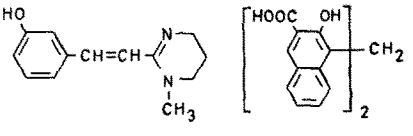
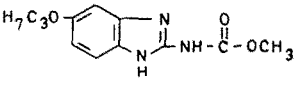
FENBENDAZOLE	
FLUBENDAZOLE	
HYCANTHONE	
LEVAMISOLE	
OMPI	
MEBENDAZOLE	
METHYRIDINE	
METRIFONATE	
MORANTEL	
NICLOSAMIDE	
NOCODAZOLE	
OXAMNIQUINE	
OXANTEL PAMOATE	
OXIBENDAZOLE	

Table 1—*Continued*

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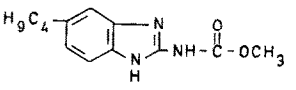
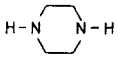
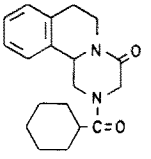
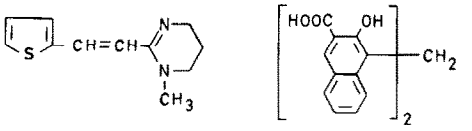
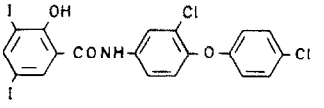
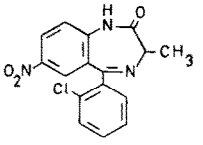
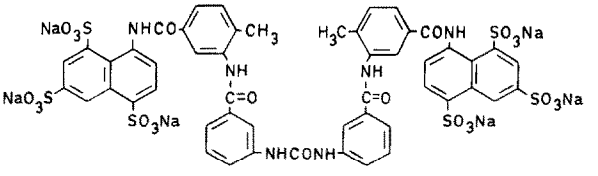
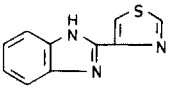
PARBENDAZOLE	
PIPERAZINE	
PRAZIQUANTEL	
PYRANTEL PAMOATE	
RAFOXANIDE	
Ro 11-3128	
SURAMIN	
THIABENDAZOLE	

tively, at the end of the year [14]. It is of interest to note that levamisole, besides its anthelmintic activity, also restores host defence in immunocompromised patients. For this reason levamisole is being used in a variety of immunodeficiency diseases, including rheumatoid arthritis, where it is thought to act through the free sulphydril group of its major metabolite OMPI [15, 16].

Broad-spectrum anthelmintics, like mebendazole [17] and flubendazole [18], are available for large scale chemotherapy of the soil-transmitted nematodal infections caused by the classic triad: *Ascaris*, hookworms and *Trichuris*. Mebendazole is successful in the treatment of intestinal capillariasis [19] and shows great promise in the treatment of trichinosis [20–22]. Mebendazole also may be the opening to the chemotherapy of hydatid disease [23–26].

Anticestodal drugs of special interest are niclosamide and bunamidine. The novel antischistosomal drug, praziquantel, proves to be effective against mature cestodes in animals and man [27, 28]. This pyrazinoisoquinolinone derivative is active against

Schistosoma mansoni, *S. haematobium* and *S. japonicum*. The activity against *S. japonicum* is noteworthy. If this can be confirmed in further clinical trials, population-based chemotherapy of *S. japonicum* by a non-metal anthelmintic may become a reality in the future [29, A. Davis, personal communications].

Oxamniquine is a promising single-dose oral agent for the treatment of *S. mansoni* infections [30]. In South America, this hydroxytetrahydroquinoline derivative is considered to be the drug of choice against this infection [31].

The cheap organophosphorus cholinesterase inhibitor, metrifonate, is effective in the treatment of *S. haematobium* but lacks activity in humans infected with *S. mansoni* [32].

Thus some effective compounds are currently available for treatment of schistosomiasis. However, there is still need for a cheap broad-spectrum anti-schistosomal drug.

Promising results are obtained with relatively high doses of praziquantel in the treatment of clonorchiasis, paragonimiasis and metagonimiasis [33]. No satisfactory agent was available before. Certainly much more studies are needed to achieve an inexpensive, simple treatment of these distomiases.

In 1948, Hewitt *et al.* [34] discovered significant antifilarial action in a series of piperazine derivatives. One of these compounds, diethylcarbamazine (DEC), is still the only drug known that will reliably kill the microfilariae of the mosquito-transmitted *W. bancrofti* and *B. malayi* [31]. DEC also is microfilaricidal against *O. volvulus* [35].

Suramin, a compound introduced by Bayer in 1920, is macrofilaricidal against *O. volvulus* but its toxicity is such as to preclude its use in both brugian and bancroftian filariasis [36]. DEC and suramin are far from ideal. They can only be administered under strict medical supervision [37]. Therefore a new drug is certainly needed. Recently, filaricidal activity was found in some benzimidazole carbamates, e.g. mebendazole, flubendazole, albendazole, and in avermectins [38]. Based on these results, clinical studies with mebendazole and flubendazole are proceeding.

Most, if not all, of the available anthelmintics had their origin in a rational exploitation of massive empirical screening. I believe that for a given time, this approach will keep playing an important role in the search for new tools for better control of worm populations. However, it is difficult to imagine that the harvest of new information originating from immunologists, molecular biochemists, cell biologists or from what C. de Duve calls, the enthusiasts of the 'new biology' [39] will not fertilize, sooner or later, the search for tools that interfere with the host–parasite interface. Therefore, I also believe that screening will be increasingly helped by and even orientated towards the so-called rational approach.

Comparative biology and mode of action studies

Comparative biology studies are an essential part of investigations relevant to chemotherapy. These studies are aimed at uncovering differences in metabolism, structure and function of cell membranes, forces that govern the development and differentiation of tissues, electrophysiological processes and at elucidating the mechanisms that the helminths may use to escape the host immune response. Another type of investigation relevant to chemotherapy is concerned with the question "how does that anthelmintic work?". Such studies may provide insight into the way drugs reach and affect their target in the parasite. This not only opens the way to new screening systems but, and perhaps at the moment still more important and urgent, it also increases our knowledge of the molecular biology of the invading organisms.

In this commentary we will focus on general sites of action of some anthelmintics.

Anthelmintics and neuromuscular systems

The survival of parasitic helminths greatly depends on their ability to remain *in situ* when exposed to peristalsis in the case of intestinal parasites or to the flow of lymph or blood for some systemic helminths [40].

There is sufficient evidence available to demonstrate that piperazine induces reversible paralysis by acting on the neuromuscular system (for reviews see refs. 41 and 42). Summing up, piperazine has been found to increase the resting potential of the *Ascaris* muscle. Since the degree of muscle hyperpolarization induced by piperazine depends upon the extra-cellular chloride concentration, del Castillo and colleagues draw the conclusion that the hyperpolarization is brought about by a specific increase in the permeability of the cell surface membrane to chloride ions [41].

The experiments of Kaushik *et al.* [43] on *Ascaridia galli* and those of Natarajan *et al.* [44] on micro- and macrofilariae of *Breinlia sergenti* indicate that the piperazine derivative, diethylcarbamazine, also affects parasite motility.

However, it is not certain whether this piperazine-like effect is at the basis of diethylcarbamazine's antifilarial activity. In patients infected with *O. vulvulus* and treated with diethylcarbamazine, acute nests of inflammation around dead and degenerating microfilariae are found in the dermis [45, 46]. Recent electron microscopic research has shown that the cuticle of the microfilariae collected from patients after treatment with this piperazine derivative is changed [47]. On the basis of these observations, the following hypothesis was advanced: diethylcarbamazine would alter the microfilariae to such an extent that they are regarded by the host as alien bodies and hence can be destroyed by the host's defence system [47]. This has become an appealing hypothesis, especially after the study of Martinez-Palomo, in which it was shown that the immunogenic determinants of the *Onchocerca* microfilaria are protected by an acellular cuticle [48].

Anthelmintics whose interference with the neuromuscular system certainly represents an important facet of their mode of action are tetramisole and its levorotatory isomer levamisole.

In vitro, levamisole is immediately and almost completely absorbed by *Ascaris suum* via a transcuticular mechanism [49]. This is consistent with the rapid occurrence of a levamisole-induced spastic contraction of the *Ascaris* muscle [50]. In incubated larval and adult nematodes, the presence of lev-

amisole causes a contraction followed by tonic paralysis [42]. Whether this effect is reversible depends on the worm species, the stage in the cycle, the concentration of levamisole and the incubation circumstances [42, 51]. Pharmacological experiments have demonstrated that levamisole acts as a ganglion-stimulating compound [50, 51] and that afterwards it induces a neuromuscular inhibition of the depolarizing type [51]. By using isolated *Ascaris* muscles it could be shown that levamisole decreases the resting potential from a normal −30 mV to −15 mV (unpublished results).

Bephenium hydroxynaphtoate, methyridine, pyrantel and its analogues morantel and oxantel are, as levamisole, anthelmintics that paralyse nematodes [41, 42]. Pyrantel causes depolarization of muscle cells and spastic contraction of *A. suum* which is inhibited by two cholinergic blocking agents, tubocurarine and piperazine [51, 52]. *Nippostrongylus brasiliensis* is protected from levamisole-induced spastic paralysis by pyrantel and methyridine, suggesting that levamisole, pyrantel and methyridine act at the same site [51, 53]. However, unlike the pyrantel-induced contraction, the levamisole-induced one is not inhibited by piperazine and tubocurarine, suggesting pharmacological differences [51, 52].

Much more knowledge of the neurophysiology and underlying biochemical processes is required before the mode of action of the above-mentioned anthelmintics can be better understood.

Not only antinematodal drugs affect parasite motility, important antischistosomal drugs also do. The organophosphorus compound, metrifonate, has a reversible paralysing effect on *S. mansoni* and *S. haematobium* [54]. This paralysis originates from cholinesterase inhibition [55]. Paralysing *S. haematobium* causes the flukes to relax, thus resulting in a shift to the lungs from which relocation to the urinary bladder veins may be impossible. In the case of *S. mansoni* paralysis will result in a 'hepatic shift'. As the effect of the drug diminishes, the *S. mansoni* can relocate itself in the mesenteric veins [40]. This may be at the origin of the lower susceptibility.

Numerous clinical trials with the thioxanthenone derivative, hycanthone, indicate high efficacy against both *S. mansoni* and *S. haematobium*. It is a non-competitive inhibitor of the schistosomal acetylcholinesterase activity [56]. The effectiveness of such inhibition is related to the species. It is very weak against eel or bovine esterase and relatively strong against that of schistosomes [56, 57]. Physostigmine, a strong inhibitor in mammalian systems, is much less active against the cholinesterase in schistosomes [56]. Although the mechanism of the therapeutic action of hycanthone may not be related to this acetylcholinesterase inhibitory capacity, the differences between parasite and host enzymes may be exploited for new antischistosomal compounds.

The antischistosomal activity of hycanthone has also been related to inactivation of the acetylcholine receptor site [56, 58]. It was found that hycanthone blocks the paralysis of *S. mansoni* that normally occurs when carbachol (carbamylcholine) is applied to worms [56]. Using a histochemical technique in which schistosomes are labelled with a dansylated analogue of acetylcholine, hycanthone was found to be an effective blocker of this labelling. This results suggest that hycanthone has an anticholinergic activity in schistosomes. However, hycanthone has no cholinomimetic or anticholinergic activity in mammals. This, together with the fact that atropine and *d*-tubocurarine, two of the most active cholinergic blocking agents on mammalian synapses, only affect the neuromuscular activity of trematodes at high concentrations [40, 56, 59], further proves that the acetylcholine receptor may be substantially different from those found in mammalian systems.

Oxamniquine is another antischistosomal drug that, like hycanthone, possesses an alkylamino ethylamino group *para* to a hydroxymethyl group. Like hycanthone, oxamniquine has anticholinergic activity [60]. However, here too, many more studies are needed.

Other drugs whose primary target could be the neuromuscular system are praziquantel and the benzodiazepine derivative, Ro 11-3128. At low concentrations these drugs will produce a rapid and marked spastic paralysis of the musculature of schistosomes [61–63]. This drug-induced spastic paralysis appeared to be independent of any action that the drug may have on *S. mansoni's* neurotransmitter receptors. Thus it was observed that the praziquantel or Ro 11-3128-induced response could not be blocked by compounds such as dopamine, 5-hydroxytryptamine, carbachol, spiroperidol, bromlysergic acid diethylamide and atropine. All of these drugs are known to act at neuroreceptive sites in *S. mansoni* [63].

The addition of praziquantel or Ro 11-3128 to *S. mansoni* preparations causes a rapid depolarization of the muscle cells of the schistosome [64]. Assuming that the resting membrane potential of *S. mansoni* muscle cells is maintained in a manner similar to that in other animals, one would predict a sodium ion influx and a potassium ion efflux in these cells. The results obtained by Pax *et al.* [63] show that both drugs stimulate the uptake of $^{22}Na^{+}$ but inhibit the uptake of $^{42}K^{+}$. A rapid depolarization can also be explained by an increased permeability for calcium ions. Uptake studies of calcium by male schistosomes indicate that both compounds stimulate the influx of $^{45}Ca^{2+}$ [63]. These studies indicate that the interference with inorganic ion transport mechanisms by Ro 11-3128 and praziquantel may be related to their antischistosomal action.

Praziquantel at concentrations as low as 1 ng/ml also stimulates movement in hymenolepid cestodes and pre-adult *Echinococcus* [65]. Concentrations of 0.01–0.1 and 1–10 μg/ml cause contraction in the relaxed state and paralysis in the contracted state within 10 min and 10–30 sec, respectively [65].

'Metabolic blockers'

Praziquantel affects glucose uptake and lactate production [65–66]. However, this may be secondary to the effects on the musculature.

It has also been suggested that the praziquantel-induced inhibition of glucose uptake by *Hymenolepis diminuta* might be mediated through modulation of mitochondrial enzymes [65]. Praziquantel diffuses into the *Ascaris* muscle mitochondria where it

inhibits the various mitochondrial NADH-oxidizing activities including NADH oxidase and NADH fumarate reductase systems [65, 67]. Since in *Ascaris* muscle mitochondria the major respiratory-chain-linked phosphorylation activity is related to a NADH-linked reduction of fumarate to succinate, this inhibitory effect will result in a decreased ATP synthesis.

The fumarate reductase system is an essential component of carbohydrate metabolism in many parasitic stages of helminths (for reviews see refs. 42, 68–70). It is well known that the succinate dehydrogenase–fumarate reductase complex from aerobic organisms has a high rate of succinate oxidation relative to fumarate reduction. For mammalian cells this ratio is about 60 [65]. On the other hand, the succinate oxidation/fumarate reduction for the facultative anaerobe *Propionibacterium* is about 3 [71], whereas for the obligate anaerobe *Micrococcus lactilyticus*, a ratio of 0.03 is found [72]. This ratio is about 8.3 in *Trichinella pseudospiralis*, thus resembling that of the facultative anaerobe [73]. Similar ratios were found with preparations of *Syphacia muris* [74], *Haemonchus contortus* larvae [75] and *F. hepatica* [76].

This substantial difference between the energy metabolism in mitochondria from mammalian or worm sources provides an important target for chemotherapeutic attack.

The first anthelmintics found to inhibit the fumarate reductase system in *Ascaris* muscle mitochondria were tetramisole and its isomer levamisole [42, 67, 77–79]. It is interesting to note that the laevoisomer proved to be a more potent inhibitor of the enzyme system, a fact compatible with its more potent anthelmintic action [79]. Tetramisole also inhibits the fumarate reductase system in *A. galli*, *Toxocara cati*, *Dictyocaulus viviparus*, *H. contortus* larvae and adults, *T. spiralis* and *F. hepatica* [42].

In addition to praziquantel, tetramisole and levamisole, thiabendazole cambendazole, morantel tartrate and disophenol also seem to affect the fumarate reductase system [42, 80]. The fumarate reductase from thiabendazole-resistant *Haemonchus contortus* is resistant to thiabendazole and cambendazole [81, 82]. This apparently supports the suggestion that both benzimidazoles act by inhibiting the fumarate reduction system. However, cross-resistance occurs between thiabendazole and mebendazole in some *H. contortus* strains [83, 84], although mebendazole does not inhibit the reduction of fumarate. Furthermore, the benzimidazole-tolerant strains did not so far show cross-resistance to the non-benzimidazoles levamisole, morantel and disophenol [83, 85–87]. This suggests that the effect of the described anthelmintics on the fumarate reductase system in parasitic helminths represents only one aspect of their mode of chemotherapeutic action. Studies of Köhler and Bachmann [67] indicate that levamisole, thiabendazole and praziquantel inhibit various NADH pathways in *Ascaris* muscle mitochondria. However, succinate oxidase and succinate–cytochrome *c* reductase activities were only significantly inhibited by thiabendazole. This would indicate at least an additional site of inhibition by thiabendazole.

A better knowledge of the organization and function of mitochondrial electron transport in parasitic helminths may provide us with a chemoreceptor which has no analogue in the organs of the host and may thus lead to the development of more selective inhibitors.

Mebendazole is capable of exerting a number of effects which, taken together, may account for its broad spectrum anthelmintic activity (for reviews see refs. 42, 88, 89). Progressive time-related morphological changes in cestodes and nematodes after treatment of the hosts with mebendazole have been reported [90–92]. The first effect observed is a disappearance of the cytoplasmic microtubules of the tegumental or intestinal cells of the parasitic worms. Similar results were obtained with the fluorine analogue of mebendazole, flubendazole [91]. The disappearance of microtubules and the subsequent block in transport of secretory vesicles may lead to impaired coating of the membranes, followed by a decreased digestion and absorption of nutrients. In fact, mebendazole affects *in vitro* and *in vivo* the glucose uptake by nematodes and cestodes. This is followed by an increased utilization of endogenous glycogen and/or decreased synthesis of this polysaccharide [42, 93]. Mebendazole was also found to have a number of effects on energy metabolism in helminths. For example, it affects malate-induced phosphorylation in *Ascaris* mitochondria [42] and *in vitro* and *in vivo* it decreases ATP synthesis in *Moniezia expansa* [93, 94].

Another benzimidazole carbamate, fenbendazole, also interferes with the absorption of glucose and especially with the incorporation of glucose into glycogen by *Ascaris* [95]. Fenbendazole shares with mebendazole and other benzimidazole derivatives, e.g. albendazole, nocodazole, oxibendazole, parbendazole, benomyl, carbendazin and cambendazole, the ability to bind to mammalian tubulin and to inhibit the assembly of microtubules [96–98]. At high concentrations, thiabendazole also affects the polymerization of bovine brain tubulin. Fifty per cent inhibition was achieved at 5.49×10^{-4} M [98]. Thiabendazole also binds to fungal tubulin [99], a property common to carbendazin [99] and nocodazole [100]. A high degree of homology between fungal and mammalian tubulin can be derived from their property to co-polymerize [100, 101]. However, the inhibitor constant K_i of nocodazole for fungal tubulin has shown to be 8×10^{-8} M [102]. This is about 120 times lower than the K_i obtained for rat brain tubulin [96], therefore indicating that distinct differences may be present. In current work, P. A. Friedman finds that the inhibition constant of mebendazole for *Ascaris* embryonic tubulin is about 380 times less than that of mebendazole for brain tubulin (Friedman, personal communication). These observations further suggest that tubulin from different phylogenetic sources may have differential binding affinities and may explain, at least partly, the selective action of the benzimidazole derivatives. However, studies on tubulin from *Ascaris* intestinal cells are needed before definite conclusions can be drawn. A better characterization and a greater understanding of the biochemistry of helminth tubulin may lead to the finding of new targets for specific chemotherapy.

Other examples of important targets are the phosphofructokinase, the limiting enzyme of the glycolytic pathway of schistosomes (for review see refs. 40, 60) and the fixation of CO_2 into phosphoenolpyruvate, a reaction catalysed by the phosphoenolpyruvate carboxykinase (for reviews see refs. 70, 103, 104). The latter enzyme is a key enzyme in the catabolism of carbohydrates in, for example, *Ascaris* [105], *F. hepatica* [106] and *M. expansa* [93]. In mammalian cells this enzyme is biologically active in the direction of phosphoenolpyruvate formation. Here it is a key enzyme in the biosynthesis of glycogen.

A series of important anthelmintics belongs to the salicylanilides, of which niclosamide and rafoxanide are well-known examples. A recently developed salicylanilide is closantel, an intramuscularly and orally active anthelmintic drug, efficacious in cattle and sheep against *F. hepatica*, *F. gigantica*, bloodsucking nematodes and the insect larval stages of *Oestrus ovis*, *Dermatobia sp.* and *Hypoderma sp.* (D. Thienpont *et al.*, personal communication). Experimental evidence obtained thus far indicates that closantel, *in vitro* and *in vivo*, disturbs the mitochondrial phosphorylation in *F. hepatica* and can be classified as an antiparasitic hydrogen ionophore [107].

Although this salicylanilide also uncouples mammalian mitochondria *in vitro*, it has no effect *in vivo* on liver mitochondria from uninfected rats and on heart mitochondria from both normal and infected rats [107]. Liver mitochondria isolated from rats with adult liver flukes seemed to be completely uncoupled. When these rats were injected i.m. with 5 mg closantel/kg body wt, the liver mitochondria returned to the more tightly coupled state and became fully comparable to controls [107].

Since mitochondria from the parasite and host are equally sensitive *in vitro* to closantel and other salicylanilides, whereas *in vivo* at therapeutic doses only the parasite mitochondria are affected, it would be fair to assume that the selectivity is associated with binding to proteins and distribution within the host and differential absorption by *Fasciola* [42, 80]. This certainly calls attention to the role of pharmacokinetic studies in the development of anthelmintics. Here also much groundwork remains to be done.

Conclusion

The studies discussed here indicate that the door is ajar for a glance at the physiological world of parasitic helminths. However, many more questions than answers are visible. In fact, thus far the field of parasitic diseases has received only crumbs of the "new biology's cake".

The World Health Organization has dedicated its efforts to the attainment, by the year 2000, of a level of health by all peoples of the world that will permit them to lead a socially and economically productive life. The provision of primary health care is seen as the fundamental vehicle for achieving this goal [108].

Although socioeconomic improvement and a more rational utilization of existing effective drugs will already be of great help, we have to kick open the door as far as possible to meet the challenge.

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REFERENCES

1. M. A. Ruffer, *Br. med. J.* **1**, 16 (1910).
2. M. Gentilini, B. Duflo, B. Lagardère and M. Danis, *Médecine Tropical*. Flammarion Médecine-Sciences, Paris (1977).
3. E. Chernin, *Tropical Medicine from Romance to Reality* (Ed. C. Wood), p. 114. Academic Press, London (1978).
4. *Tech. Rep. Ser. Wld. Hlth. Org.*, No. 597 (1976).
5. *Tech. Rep. Ser. Wld. Hlth. Org.*, No. 542 (1974).
6. P. G. Janssens and P. J. Zuidema, *Importziekten*. Stafleu's Wetenschappelijke Uitgeversmaatschappij, Leiden (1973).
7. *WHO Chron.* **33**, 399 (1979).
8. A. Davis, *Drug Treatment in Intestinal Helminthiasis*. World Health Organization, Geneva (1973).
9. K. S. Warren and A. A. F. Mahmoud, *Geographic Medicine for the Practitioner*. University of Chicago Press, Chicago (1978).
10. *Tech. Rep. Ser. Wld Hlth Org.*, No. 637 (1979).
11. R. Palmer, *World Medicine* (Feb. 8), 39 (1978).
12. R. M. Matossian, M. D. Richard and J. D. Smyth, *Bull. Wld Hlth Org.* **55**, 499 (1977).
13. G. D. Schmidt and L. S. Roberts, *Foundations of Parasitology*. C. V. Mosby, St. Louis (1977).
14. F. Gatti, F. Krubwa, J. Vandepitte and D. Thienpont, *Annls Soc. belge Méd. trop.* **52**, 19 (1972).
15. J. Symoens and Y. Schuermans, *Clin. rheum. Dis.* **5**, 603 (1979).
16. A. G. Mowat and T. L. Vischer, *Levamisole. "Immunomodulation"—A New Approach to Basic Therapy of Rheumatoid Arthritis*. Eular, Basle (1979).
17. J. S. Keystone and J. K. Murdoch, *Ann. intern. Med.* **91**, 582 (1979).
18. J. P. Nozais, *Méd. Afr. noire* **25**, 473 (1978).
19. C. N. Singson, T. C. Banzon and J. H. Cross. *Am. J. trop. Med. Hyg.* **24**, 932 (1975).
20. D. Thienpont, *La nouv. Presse Méd.* **5**, 197 (1976).
21. J. J. Sonnet and D. Thienpont, *Acta clin. belg.* **32**, 297 (1977).
22. M. Vujošević, A. Kostić, L. Ilić, S. Žerjav and S. Mišic, 4th Yugoslav and Italian Meeting on Infectious Diseases, Perugia (1977).
23. A. Bekhti, J.-P. Schaaps, M. Capron, J.-P. Dessaint, F. Santoro and A. Capron, *Br. med. J.* **2**, 1047 (1977).
24. T. C. Beard, M. D. Richard and H. T. Goodman, *Med. J. Aust.* **1**, 633 (1978).
25. P. Kern, M. Dietrich and K. J. Volkmer, *Tropenmed. Parasit.* **30**, 65 (1979).
26. A. Bekhti, Thésis, University of Liège (1979).
27. H. Thomas and P. Andrews, *Pestic. Sci.* **8**, 556 (1977).
28. H. Thomas and R. Gönnert, *Res. vet. Sci.* **24**, 20 (1978).
29. R. Gönnert and P. Andrews, *Z. ParasitKde.* **52**, 129 (1977).
30. M. J. Miller, in *Progress in Drug Research* (Ed. E. Jucker), Vol. 20, p. 433. Birkhäuser, Basel (1977).
31. W. Peters, in *Pharmaceutical for Developing Countries*, p. 59. Div. Int. Health, Institute of Medicine, National Academy of Science, Washington (1979).
32. L. M. Werbel, in *Topics in Medicinal Chemistry* (Eds. J. L. Rubinowitz and R. M. Meyerson), Vol. 3, p. 125. Wiley, New York (1970).
33. Hang-Jong Rim, *Short communications ICOPA* IV, Section D, Warszawa, p. 24 (1978).
34. R. I. Hewitt, E. White, S. Kushner, W. S. Wallace, H. W. Stewart and Y. Subbarow, *Ann. N.Y. Acad. Sci.* **50**, 128 (1948).
35. F. Hawking, *Wld Hlth Org.* ONCHO **78**, 142 (1978).

36. D. A. Denham, *J. Helminth.* **53**, 175 (1979).
37. M. L. Bazin, *Wld Hlth* 5 (1978).
38. WHO Scientific Working Group on Filariasis Report No.: TDR/FIL—SWG (3) 79.3 (1979).
39. C. de Duve, *Annls Soc. belge Méd. trop.* **58**, 281 (1978).
40. T. E. Mansour, *Science* **205**, 462 (1979).
41. J. del Castillo, in *Chemical Zoology* (Eds. M. Florkin and B. T. Scheer), Vol. 3, p. 521. Academic Press, New York (1969).
42. H. Van den Bossche, in *Biochemistry of Parasites and Host–Parasite Relationships* (Ed. H. Van den Bossche), p. 553. Elsevier/North Holland Biomedical Press, Amsterdam (1976).
43. R. K. Kaushik, J. C. Katiyar and A. B. Sen, *Indian J. med. Res.* **62**, 1367 (1974).
44. P. N. Natarajan, V. Zaman and T. S. Yeah, *Int. J. Parasit.* **3**, 803 (1973).
45. M. Martinez-Baez, R., *Revta. Int. Salubr. Enferm. trop. Méx.* **10**, 95 (1949).
46. F. Hawking, *Br. med. J.* **1**, 992 (1952).
47. D. W. Gibson, D. M. Connor, H. L. Brown, H. Fuglsang, J. Anderson, B. O. L. Duke and A. A. Buck, *Am. J. trop. Med. Hyg.* **25**, 74 (1976).
48. A. Martínez-Paloma, *J. Parasit.* **64**, 127 (1978).
49. H. L. E. Verhoeven, G. Willemsens and H. Van den Bossche, in *Biochemistry of Parasites and Host–Parasite Relationships* (Ed. H. Van den Bossche), p. 573. Elsevier/North Holland Biomedical Press, Amsterdam (1976).
50. J. M. Van Nueten, in *Comparative Biochemistry of Parasites* (Ed. H. Van den Bossche), p. 101. Academic Press, New York (1972).
51. G. C. Coles, J. M. East and S. N. Jenkins, *Gen. Pharmac.* **6**, 309 (1975).
52. M. L. Aubry, P. Cowell, M. J. Davey and S. Shevde, *Br. J. Pharmac.* **38**, 332 (1970).
53. G. C. Coles, J. M. East and S. N. Jenkins, *Experientia* **30**, 1265 (1974).
54. D. A. Denham and R. J. Holdsworth, *Trans. R. Soc. trop. Med. Hyg.* **65**, 696 (1971).
55. E. Bueding, C. L. Lin and S. H. Rogers, *Br. J. Pharmac.* **46**, 480 (1972).
56. A. W. Senft and G. R. Hillman, in *Biochemistry of Parasites and Host–Parasite Relationships* (Ed. H. Van den Bossche), p. 619. Elsevier/North Holland Biomedical Press, Amsterdam (1976).
57. G. R. Hillman, *Short communication ICOPA* IV, Section D, Warszawa, p. 9 (1978).
58. G. R. Hillman, W. B. Gibler and J. B. Anderson, *Am. J. trop. Med. Hyg.* **26**, 238 (1977).
59. G. R. Hillman, A. M. Gibler and J. W. Anderson, *J. Pharmac. exp. Ther.* **207**, 992 (1978).
60. N. M. Woolhouse, *Biochem. Pharmac.* **28**, 2413 (1979).
61. H. R. Stohler, in *Current Chemotherapy* (Eds. W. Siegenthaler and R. Lüthy), Vol. 1, p. 147. American Society for Microbiology, Washington (1978).
62. G. C. Coles, in *Perspectives in the Control of Parasitic Disease in Animals in Europe* (Eds. D. W. Jolly and J. M. Somerville), p. 53. Association of Veterinarians in Industry, Windlesham, Surrey (1977).
63. R. Pax, J. L. Bennett and R. Fetterer, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.* **304**, 309 (1978).
64. R. A. Pax, R. E. Fetterer and J. L. Bennett, *Short communication ICOPA* IV, Section D, Warszawa, p. 12 (1978).
65. P. Andrews and H. Thomas, *Tropenmed. Parasit.* **30**, 391 (1979).
66. P. Andrews, *Short communication ICOPA* IV, Section D, Warszawa, p. 5 (1978).
67. P. Köhler and R. Bachmann, *Molec. Pharmac.* **14**, 155 (1978).
68. T. von Brand, *Biochemistry of Parasites*, 2nd Edn, p. 134. Academic Press, New York (1973).
69. J. Barrett, in *Biochemistry of Parasites and Host–Parasite Relationships* (Ed. H. Van den Bossche), p. 67. Elsevier/North Holland Biomedical Press, Amsterdam (1976).
70. F. F. Soprunov, *Biochemie der Helminthen* I. *Energiehaushalt der Helminthen. Parasitologische Schriftenreiche* (Eds. W. Eichler, K. Odening and F. F. Soprunov), Band 23. VEB Gustav Fisher, Jena (1978).
71. F. J. S. Lara, *Biochim biophys. Acta* **33**, 565 (1959).
72. M. G. Warringa, O. H. Smith, A. Guiditta and T. P. Singer, *J. biol. Chem.* **230**, 97 (1958).
73. K. Boczon, *Short communication ICOPA* IV, Section D, Warszawa, p. 10 (1978).
74. H. Van den Bossche, J. Schaper and M. Borgers, *Comp. Biochem. Physiol.* **38B**, 43 (1971).
75. C. W. Ward and P. J. Schofield, *Comp. Biochem. Physiol.* **23**, 335 (1967).
76. J. Barrett, *Parasitology* **76**, 269 (1978).
77. H. Van den Bossche and P. A. J. Janssen, *Life Sci.* **6**, 1781 (1967).
78. H. Van den Bossche and P. A. J. Janssen, *Biochem. Pharmac.* **18**, 35 (1969).
79. H. Van den Bossche, in *Comparative Biochemistry of Parasites* (Ed. H. Van den Bossche), p. 117. Academic Press, New York (1972).
80. G. C. Coles, *Pestic. Sci.* **8**, 536 (1977).
81. M. F. Malkin and R. M. Camacho, *J. Parasit.* **58**, 845 (1972).
82. R. K. Prichard, *Int. J. Parasit.* **3**, 409 (1973).
83. M. L. Colglazier, K. C. Kates and F. D. Enzie, *J. Parasit.* **61**, 778 (1975).
84. J. D. Kelly, *Short communication ICOPA* IV, Section D, Warszawa, p. 15 (1978).
85. V. J. Theodorides, G. C. Scott and M. Laderman, *Am. J. vet. Res.* **31**, 859 (1970).
86. K. C. Kates, M. L. Colglazier, F. D. Enzie, I. L. Lindahl and G. Samuelson, *J. Parasit.* **57**, 356 (1971).
87. M. L. Colglazier, K. C. Kates and F. D. Enzie, *Proc. helminth. Soc. Wash.* **39**, 28 (1972).
88. H. Van den Bossche, *Nature, Lond.* **273**, 626 (1978).
89. C. A. Behm and C. Bryant, *Vet. Parasit.* **5**, 39 (1979).
90. M. Borgers, S. De Nollin, M. De Brabander and D. Thienpont, *Am. J. vet. Res.* **36**, 1153 (1975).
91. M. Borgers, S. De Nollin, A. Verheyen, M. De Brabander and D. Thienpont, in *Microtubules and Microtubular Inhibitors* (Eds. M. Borgers and M. De Brabander), p. 497. Elsevier/North Holland Biomedical Press, Amsterdam (1975).
92. A. Verheyen, M. Borgers, O. F. Vanparijs and D. Thienpont, in *Biochemistry of Parasites and Host–Parasite Relationships* (Ed. H. Van den Bossche), p. 605. Elsevier/North Holland Biomedical Press, Amsterdam (1976).
93. M. S. Rahman and C. Bryant, *Int. J. Parasit.* **7**, 403 (1977).
94. M. S. Rahman, R. A. Cornish, R. A. F. Chevis and C. Bryant, *N.Z. vet. J.* **25**, 79 (1977).
95. D. Düwel, *Pestic Sci.* **8**, 550 (1977).
96. J. Hoebeke, G. Van Nijen and M. De Brabander, *Biochem. biophys. Res. Commun.* **69**, 319 (1976).
97. P. A. Friedman and E. G. Platzer, *Biochim. biophys. Acta* **544**, 605 (1978).
98. C. M. Ireland, K. Gull, W. E. Gutteridge and C. I. Pogson, *Biochem. Pharmac.* **28**, 2680 (1979).
99. L. C. Davidse and W. Flach, *Biochim. biophys. Acta* **543**, 82 (1978).
100. L. C. Davidse, Thesis, Wageningen, The Netherlands (1976).
101. G. Scheir-Neiss, R. V. Nardi, M. A. Gealt and W. R. Morris, *Biochem. biophys. Res. Commun.* **69**, 285 (1976).

102. L. C. Davidse and W. Flach, *J. Cell Biol.* **72**, 174 (1977).
103. C. Bryant, in *Advances in Parasitology* (Ed. B. Dawes), Vol. 13, p. 35. Academic Press, New York (1975).
104. C. Bryant, in *Advances in Parasitology* (Eds. W. H. R. Lumsden, R. Muller and J. R. Baker), Vol. 16, p. 311. Academic Press, New York (1978).
105. H. Van den Bossche, *Comp. Biochem. Physiol.* **31**, 789 (1969).
106. R. K. Prichard, *Int. J. Parasit.* **6**, 227 (1976).
107. H. Van den Bossche, H. Verhoeven, O. Vanparijs, H. Lauwers and D. Thienpont, *Archs int. Physiol. Biochim.* **87**, 851 (1979).
108. *WHO Chron.* **33**, 399 (1979).