Acta Parasitologica, 2005, 50(1), 1–18; ISSN 1230-2821

REVIEW ARTICLE

Biochemical targets in filarial worms for selective antifilarial drug design

Sapna Gupta and Arvind K. Srivastava*

Division of Biochemistry, Central Drug Research Institute, Lucknow-22 6001, India

Abstract

Filarial nematodes infect more than 150 million people worldwide and are responsible for diseases including elephantiasis, river blindness and tropical pulmonary eosinophilia. Antifilarial agents that can kill all the stages in the life cycle of causative filariae have yet to be developed. Very little effort has been made towards rational drug design, employing knowledge gained from studies of the biochemistry and physiology of filarial worms and of their interactions with their specific vertebrate hosts. In this review, we highlight the research and development of rational antifilarial agents and we discuss the pitfalls since the discovery of diethylcarbamazine, the only drug of choice for controlling filariasis, despite its adverse side effects.

Key words

Filariasis, antifilarial targets, antifilarial drugs, filarial metabolism

Introduction

Filarial nematodes are responsible for health and economical blight. Lymphatic filariasis has been identified by the World Health Organization (WHO) as the second leading cause of permanent and long-term disability (WHO 1997a, b) whereas blindness is caused by the filarial species *Onchocerca volvulus* (WHO 2000).

Current filariasis control strategies are not entirely successful and filarial infections are on the rise. In the absence of availability of antifilarial vaccines, chemotherapy remains the mainstay for treatment of the diseases caused by filarial nematodes. However, the precise primary effects of different chemical classes of compounds currently used as antifilarials are still unclear (Table I). A clear understanding of the mode of action of antifilarials awaits greater knowledge of the biochemical pathways operating in filarial parasites. The present review contrasts filarial and human metabolism so that strategic differences can be harnessed for newer developments in chemotherapy of filariasis.

Carbohydrate metabolism

Carbohydrates play a significant role in providing energy to filarial species. Filarial parasites have active glycogenic and glycolytic pathways and a somewhat submissive tricarboxylic acid cycle (TCA) (Saz 1981, Barrett 1983). This is in contrast to mammals, which have active TCA and electron transport systems. The pathways of energy generation in filarial parasites are summarized in Figure 1. Most adult filarial parasites use the glycolytic breakdown of carbohydrate to lactate as a preferred route to supply their energy requirements (Saz 1981, Barrett 1983, Barrett *et al*. 1986, Dunn *et al*. 1988, Köhler 1991). Microfilariae (Mf) exhibit an aerobic carbohydrate catabolism, requiring oxygen at least for motility, but apparently not for survival (Rew and Saz 1977). There are also minor

*Corresponding author: drarv1955@yahoo.com

catabolic routes, similar to those present in adults that result in acetate and succinate formation by Mf (Srivastava *et al*. 1988).

The complete sequence of glycolytic enzymes involved has been demonstrated with high activity levels in *Chandlerella hawkingi*, *Dirofilaria immitis*, *Litomosoides carinii*, *Setaria cervi*, *Brugia pahangi*, *Acanthocheilonema viteae* and *O. volvulus* (Srivastava *et al*. 1970, Srivastava and Ghatak 1971, Saz and Dunbar 1975, Anwar *et al*. 1977, Middleton and Saz 1979, Walter and Schulz-Key 1980). Enzymes of the TCA cycle have also been demonstrated in adult *B. pahangi*, *D. immitis*, *C. hawkingi*, *Dipetalonema viteae*, *L. carinii*, *O. volvulus* and *S. cervi* (Srivastava and Ghatak 1971, Anwar *et al*. 1977, Middleton and Saz 1979, Walter and Shulz-Key 1980, Agarwal *et al*. 1986, Dunn *et al*. 1988). However, it was concluded that the TCA cycle does not play a significant role in energy production (Wang and Saz 1974, Walter and Van den Bossche 1980, Dunn *et al*. 1988). The activities of the TCA cycle enzymes were found to be relatively lower in filarial species (Barrett *et al*. 1986, Dunn *et al*. 1988). Filariae possess mitochondrial electron transport system; however, the nature of the terminal oxidase is still uncertain (Barrett 1983). Neither cytochrome *c* nor cytochrome oxidase were detected in *L. carinii*, *B. pahangi* and *D. viteae* (Bueding and Charms 1951, 1952; Wang and Saz 1974). However, cytochrome oxidase activity has been detected in the Mf of *B. pahangi* (Rew and Saz 1977, Mendis and Townson 1985) and cytochromes *c*, *b* and *a* have been detected in adult *D. immitis* (Hayashi and Oya 1978). Hayashi and Oya (1978) suggested that *D. immitis* might have a branched cytochrome chain, with an *o*-type cytochrome as the alternative oxidase.

Litomosoides carinii has a relatively exceptional type of energy metabolism (Wang and Saz 1974, Ramp and Köhler 1984). These filariae show absolute oxygen requirement for maintenance of motility and survival but the TCA cycle does not constitute a significant energy-yielding pathway. Aerobic requirement may reside completely in the oxidative decarboxylation of pyruvate to acetate and CO_2 (Wang and Saz 1974).

Table I. Possible sites of action of antifilarial compounds (modified from Subrahmanyam 1987)

Antifilarial agent	Metabolism/system effected
Diethylcarbamazine	neuromuscular system, carbohydrate and folate metabolism
Ivermectin	neuromuscular system
Suramin	carbohydrate and folate metabolism
Benzimidazoles	microtubular system and carbohydrate metabolism
Isothiocyanates	carbohydrate, nucleic acid and protein metabolism
Levamisole	neuromuscular system and carbohydrate metabolism
Arsenicals	carbohydrate and glutathione metabolism
Antimonials	carbohydrate metabolism

Respiratory metabolism as a target

Since the pathways of respiratory metabolism in the parasites differ from those of their hosts, this promises to be an important antifilarial target (Behm and Bryant 1979). One question that arises is would the chemotherapeutic blockage of one enzyme alone be adequate to produce the lethal effect? A variety of antifilarial drugs have been shown to inhibit some enzymes of these pathways as follows.

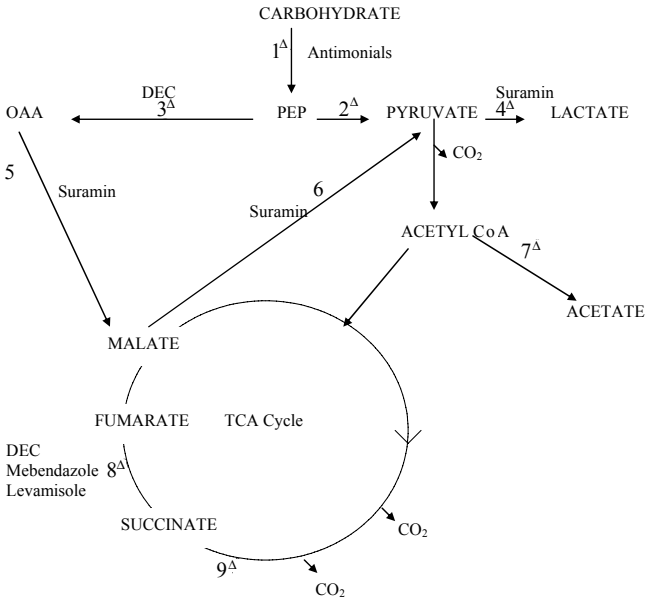

Fig. 1. The pathways of energy generation in adult filarial parasites. Possible sites of action of antifilarials are also indicated. (1) phosphoglycerate kinase, EC 2.7.2.3; (2) pyruvate kinase, EC 2.7.1.40; (3) phosphoenolpyruvate carboxykinase, EC 4.1.1.38; (4) lactate dehydrogenase, EC 1.1.1.27; (5) malate dehydrogenase, EC 1.1.99.16; (6) malic enzyme, EC 1.1.1.40; (7) mechanism of possible energy generation is unclear; (8) fumarate reductase, EC 1.3.99.1; (9) succinyl-CoA synthetase, EC 6.2.1.5 (modified from Köhler 1991, Subrahmanyam 1987). DEC – diethylcarbamazine, OAA – oxaloacetate, PEP – phosphoenol pyruvate. The major pathway is underlined and Δ represents sites of energy generation

Phosphofructokinase as a target

Saz and Dunbar (1975) demonstrated that the antimonial stibophen blocked glycolysis in filariae, *L. carinii*, *D. viteae* and *B. pahangi* by significant inhibition of the phosphofructokinase (PFK) activity as compared to the isofunctional mammalian enzyme. In homogenates from all three parasites, the PFK activities were inhibited significantly down to a stibophen concentration of 2×10^{-6} M. In contrast, concentrations up to 10^3 times greater were required before a low level of inhibition of PFK could be demonstrated in hamster liver.

Lactate dehydrogenase as a target

Walter and Schulz-Key (1980) have suggested that the efficacy of suramin in the treatment of human onchocerciasis may

reside in its ability to inactivate the *O. volvulus* lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and malic enzyme (Walter and Albiez 1981). However, complete validation of LDH as a viable chemotherapeutic target could not be achieved due to lack of a suitably specific inhibitor of LDH, effective at low concentrations.

Fructose 1,6-bisphosphate aldolase as a target

Fructose 1,6-bisphosphate aldolase has been characterized in various filarial species (Barrett *et al*. 1986, Dunn *et al*. 1988). McCarthy *et al*. (2002) suggested that the immunogenic component of the filarial aldolase is quite distinct from its mammalian counterparts, thus identifying it as a potential vaccine target, but the extent to which it is significant as an antifilarial chemotherapeutic target is still an area of active research.

Other respiratory enzymes and glucose uptake, transport, incorporation and utilization as targets

Diethylcarbamazine (DEC) alters glucose uptake and inhibits phosphoenolpyruvate carboxykinase, fumarate reductase and succinate dehydrogenase (Subrahmanyam 1987). Benzimidazoles inhibit fumarate reductase (Prichard 1973) and glucose transport (Van den Bossche and de Nollin 1973). Isothiocyanates and their derivatives also affect the energy metabolism of the parasites. Glucose uptake and incorporation into glycogen were inhibited in *L. carinii* and *B. pahangi* after treatment of infected jirds with amoscanate (Nelson and Saz 1984). Levamisole, a broad spectrum antinematodal agent was reported to affect carbohydrate metabolism by decreasing utilization of glucose together with a shift towards homolactate fermentation (Rew and Saz 1977). The arsenicals affect glucose uptake (Subrahmanyam 1987).

Trehalose metabolism as a target

Trehalose is a sugar present in many nematode species but absent in mammals. The synthesis, accumulation and utilisation of trehalose by nematodes are important in interaction with their external environment, in osmoregulation, in resistance to desiccation, in cryopreservation and in egg-hatching. Trehalose also functions as a reserve carbohydrate fuel for energy metabolism. The combined action of two enzymes, i.e. trehalose 6-phosphate synthase and trehalose 6-phosphate phosphatase, catalyse the synthesis of trehalose in most organisms. These enzymes have been detected in nematodes but the processes regulating their activity are unknown (Behm 1997). Trehalose metabolism may provide new targets for attacking nematodes parasitic in mammals. In filariids, trehalose has been detected in adult *L. carinii*, *B. pahangi* and *D. viteae* and studies indicate that trehalose is synthesized from glucose (Fairbairn 1958; Powell *et al*. 1986a, b).

Chitin metabolism as a target

Chitin metabolism has also been proposed to be a parasite unique target, as the vertebrate host does not contain chitin (Gooday *et al*. 1988, Shahabuddin *et al*. 1993). Chitinases have been identified in three separate stages in the filarial life cycle (Gooday *et al*. 1988, Fuhrman *et al*. 1992, Raghavan *et al*. 1994). In adult females of *Onchocerca gibsoni* this enzyme plays a role in embryogenesis (Gooday *et al*. 1988). The biological role of this enzyme in Mf and third stage larvae is not yet clear (Fuhrman 1995). Two isoforms of chitinase have been purified from Mf of *B. malayi* (Fuhrman *et al*. 1992). Raghavan *et al*. (1994) characterized a chitinase like antigen expressed in the third stage larvae of *Wuchereria bancrofti*. Filarial chitinase can be inhibited by allosamidin, a competitive inhibitor of the enzyme (Sakuda *et al*. 1987).

Lipid metabolism

Lipid metabolism is a relatively untouched aspect of the biology of filarial worms. Recent investigations have been restricted mainly to analyses of lipid composition and studies on lipid synthesis (Barrett 1983). The incorporation of mevalonate into free fatty acids suggests the presence of a fatty acid chain expansion system (Comley and Jaffe 1981). Filarial worms readily incorporate labelled lipid precursors into their neutral and phospholipid fractions (Jaffe and Doremus 1970, Hutchison and Turner 1979, Turner and Hutchison 1979). All the major classes of neutral lipids viz. triacylglycerols, diacylglycerols, sterols, sterol esters, hydrocarbons and traces of free fatty acids have been verified in *D. immitis* (Hutchison *et al*. 1976, Turner and Hutchison 1979, Comley and Jaffe 1981, Comley *et al*. 1981). Triacylglycerols and sterols have been demonstrated in adult *S. cervi* (Ansari *et al*. 1973, Rathaur *et al*. 1980) and *B. pahangi* was reported to have triacylglycerols, sterols, free fatty acids and hydrocarbon fractions (Comley *et al*. 1981). All the major phospholipid classes have also been identified in adult filarial worms of *D. immitis*, *B. pahangi*, *L. carinii* and *S. cervi* (Hack *et al*. 1962, Subrahmanyam 1967, Ansari *et al*. 1973, Comley and Jaffe 1981). Total fatty acid profiles are accessible for adult filarial worms of *D. immitis*, *D. viteae* and *L. carinii* (Ueda and Sawada 1968, Hutchison *et al*. 1976). Srivastava *et al*. (1987) demonstrated the participation of glycerol-3-phosphate in the formation of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerol (PG) and cardiolipin (CL) in adult *B. pahangi* and *B. patei*. The presence of sn-glycerol-3-phosphate acyltransferase, phosphatidic acid phosphatase, choline phosphotransferase, ethanolamine phosphotransferase, PE methyl transferase, PS decarboxylase, PI synthetase, PGP synthetase, and base exchange enzymes of ethanolamine, serine and inositol for the base components of preformed phospholipids, were detected in crude extracts of these worms. These findings along with previous studies (Srivastava *et al*. 1985; Srivastava and Jaffe 1985, 1986, 1987) suggest that filarial worms can synthesize PC by two pathways, PE by three pathways, and PI by two pathways and fabricate PS, PG and CL (Srivastava *et al*. 1987).

Isoprenoid metabolism

Nematodes do not synthesize the sterol ring, *de novo*, but the early stages of the pathway necessary to provide the polyiso-

prenoid precursors of sterols are present (Comley and Jaffe 1981). The filarial isoprenoid biosynthetic pathway leads to the formation of quinones, dolichols, geranyl geraniol, juvenile hormones and purine derivatives of isopentenyl pyrophosphate (Comley 1985). Quinones have their role in filarial electron transport (Comley *et al*. 1981), dolichols are involved in glycoprotein synthesis (Comley *et al*. 1982, Walter *et al*. 1985), juvenile hormones are regulators of larval development (Mendis *et al*. 1983) and isopentenyl adenosine is known as the constituent of tRNA (Brown and Goldstein 1980). However, the role of geranyl geraniol is still not clear.

HMG-CoA reductase as a target

Considering the variety of important biochemical roles attributed to isoprenoids, it is possible that selective inhibition of isoprenoid biosynthesis at the HMG-CoA reductase catalysed rate limiting stage could have drastic consequences on filariae. Mevinolin, a known inhibitor of HMG-CoA reductase, has been shown to significantly reduce the biosynthesis of geranyl geraniol, ubiquinone and dolichols in *B. pahangi in vitro* (Comley and Lancaster 1983).

Amino acid and protein metabolism

Amino acid metabolism has also received little attention in filarial parasites. Data are restricted mainly to amino acid composition, uptake and incorporation into proteins (Barrett 1983). Decarboxylation and transamination reactions have also been examined but only limited attempts have been made to elucidate pathways of amino acid catabolism (McManus 1986). Parasitic helminths are able to take up amino acids from their surroundings through their tegument (Asch and Read 1975). The functional importance of the free amino acid pool has been indicated in intracellular osmoregulation and protein synthesis (Kurelec and Rijavec 1966). The incorporation of amino acids into proteins has been demonstrated in a number of filarial worms such as adults of *L. carinii*, *S. cervi* and Mf of *D. immitis* (Jaffe and Doremus 1970, Anwar *et al*. 1978, Akinwande and Akinrimisi 1980). Serine hydroxymethyl transferase, an enzyme responsible for the conversion of serine into glycine and *vice versa* has also been demonstrated in adult *D. immitis* and *B. pahangi* (Jaffe and Chrin 1981).

Sulphur amino acid metabolism of filarial parasites has attracted attention. *D. immitis* and *B. pahangi* possess three enzymes from the methionine cycle viz. methionine adenosyltransferase, S-adenosylmethionine methyltransferase and S-adenosylhomocysteine hydrolase for the conversion of methionine to homocysteine, but apparently lack the final enzymes viz. methionine synthetase and/or betaine-homocysteine transmethylase for the conversion of homocysteine to methionine (Jaffe 1980a) (Fig. 2). Related studies have confirmed that *B. pahangi* is incapable of resynthesizing methionine from homocysteine and appears to rely on methionine uptake from the mosquito host (Jaffe and Chrin 1979a, b).

In vertebrates, the trans-sulphuration sequence constitutes the final two steps in the pathway of cysteine formation from

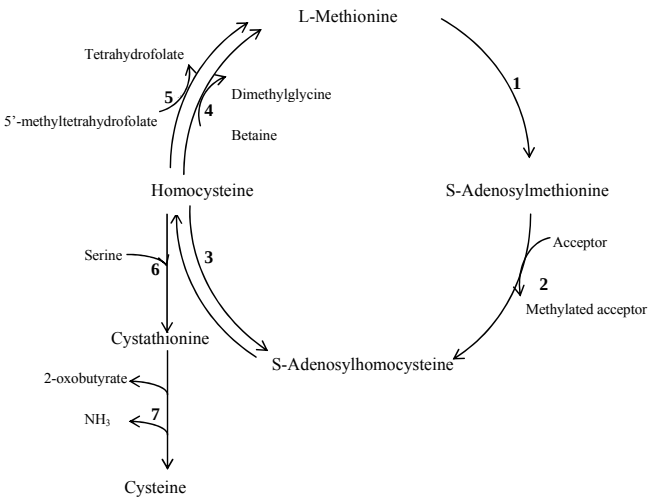

Fig. 2. Generalized scheme of methionine metabolism in filarial species. (1) methionine adenosyltransferase, EC 2.5.1.6; (2) various S-adenosylmethionine methyltransferases, EC 2.1.1.?; (3) S-adenosylhomocysteine hydrolase, EC 3.3.1.1; (4) betaine-homocysteine methyltransferase, EC 2.1.1.5; (5) 5'-methyltetrahydrofolate-homocysteine transmethylase (methionine synthase), EC 2.1.1.13; (6) cystathionine β-synthase, EC 4.2.1.22; (7) γ-cystathionase, EC 4.4.1.1 (modified from Walker and Barrett 1997).

dietary methionine catalysed by cystathionine-β-synthase and γ-cystathionase (Cooper 1983, Bender 1985, Griffith 1987) and is the predominant pathway for methionine catabolism (Cooper 1983). All the enzymes involved in the methionine to cysteine pathway have been identified in the filarial nematodes *B. pahangi* and *D. immitis* (Jaffe 1980a) (Fig. 2). However, the very low cystathionine catabolizing activity in helminths may represent a significant bottleneck in this pathway (Gomez-Bautista and Barrett 1988; Walker and Barrett 1991a, b; Bankov *et al*. 1996).

Sulphur amino acid metabolism as a target

The identification of a novel, non-mammalian form of cystathionine-β-synthase in nematodes may facilitate the selective inhibition of a parasite-specific enzyme (Walker and Barrett 1997). Cystathionine synthesizing activity has been found in the filarial nematodes *B. pahangi* and *D. immitis* (Jaffe 1980a). Further studies towards non-mammalian "activated serine sulphydrase" activity of cystathionine-β-synthase and its biological role in filarial worms may offer opportunities for selective inhibition.

Collagen biosynthesis as a target

Prolyl-4-hydroxylase as a target

The cuticle forms the external surface of parasitic nematodes, thus forming the interface between the parasite and the host. The nematode cuticle consists of a network of collagen molecules, which are primarily held together by intermolecular

disulfide bonds (Cox 1992). The central enzyme involved in the biosynthesis of collagen is prolyl-4-hydroxylase (Kivirikko *et al*. 1989) which catalyses the post-transcriptional oxidation of proline to 4-hydroxyproline in nascent collagen chains. This enzyme has been a subject of acute concern as a potential chemotherapeutic target, because molecules inhibiting its activity might be expected to be relatively specific inhibitors of collagen biosynthesis (Hanauske-Abel 1991). Both α and β subunits of this enzyme from *O. volvulus* have been cloned and expressed in a biologically active form. Protein disulfide isomerase functions to catalyse the formation of correct disulfide bonds in nascent proteins, and also acts as one of the subunits of prolyl-4-hydroxylase (Wilson *et al*. 1994).

Transglutaminase as a target

Transglutaminase catalysed reactions play an important role in the growth, development and maturation of nematodes. Because of its role in cuticle biosynthesis and lack of homology with mammalian transglutaminases, this parasite enzyme represents a target in the development of an effective chemotherapeutic drug (Chandrashekar and Mehta 2000). Adult worms of *B. malayi*, *B. pahangi* and *O. volvulus* contain high levels of transglutaminase activity (Mehta *et al*. 1990, 1992; Lustigman *et al*. 1995). Monodansylcadaverine, a pseudosubstrate of this enzyme was found to affect the release of Mf from *B. malayi* as well as causing a dose dependent decrease in the MTT reduction potential of the worms (Rao *et al*. 1991). Several physicochemical properties such as thermostability, substrate specificity, effect of various reagents and inhibitors suggest that this enzyme from *D. immitis* is very similar to transglutaminase purified from *B. malayi*, but quite distinct from mammalian transglutaminases (Singh *et al*. 1995).

Aminoacyl-tRNA synthetase as a target

Aminoacyl-tRNA synthetases (AARS) are also one of the verified antifilarial targets. Divergence of host and parasite enzyme structures surrounding the catalytic site can be utilized to design drugs (Cusack 1997). Biologically active AARS have been cloned, expressed and purified from *B. malayi* and humans. Synthetic aminoacyl adenylate analogues, which mimic the normal intermediate formed by AARS, are potent AARS inhibitors (Lazdins and Kron 1999).

Nucleic acid metabolism

Nucleic acids are essential components of all living organisms but relatively little work had been done on nucleotide and nucleic acid synthesis in parasitic filariids. Filarial worms possess both *de novo* and salvage pathways for purines and pyrimidines. Synthetic pathways are usually under tight metabolic control (Barrett 1983). Incorporation of uridine and uracil into nucleic acids has been reported in adult *D. immitis* (Jaffe *et al*. 1972), whilst the Mf of *D. immitis* incorporate uridine, uracil, adenine and adenosine into RNA (Jaffe and Doremus 1970). Jaffe and Doremus (1970) reported incorporation of orotate derivatives into RNA by the Mf of *D. immitis*. Incubation of adult *B. pahangi* with 5-^{14}C methyltetrahydrofolate resulted in ^{14}C-labelled adenosine and guanine ribonucleotides as well as ^{14}C-labelled inosine monophosphate (Jaffe and Chrin 1981). The incorporation of label from glycine into DNA and RNA and of inorganic phosphate into RNA has also been reported in adult *L. carinii* (Akinwande and Akinrimisi 1980).

DNA topoisomerase II as a target

It has been shown that DNA topoisomerase II is an essential enzyme, which has an important role in DNA replication, repair and transcription and recently, this enzyme has been identified as a target for the development of antifilarial compounds (Tripathi *et al*. 2001). The 7-O-acetamidyl-4-alkyl-2H-1-benzopyran-2-ones, glycosylated beta-amino acid derivatives and Ru (II) poly-pyridyl hydridocarbonyl complexes were prepared and screened against DNA topoisomerase II of filarial parasite *S. cervi* (Tripathi *et al*. 2001, Katiyar *et al*. 2003, Chandra *et al*. 2004).

Folate metabolism

Folate derivatives are concerned with the transport and interconversion of one carbon units for synthetic reactions. The high synthetic capacities of parasites and the differential sensitivities of certain folate metabolizing enzymes to inhibitors means that folate metabolism is a potential area for chemotherapy (Jaffe 1980b). Adult filariae possess an array of enzymes involved in the interconversion of folate analogues (Jaffe and Chrin 1980, 1981; Jaffe *et al*. 1980; Comley *et al*. 1981) (Fig. 3). The presence of this group of enzymes indicates that adult filariae can synthesize a variety of tetrahydrofolate (FH_4) cofactors, but the physiological roles of these cofactors and the factors regulating their levels in filarial cells remain to be fully defined (Jaffe and Chrin 1980).

Dihydrofolate reductase has been demonstrated in a number of adult filarial worms of *D. immitis*, *L. carinii*, *D. viteae* and *O. volvulus*, and the sensitivity of this enzyme to inhibitors has been investigated (Jaffe 1972, Jaffe *et al*. 1972). In contrast to the adults, no dihydrofolate reductase activity was detected in the Mf of *B. pahangi* or *D. immitis* (Jaffe 1972; Jaffe *et al*. 1972, 1977). Analysis of different steps in folate metabolism in the parasites revealed certain basic differences from those operating in the vertebrates. The enzyme 5, 10 methylene FH_4 reductase catalyses the irreversible formation of N^5-methylene FH_4 from N^5-N^{10}-methylene FH_4 in almost all vertebrates. However, in filarial parasites, this enzyme operates preferentially in the reverse direction, favouring the formation of N^5-N^{10}-methylene FH_4 (Jaffe 1980b, Comley *et al*. 1981). 10-formyl FH_4 dehydrogenase, which catalyses the deformylation of N^{10}-formyl FH_4 and thus regulates the endogenous concentrations of FH_4 cofactors, is more active in *B. pahangi* and *D. immitis* than in mammalian liver. Another enzyme of interest is serine hydroxymethyltransfer-

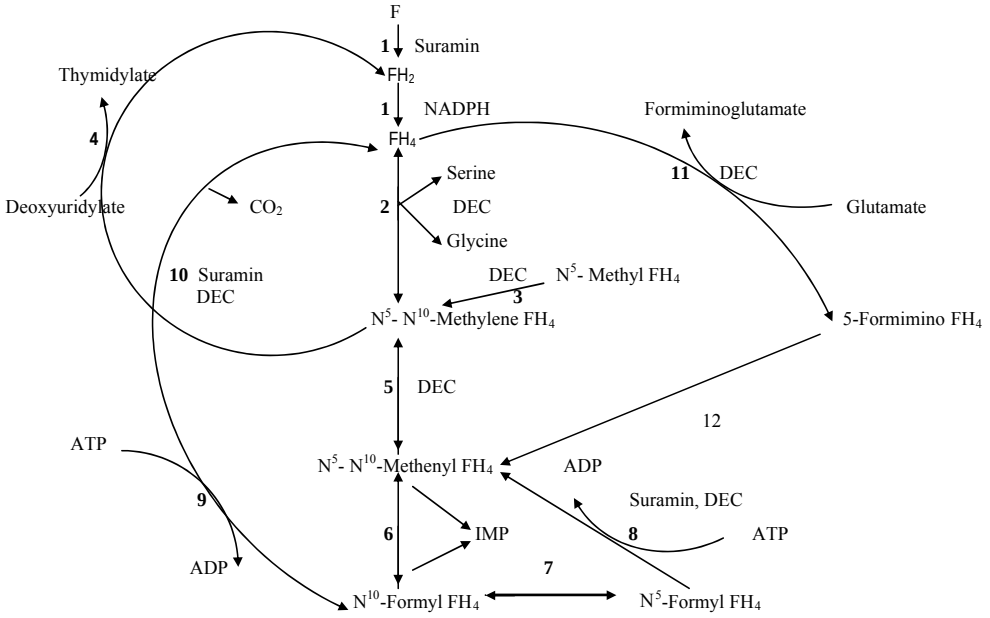

Fig. 3. Folate metabolism in filarial species with sites of action of antifilarials. (1) FH_2 reductase, EC 1.5.1.3; (2) serine hydroxymethyltransferase [glycine hydroxymethyltransferase], EC 2.1.2.1; (3) 5, 10-methylene FH_4 reductase, EC 1.7.99.5; (4) thymidylate synthase, EC 2.1.1.45; (5) methylene FH4 dehydrogenase, EC 1.5.1.5; (6) methenyl FH_4 5-cyclohydrolase, EC 3.5.4.9; (7) N^5,N^{10}-formyl FH_4 mutase; (8) 5-formyl FH_4 cycloligase, EC 6.3.3.2; (9) formate FH_4 ligase, EC 6.3.4.3; (10) 10-formyl FH_4 dehydrogenase, EC 1.5.1.6; (11) glutamate formiminotransferase, EC 2.1.2.5; (12) formiminodoyl FH_4 cyclodeaminase, EC 4.3.1.4 (modidied from Subrahmanyam 1987). F – folate, FH_2 – dihydrofolate, FH_4 – tetrahydrofolate, IMP – inosinate, DEC – diethylcarbamazine

ase which catalyses the synthesis of N^5-N^{10}-methylene FH_4 from tetrahydrofolate and requires pyridoxal phosphate (Subrahmanyam 1987). There was a virtual block in the development of infective larvae of *L. carinii* to the adult stages in pyridoxine deficient rodents, possibly because of interference with folate metabolism (Subrahmanyam 1983).

There is indirect evidence that adult filariae do not synthesize FH_2, but require a source of preformed folates (Jaffe 1980a); although during short term incubations Chen and Howells (1981) reported no uptake of folic acid by the adults, juveniles, infective larvae or Mf of *B. pahangi.* The adults of *B. pahangi* can convert N^5-methyl FH_4 to N^5-N^{10}-methylene FH_4, N^5-N^{10}-methenyl FH_4, N^5-formyl FH_4 and N^{10}-formyl FH_4 (Jaffe and Chrin 1981). The N^5-methyl FH_4 analogue is the predominant folate derivative found in mammalian tissue, but interestingly mammalian cells do not convert N^5-methyl FH_4 back to N^5-N^{10}-methylene FH_4.

Folate metabolism as a target

DEC also inhibits a number of folate metabolism enzymes (Fig. 3) but it remains to be determined whether the ability of DEC to interfere with multiple aspects of filarial folate-related metabolism is in any way related to the antifilarial action of this drug (Jaffe and Chrin 1980). Suramin inhibits the dihydrofolate reductase of *O. volvulus* and NADP-dependent 10-formyl FH_4 dehydrogenase of *B. pahangi*. 10-formyl FH_4 dehydrogenase may play an important role in regulating levels of FH_4 coenzymes within the worms in response to fluctuating physiological conditions (Jaffe 1980a, b).

Biogenic amine metabolism

Various biogenic amines viz. norepinephrine (NE), dopamine (DA), 5-hydroxytryptamine (5-HT) and histamine (Hm) have been reported to be present in Mf and adults of *L. carinii* (Saxena *et al*. 1977), *D. viteae* (Singh *et al*. 1985) and *S. cervi* (Rathaur *et al*. 1985). The amine content of Mf was found to be higher as compared to adult worms. The 5-hydroxyindole acetic acid and homovanillic acid, the catabolic products of amines, were also detected (Rathaur *et al*. 1985). The role of biogenic amines in neuromuscular activity (Gianutsos and Bennett 1977) and behavioural coordination (Croll 1975) of nematodes is well documented. Jones *et al*. (1974) also showed the involvement of histamine (Hm) in the host's immune response to nematode infections. In order to understand the role of amines and their metabolizing enzymes in the development and pathogenesis of filarial worms, studies were carried out on some of these enzymes. Dopamine-beta-hydroxylase was detected in *L. carinii*, *D. viteae* and *S. cervi* (Agarwal *et al*. 1982). Monoamine oxidase (MAO) and acetylcholinesterase, which play a role in neuromuscular transmission, were detected in both adults and Mf of *S. cervi*.

Monoamine oxidase and acetylcholinesterase as targets

Centperazine, DEC and levamisole strongly inactivated these enzymes from *S. cervi* females (Rathaur 1980). *S. cervi* MAO can be differentiated from the host enzyme (bovine) on the basis of substrate specificity, pH optima and Km value (Rathaur 1980).

Octapamine as a target

Of special attention is octapamine (OA), the only one amine with no apparent vital function in mammals. OA plays an important role in the regulation of a number of key processes in nematodes, including pharyngeal pumping, locomotion and egg laying (Brownlee *et al*. 2000). Among filariids it has been identified in *O. volvulus* and *B. pahangi* (Isaac *et al*. 1990, Aisien *et al*. 1995). Potential drugs may come from agonists and antagonists of the OA receptor (Ginger 1991).

Polyamine metabolism

The polyamines putrescine, spermidine and spermine are found in all living organisms and are involved in growth, differentiation and macromolecular synthesis (Tabor and Tabor 1984, Pegg *et al*. 1988, Svensson *et al* 1993). Measurements of polyamine in *O. volvulus*, *D. immitis*, *Brugia patei*, *S. cervi* and *L. carinii* worms have demonstrated that these parasites contain high levels of spermidine and spermine but low levels of putrescine and N-acetylated polyamines (Srivastava *et al*. 1980, Wittich *et al*. 1987). The enzymes of polyamine biosynthesis viz. ornithine decarboxylase (ODC), S-adenosyl methionine decarboxylase (SAMDC) and arginine decarboxylase (ADC) were shown to be very low or absent in filarial parasites (Wittich *et al*. 1987, Walter 1988). Moreover, uptake of polyamines from the incubation medium as well as interconversion and excretion of putrescine and N^1-acetylputrescine have been detected in filariids (Wittich *et al*. 1987, Singh *et al*. 1989). There is evidence for the existence of a complete reverse pathway generating putrescine from spermidine and spermine, respectively, in filarial worms (Wittich *et al*. 1987). A scheme of filarial polyamine metabolism is suggested in Figure 4. The presence of considerable levels of polyamine oxidase, an important enzyme in the reverse pathway of polyamines, reported earlier (Walter 1988, Sharma *et al*. 1991) strongly supports the existence of a salvage pathway for polyamines in helminths.

S-adenosyl-methionine decarboxylase as a target

S-adenosyl-methionine decarboxylase (SAMDC), a key regulatory enzyme in polyamine biosynthesis, is considered as a potentially important antifilarial target. Various inhibitors of SAMDC, such as berenil and aromatic methylglyoxal bis(guanylhydrazone) analogues, might have potential as drug candidates against filarial worms (Da'dara *et al*. 1998). The *in vitro* treatment of adult filariae with polyamine analogues and inhibitors of enzymes involved in the polyamine biosynthesis killed the parasites (Müller *et al*. 1988, 1991).

Glutathione metabolism

Glutathione (GSH) is of major importance in filarial species because it has been proposed to constitute the antioxidant system responsible for the long term existence of filarial worms in mammalian hosts by protecting them from the reactive oxygen species produced by normal metabolism and by immune cells of the host (Callahan *et al*. 1988, Brophy and Pritchard 1992). Therefore, it seems useful to develop drugs that could selectively deplete or distort glutathione stores in these para-

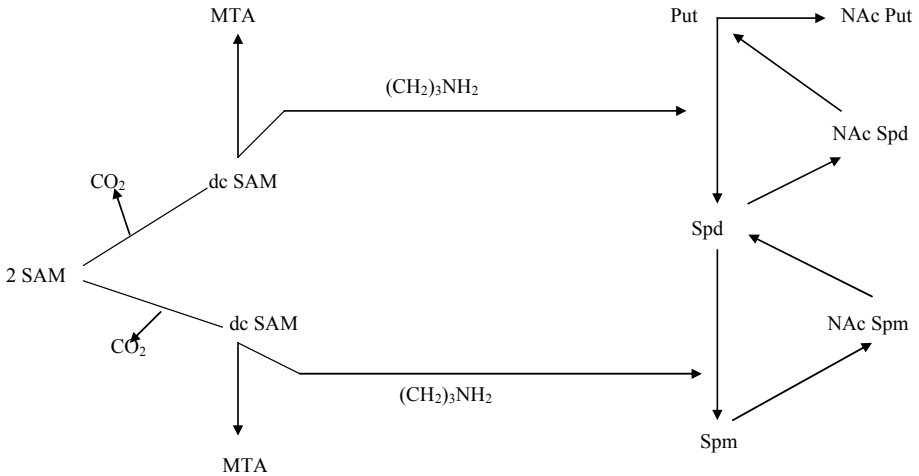

Fig. 4. Proposed scheme of the regenerative pathway of polyamines in filarial worms (from Wittich *et al*. 1987). SAM – S-adenosylmethionine, dcSAM – decarboxylated SAM, MTA – 5'-methylthioadenosine, Put – putrescine, Spd – spermidine, Spm – spermine, NAc Put – N-acetylputrescine, NAc Spd – N-acetylspermidine, NAc Spm – N-acetylspermine

sites. The GSH either acts in solo or as a substrate to various enzymes like glutathione peroxidase (GPX), phospholipid hydroperoxide GPX and glutathione-S-transferase (GST) (Zhang *et al*. 1989, Lomaestro and Malone 1995) to quench the free radicals. After GSH has been oxidized to GSSG, the enzyme glutathione reductase (GR) accomplishes the recycling of GSSG back to GSH (Carlberg and Mannervik 1985, Schirmer *et al*. 1987).

The group of six enzymes that are responsible for the synthesis and breakdown of GSH constitutes the γ-glutamyl cycle. At present only two enzymes of the γ-glutamyl cycle viz. glutamate-cysteine ligase (GCL) and γ-glutamyl transpeptidase (γ-GT) have been characterized from filarial species (Singh *et al*. 1996, Lüersen *et al*. 2000). The whole of this cycle needs to be characterized in filarial worms, although, it has been well characterized in mammalian systems (Orlowski and Meister 1973, Werf *et al*. 1975, Oppenheimer *et al*. 1979, Kozak and Tate 1982, Seelig and Meister 1982, Tate and Meister 1985). The sensitivity of filarial γ-glutamyl cycle enzymes and their counterparts from mammalian sources to known inhibitors needs to be compared. If marked differences in sensitivity exist this may provide targets for antifilarial drug development. Figure 5 represents the proposed sequence of glutathione metabolism in mammalian and filarial species.

Glutamate-cysteine ligase (GCL) as a target

Glutamate-cysteine ligase is the rate limiting enzyme of GSH biosynthesis which catalyses the ligation of the L-glutamate and L-cysteine to form L-γ-glutamyl-L-cysteine (Plummer *et al*. 1981). This enzyme has been well characterized in *O. volvulus* and *S. cervi* (Lüersen *et al*. 2000, Tiwari *et al*. 2003). The Ki values of *O. volvulus* GCL for buthionine sulfoximine (BSO) and cystamine were found to be lower than those of the corresponding mammalian enzyme (Lüersen *et al*. 2000). Hence, this enzyme represents a potential target for antifilarial drug development.

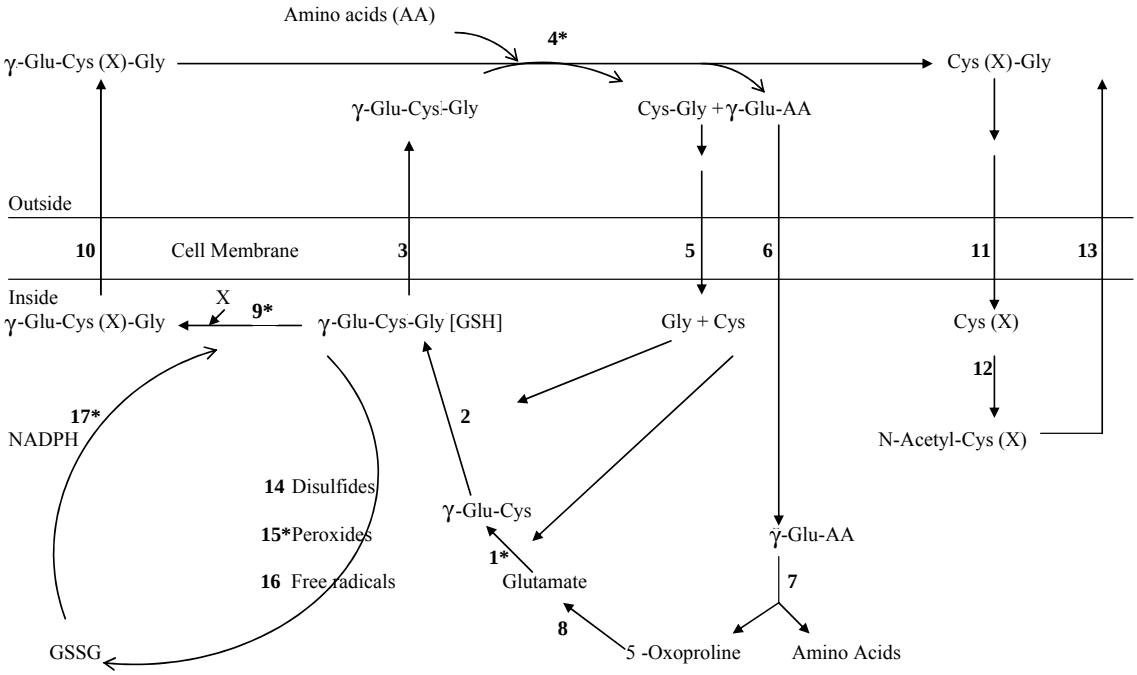

Fig. 5. Outline of GSH metabolism. The cellular turnover of GSH involves its intracellular synthesis from glutamate, cysteine and glycine catalysed by glutamate-cysteine ligase (1; EC 6.3.2.2) and GSH synthetase (2; EC 6.3.2.3), followed by transport of GSH (3) and its conversion by membrane bound γ-glutamyl transpeptidase (4; EC 2.3.2.2) to cysteinylglycine (Cys-Gly) and γ-glutamyl amino acids. Cleavage of cysteinylglycine to cysteine (Cys) and glycine (Gly) may be catalysed by membrane bound dipeptidase (followed by the transport of amino acids) or may occur intracellularly after transport of the dipeptide (5; EC 3.4.13.19). Transported γ-glutamyl amino acids (6) are converted by γ-glutamyl cyclotransferase (7; EC 2.3.2.4) to amino acids and 5-oxoproline; the latter is decyclized by 5-oxoprolinase (8; EC 3.5.2.9) to glutamate. Glutathione reacts intracellularly with a variety of compounds of exogenous and endogenous origin (X) in reactions catalysed by GSH-S-transferase (9; EC 2.5.1.18) to form GSH-S-conjugate. These are transported (10) and follow pathways similar to those involved in GSH turnover (4, 6 and 11). S-substituted derivatives of cysteine are acetylated (12) to form mercapturic acids, which are transported out of cells (13). Intracellular GSH is converted to GSSG in transhydrogenation reactions (14), in reactions catalysed by GSH peroxidase (15; EC 1.11.1.9), and by reactions with free radicals (16). GSSG is converted to GSSG by GSSG reductase (17; EC 1.6.4.2) (modified from Meister 1983). *These enzymes have been characterized in both mammals and filarial species, rest of the enzymes are characterized in mammals but need to be characterized in filariids

Glutathione reductase* (*GR*) *as a target

There is evidence to show that the mode of action of macrofilaricidal arsenicals is principally through the depletion of filarial GSH (Worthington and Rosemeyer 1974, Krohne-Ehrich *et al*. 1977, Bhargava *et al*. 1983). *L. carinii* GR was found to be much more vulnerable to inhibition with arsenical melarsen oxide than the enzyme isolated from human erythrocytes (Bhargava *et al*. 1983). Similarly, studies on the inhibition of GR by melarsen oxide revealed that human erythrocyte GR is less susceptible to inhibition by the arsenical than the enzyme from two cattle filariae viz. *S. digitata* and *Onchocerca gutturosa* along with differences in mechanisms of inhibition (Müller *et al*. 1995). These differences between host and parasite enzyme might reflect differences in the primary and secondary structures of the proteins that might be exploitable for the design of new specific filaricidal drugs.

Glutathione-S-transferase* (*GST*) *as a target

Glutathione-S-transferases are one of the major detoxification systems ubiquitous among eukaryotes and have been found in a wide range of parasitic helminths (Brophy and Barrett 1990a). Helminths lack the important cytochrome P-450 dependent detoxification system (Precious and Barrett 1989), enhancing the requirement for phase II GST activity. They perform functions ranging from catalysing the detoxification of electrophilic compounds to protecting against peroxidative damage (Armstrong 1991). The compounds that can be detoxified by GSTs include the cytotoxic products of lipid peroxidation, such as lipid hydroperoxides and reactive carbonyls. Such compounds, in the absence of GSTs, cause membrane damage. GSTs are therefore postulated to protect the parasite against host-mediated lipid peroxidation of the membrane (Brophy and Barrett 1990b, Brophy and Pritchard 1994). The inhibition of parasite GST(s) thus deprives the parasite of its major defence against oxidative stress and impairs its ability to survive. Filarial GST(s) is therefore important target for antifilarial drug design. *C. elegans*, *Ascaris suum* and *O. volvulus* GST cDNA sequences show some degree of sequence identity to mammalian pi class of GSTs (Weston *et al*. 1989; Liebau *et al*. 1994a, b, d). The cDNAs encoding two different GSTs from *O. volvulus* have been isolated and characterized (Liebau *et al*. 1994b, c; Salinas *et al*. 1994).

Glutathione peroxidase* (*GPX*) *as a target

The GPX family is well characterized in some filariids. The major soluble cuticular protein of adult *B. pahangi*, gp29 (Maizels *et al*. 1989), was identified as a secreted variant of a GPX (Cookson *et al*. 1992). Recombinant *B. pahangi* gp29 was shown to reduce fatty acid and phospholipid hydroperoxides but was not active with H_2O_2 as the substrate (Tang *et al*. 1996). The *D. immitis* homologue of *Brugia* gp29, called Di29 on the other hand possesses a low but significant activity with H_2O_2 (Tripp *et al*. 1998). The nucleotide sequences encoding homologues of this enzyme were also found in *B. malayi* and *W. bancrofti*. These enzymes belong to the group of selenium-independent GPXs. Selenium containing GPXs, a major family of enzymes in mammals appear to be absent from filarial species (Cookson *et al*. 1993, Tripp *et al*. 1998). GPXs protect the filarial worms from oxidative damage, and are thus important targets for novel chemotherapy.

Retinol-binding proteins

There is now increasing evidence of the importance of the role played by retinoids (vitamin A and its metabolites) in filarial metabolism. Parasitic nematodes appear to require retinol for a variety of their metabolic and developmental demands, such as growth, differentiation, embryogenesis, glycoprotein synthesis and as antioxidants (Sani 1990). Being highly lipophilic, retinoids require specific retinol-binding proteins (RBPs) to facilitate their transfer both within the cells and between the cells through the aqueous environment (Wolf 1991; Ross 1993a, b). Several investigators have described the occurrence and uptake of retinol, as well as its formation from β-carotene in parasites (Comley and Jaffe 1983). Sturchler *et al*. (1981, 1985) reported a retinol concentration of 12.6 IU/g in adult *O. volvulus*. This is in contrast to the much lower levels identified in *A. viteae* adult females (0.18 IU/g), males (0.2 IU/g), and Mf (0.01 IU/g) (Sturchler *et al*. 1985). Findings of Wolff and Scott (1995) in *B. malayi* adult females report retinol concentration of 6.2 IU/g. The comparative amounts of retinoic acid-binding proteins (RABPs) in filariids viz. *O. volvulus*, *O. gibsoni*, *D. viteae*, *B. pahangi* and *D. immitis* were determined to be between 2.7 and 3.1 pmol of retinoic acid bound/mg of extractable protein (Sani *et al*. 1985). Vitamin A in its phosphorylated forms, retinyl phosphate and retinyl phosphate mannose, has been suggested as an intermediate in filarial glycoprotein synthesis (Comley and Jaffe 1983). A link between vitamin A deficiency and helminthiasis has also been established (Mahalanabis *et al*. 1976, Storey 1982), indicating that the host vitamin A deficiency is concomitant with the development of onchocercal pathogenesis. Sturchler *et al*. (1981) noted that the concentration of retinol measured in tissues of *O. volvulus* was eight times higher than in the surrounding host tissue and suggested that the worms may selectively sequester retinoids. Embryogenesis of *L. carinii* was found to be delayed frequently in parasites developing in vitamin A deficient cotton rats (Storey 1982). Experiments with radiolabelled retinoic acid in *B. malayi* showed that it was taken up by worms and localized at high concentrations in early and late embryonic forms, further suggesting a role in growth and development (Wolff and Scott 1995). Studies on filarial parasites *in vitro* have also shown that synthetic retinoids can reduce motility, inhibit the release of Mf (Zahner *et al*. 1989) and moulting of the larval stages (Lok *et al*. 1990). As helminths lack a capacity for vision, the main role for retinoids has been suggested to be in the regulation of genes associated with growth and reproduction (Sani and Comley 1985, Sani 1990).

Retinol-binding proteins as targets

Several filarial parasites including *Onchocerca* spp. have been shown to contain specific parasite retinol- and retinoic acid-binding proteins, PRBP and PRABP (Sani *et al*. 1985, Sani and Vaid 1988). They differ from mammalian and avian CRBP and CRABP in physicochemical properties such as molecular weight, isoelectric pH and ligand binding including mercurial sensitivity (Sani *et al*. 1985, Sani and Vaid 1988). Ivermectin, a potent and widely used antiparasitic drug competes efficiently with retinol for retinol-binding sites on PRBP, but not for the host tissue RBP sites (Sani and Vaid 1988). Since retinoids are important molecules that may be involved in the control of normal differentiation, growth and reproduction of the parasites, and since their binding proteins may be important mediators of these biological functions, the specific biochemical differences in molecular charge, size and the mode of ligand interactions that have been observed between the host and parasite binding proteins could be exploited in selective control of parasite growth (Sani *et al*. 1985).

Filarial receptors and channels

Nematodes possess a variety of receptors, receptor subunits and channels, and their expression can be developmentally regulated. Using model organisms like *Caenorhabditis elegans* and animal parasites, it may be possible to determine which genes, encoding such receptors and their subunits, are expressed at specific life cycle stages (Lazdins and Kron 1999). By targeting such receptors it should be possible to selectively obstruct the nervous systems of nematodes.

Acetylcholine receptors as targets

Levamisole exerts its effect at the nicotinic acetylcholine receptor (nAChR). The most convincing evidence that levamisole acts as a cholinergic agonist at the neuromuscular junction, comes from the study on cholinergic receptors of *C. elegans* (Lewis *et al*. 1980). Further support for a cholinergic mechanism for the paralyzing action of levamisole is provided by the observation that mutants of *C. elegans*, highly resistant to the paralyzing effects of levamisole, respond very poorly to cholinergic agonists (Lewis *et al*. 1980). Since excitatory neuromuscular transmission in nematodes is cholinergic, acetylcholine esterase (AchE) is required for the postsynaptic inactivation of acetylcholine. Inhibition of AchE resulted in continued depolarization of postsynaptic junctions with resultant paralysis (Lee and Hodsden 1963, Hart and Lee 1966). Organophosphates such as crufomate, dichlorovos, haloxon and trichlorophon are potent inhibitors of both nematode and mammalian AchE and may result in toxic responses (Hart and Lee 1966, Jamnadas and Thomas 1979).

Glutamate gated chloride channels as targets

Glutamate gated chloride channels (Glu-Cl) are the principal targets through which the avermectins act (Martin 1996, Adelsberger *et al*. 1997, Pemberton *et al*. 2001), although Brownlee *et al*. (1997) also suggested possible interaction with GABA (γ-amino butyric acid) receptors. The distribution of the Glu-Cl subunits reported for *C. elegans* and *H. contortus* suggests that the Glu-Cls have critical roles in controlling pharyngeal function, locomotion, and possibly sensory perception and processing in nematodes, providing evidence that these receptors can be harnessed as drug targets in nematodes (Yates *et al*. 2003).

The microtubular system

The microtubular system has been identified in filarial nematodes *B. malayi* and *B. pahangi* (Helm *et al*. 1989, Guenette *et al*. 1991, Geary *et al*. 1998). Four to five β-tubulin isoforms have also been identified in tubulin-enriched extracts of *B. pahangi* adult worms (Tang and Prichard 1989). Immunogold labelling of adult *B. pahangi* sections with monoclonal antibody (mAb) revealed the presence of β-tubulin isoforms in the cuticle, hypodermal layer and somatic muscle blocks (Bughio *et al*. 1993). In Mf, mAbs were bound to myofibril structures under the hypodermal layer and within cell nuclei (Helm *et al*. 1989).

The microtubular system as a target

Tubulin is of particular interest in parasitic nematodes, since it is the target of benzimidazole carbamates, a class of microtubule depolymerizing drugs (Lacey 1988). The β-tubulin chains are of special interest because benzimidazole resistant strains of *Physarum polycephalum* and *C. elegans* have altered β-tubulins (Foster *et al*. 1987, Driscoll *et al*. 1989). *Brugia* nematodes express β-tubulin molecules that contain binding sites for the benzimidazole carbamates (Tang and Prichard 1989). These drugs act by disrupting the assembly of tubulin dimers into microtubules (Lacey and Pritchard 1986).

Developmental hormones

Filarial parasites pass through a series of five moults, during the whole course of their life cycle. Understanding how the development of these parasitic nematodes is regulated may suggest mechanisms to control and treat infection (Boreham and Atwell 1988, Crossgrove *et al*. 2002). The signals required for moulting are of particular interest in understanding filarial development. One possible candidate for a signal that controls moulting in nematodes is the steroid hormone 20-OH ecdysone (Barker *et al*. 1990). Warbrick *et al*. (1993) showed that ecdysone, or the non-steroidal agonist RH5849, accelerated the initiation of moulting of L3 to L4 larvae *in vitro*. Crossgrove *et al*. (2002) have identified a nuclear receptor gene, *Di-nhr*-7, from the parasitic nematode *D. immitis*, which is the orthologue of the *Drosophila* ecdysone-regulated *E*78 gene. *Di-nhr*-7 also exists in the closely related filarial parasites *B. malayi*, *O. volvulus* and *B. pahangi* (Unnasch *et al*. 1999). Ecdysone has also been shown to affect Mf release in *B. pahangi* and meiotic re-initiation in *D. immitis* oocytes (Barker

et al. 1991). Further, normal development of filarial larvae proceeds only if the worms are located in the correct organ of the arthropod vector or vertebrate host. These requirements suggest that host factors may trigger or are required for further development of the parasites. Filarial parasites possess the ability to respond to mammalian EGF and mammalian growth factors may regulate developmental maturation of filarial parasites (Franke *et al*. 1987, Dissanayake *et al*. 1992, Dissanayake 2000).

Eicosanoid metabolism

Filarial parasites constitutively synthesize and release the eicosanoids, which may act locally in the host and modify host-parasite interactions (Liu *et al*. 1990, 1992; Kanesathasen *et al*. 1991; Kaiser *et al*. 1992). The production of these lipid mediators may be one of the strategies for evading host immune responses (Bianco and Maizels 1989). Their continuous synthesis is therefore, essential for the survival of the parasites. Eicosanoids include prostaglandins (PG) and leukotrienes, and are produced by the enzymatic oxygenation of arachidonic acid (AA) and other polyunsaturated fatty acids (Needleman *et al*. 1986). Filarial parasites cannot produce AA and other polyunsaturated fatty acids *de novo* (Frayha and Smyth 1983), but essential fatty acids are available to Mf in human plasma. Mf avidly incorporate exogenous AA into their phospholipids (Longworth *et al*. 1985, 1987), and can also form AA from exogenous linoleic acid (Liu and Weller 1989).

Parasite elaboration of prostacyclin may enable Mf to inhibit platelet aggregation onto their surfaces (Willis 1987). Mf may release the vasodilatory prostaglandins, prostacyclin and PGE_2, to ease their passage through the microcirculation. PGE_2 inhibits T cell activation, lymphokine production and also appears to induce B cell tolerance and/or unresponsiveness (Goodwin and Ceuppens 1983, Goldings 1986, Schad and Phipps 1989). PGD_2, detected in small amounts, also inhibits platelet aggregation and is a weak vasodilator (Willis 1987).

Eicosanoid metabolism as a target

Filarial AA metabolism may be relevant to the action of DEC. DEC is not directly toxic to filariae (Kanesa-thasan *et al*. 1991), but somehow enables host phagocytes, neutrophils and eosinophils to attack Mf by unknown mechanisms (Mackenzie and Kron 1985, Medina-De la Garza *et al*. 1990, Gutierrez-Pena *et al*. 1996). The inhibition of release of eicosanoids would withdraw from the Mf a capacity to modulate the host's humoral and cellular immune responses, which may render the Mf prone to attacks (Elkhalifa *et al*. 1991, Liu and Weller 1992). It should be the objective of future research to identify the enzymatic systems of parasite involved in eicosanoid biosynthesis. Characterization of these enzymes may allow the development of specific inhibitors of parasite cyclooxygenase and lipoxygenase (Belley and Chadee 1995).

Immunomodulators

Filarial worms survive for long periods within their hosts, during which adult female filariae continuously produce Mf. Active modulation of the host's immune response is part of the parasites' strategies for long-term survival (Maizels *et al*. 1993). Several immunomodulatory components of nematodes were recently described and these studies suggest that secreted parasite products interfere with different arms of the immune system by manipulating the cytokine network, signal transduction pathways or inhibitors of essential enzymes (Hartmann *et al*. 1997, Harnett *et al*. 1999, Gomez-Escobar *et al*. 2000, Schonemeyer *et al*. 2001). Elucidation of these mechanisms will be very useful in the development of immunotherapeutic agents in the future.

Among the secreted nematode immunomodulators, cysteine protease inhibitors (cystatins) are shown to be of major importance. Nematode cystatins inhibit the proteases involved in antigen processing and presentation, which leads to a reduction in T cell responsiveness. At the same time nematode cystatins modulate cytokine responses, the most prominent trait being the upregulation of IL-10 by macrophages. These properties contribute to induction of an antiinflammatory environment, concomitant with a strong inhibition of cellular proliferation. This setting is believed to favour the survival of worms (Hartmann and Lucius 2003). A number of filarial cystatins have now been characterized in *O. volvulus*, *A. viteae*, *B. malayi* and *Litomosoides sigmodontis* (Lustigman *et al*. 1992, Hartmann *et al*. 1997, Manoury *et al*. 2001, Schonemeyer *et al*. 2001, Pfaff *et al*. 2002). In this context, a serine protease inhibitor (serpin) of filariae was shown to inhibit cathepsin G, a serine protease of neutrophil granulocytes with several immunostimulatory properties (Zang *et al*. 1999, Zang and Maizels 2001). Phosphorylcholine, a phospholipid component that is associated with many nematode proteins inhibits the proliferation of T and B cells by interference with the signals that lead to cellular activation and also upregulates the production of IL-10 by B1 cells. This action polarizes the T-helper cell response towards a Th2 type (Al-Qaoud *et al*. 1998, Harnett *et al*. 1999, Harnett and Harnett 2001). Filarial parasites also produce cytokine homologues that are able to bind to and activate immune cells. A functionally active macrophage-migration-inhibitory factor (Pastrana *et al*. 1998) and a transforming growth factor-β homologue (Gomez-Escobar *et al*. 1998, 2000) have been characterized.

Conclusions

Limited knowledge of the biochemical pathways of filariids is still the major impediment to identify promising novel chemotherapeutic targets. Moreover, relatively little work has been done on the biochemistry of filarial worms that are parasitic in humans and the extrapolation of results from one species of filariid to another may not be always justified.

Certain aspects, like carbohydrate, folate and glutathione metabolism of filarial worms have been studied in detail and most of the currently available drugs have been found to interfere with such metabolic pathways. Nevertheless, all the major pathways of filarial lipid, amino acid and nucleic acid metabolism need to be elucidated, if we are to develop new generations of anthelmintics. Among lipids isoprenoid metabolism is one of the best studied and some preliminary studies have been carried out to target it. No truly unique targets for drug development have been identified in filarial amino acid metabolism. As far as protein synthesis is concerned collagen biosynthesizing enzymes are being targeted. DNA topoisomerases are the newly explored targets in nucleic acid metabolism. Our knowledge about filarial amine and polyamine metabolism is still very poor but studies are being pursued to target the key regulatory enzymes. Retinol-binding proteins can be targeted for the treatment of onchocerciasis. Levamisole and avermectins exert their effects on receptors and channels in the nematode nervous system and benzimidazoles disrupt the microtubular system. Filarial steroid hormone ecdysone and mammalian epidermal growth factor are of relevance to filarial growth and moulting, and thus can be targeted also, as can eicosanoids and other immunomodulators, with the aim of interrupting the host-parasite relationship. It is quite clear that filariids differ from their hosts in various aspects of metabolism. Therefore, to continue to exploit filarial metabolism as a tool for a rational approach to chemotherapy, all the major steps in the metabolic pathways of filarial worms must first be identified.

Acknowledgements. Financial assistance from Council for Scientific and Industrial Research, New Delhi (India) in the form of Senior Research Fellowship to SG is greatly acknowledged.

References

Adelsberger H., Scheur T., Dudel J. 1997. A patch clamp study of a glutamatergic chloride channel on pharyngeal muscle of the nematode *Ascaris suum*. *Neuroscience Letters*, 230, 183–186.

Agarwal A., Mishra S.K., Rathaur S., Chatterjee R.K., Katiyar J.C., Ghatak S. 1982. Dopamine-β-hydroxylase in parasitic nematodes. *Indian Journal of Parasitology*, 6, 223–225.

Agarwal A., Saxena J.K., Ghatak S. 1986. Enzymes of tricarboxylic acid cycle in microfilarial and adult stages of *Setaria cervi*. *Indian Journal of Medical Research*, 84, 260–263.

Aisien S.O., Hellmund C., Walter R.D. 1995. Characterization of the arylalkylamine N-acetyltransferase in *Onchocerca volvulus*. *Parasitology Research*, 82, 369–371.

Akinwande A.I., Akinrimisi E.O. 1980. Comparative study of the rates of DNA, RNA and protein synthesis in *Litomosoides carinii* and host tissue. *IRCS Medical Science*, 8, 565–566.

Al-Qaoud K.M., Fleischer B., Hoerauf A. 1998. The Xid defect imparts susceptibility to experimental murine filariosis-association with a lack of antibody and IL-10 production by B cells in response to phosphorylcholine. *International Immunology*, 10, 17–25.

Ansari A.A., Yusufi A.N., Siddiqi M. 1973. Lipid composition of the filarial parasite *Setaria cervi*. *Journal of Parasitology*, 59, 939–940.

Anwar N., Ansari A.A., Ghatak S., Krishna Murti C.R. 1977. *Setaria cervi*: enzymes of glycolysis and PEP-succinate pathway. *Zeitschrift für Parasitenkunde*, 51, 275–283.

Anwar N., Srivastava A.K., Ghatak S. 1978. Effect of antifilarials on the metabolic activity of *Setaria cervi*. *Indian Journal of Parasitology*, 2, 101–105.

Armstrong R.N. 1991. Glutathione-S-transferases: reaction mechanism, structure and function. *Chemical Research in Toxicology*, 4, 131–140.

Asch H.L., Read C.P. 1975. Transtegumental absorption of amino acids by male *Schistosoma mansoni*. *Journal of Parasitology*, 61, 378–379.

Bankov I., Timanova A., Barrett J. 1996. Methionine and cysteine metabolism in *Fasciola hepatica*. *International Journal for Parasitology*, 26, 1401–1404.

Barker G.C., Chitwood D.J., Rees H.H. 1990. Ecdysteroids in helminthes and annelids. *Invertebrate Reproduction and Development*, 18, 1–11.

Barker G.C., Mercer J.G., Rees H.H., Howells R.E. 1991. The effect of ecdysteroids on the microfilarial production of *Brugia pahangi* and the control of meiotic reinitiation in the oocytes of *Dirofilaria immitis*. *Parasitology Research*, 77, 65–71.

Barrett J. 1983. Biochemistry of filarial worms. *Helminthological Abstracts*, *Ser. A*, *Animal and Human Helminthology*, 52, 1–18.

Barrett J., Mendis A.H., Butterworth P.E. 1986. Carbohydrate metabolism in *Brugia pahangi* (Nematoda: Filarioidea). *International Journal for Parasitology*, 16, 465–469.

Behm C.A. 1997. The role of trehalose in the physiology of nematodes. *International Journal for Parasitology*, 27, 215–229.

Behm C.A., Bryant C. 1979. Anthelmintic action – a metabolic approach. *Veterinary Parasitology*, 5, 39–49.

Belley A., Chadee K. 1995. Eicosanoid production by parasites: From pathogenesis to immunomodulation? *Parasitology Today*, 11, 327–334.

Bender D.A. 1985. Amino acid metabolism. Wiley, New York.

Bhargava K.K., Le Trang N., Cerami A., Eaton J.W. 1983. Effect of arsenical drugs on glutathione metabolism of *Litomosoides carinii*. *Molecular and Biochemical Parasitology*, 9, 29–35.

Bianco A.E., Maizels R.M. 1989. Parasite development and adaptive specialization. *Parasitology*, 99, Suppl., S113–S123.

Boreham P.F.L., Atwell R.B. 1988. Dirofilariasis. CRC Press Inc., Boca Raton, Florida.

Brophy P.M., Barrett J. 1990a. Glutathione transferase in helminths. *Parasitology*, 100, 345–349.

Brophy P.M., Barrett J. 1990b. Detoxification of secondary products of lipid peroxidation in the cytosol of a mouse fibroblast cell line. *Biochemistry and Cell Biology*, 68, 1288–1291.

Brophy P.M., Pritchard D.I. 1992. Immunity to helminths: Ready to tip the biochemical balance? *Parasitology Today*, 8, 419–422.

Brophy P.M., Pritchard D.I. 1994. Parasitic helminth glutathione S-transferases: an update on their potential as targets for immuno- and chemotherapy. *Experimental Parasitology*, 79, 89–96.

Brown M.S., Goldstein J.L. 1980. Multivalent feedback regulation of HMG-CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. *Journal of Lipid Research*, 21, 505–517.

Brownlee D.A., Holden-Dye L., Walker R.J. 1997. Action of the anthelmintic ivermectin on the pharyngeal muscle of the parasitic nematode *Ascaris suum*. *Parasitology*, 115, 553–561.

Brownlee D.A., Holden-Dye L., Walker R. 2000. The range and biological activity of FMRC amide-related peptides and classical neurotransmitters in nematodes. *Advances in Parasitology*, 45, 109–180.

Bueding E., Charms B. 1951. Respiratory metabolism of parasitic helminths without participation of the cytochrome system. *Nature*, 167, 149.

Bueding E., Charms B. 1952. Cytochrome *c*, cytochrome oxidase and succinoxidase activities of helminths. *Journal of Biological Chemistry*, 196, 615–627.

Bughio N.I., Faubert G.M., Prichard R. 1993. Characterization and biological activities of anti-*Brugia pahangi* tubulin monoclonal antibodies. *International Journal for Parasitology*, 23, 913–924.

Callahan H.L., Crouch R.K., James E.R. 1988. Helminth anti-oxidant enzymes: A protective mechanism against host oxidants? *Parasitology Today*, 4, 218–225.

Carlberg I., Mannervik B. 1985. Glutathione reductase. *Methods in Enzymology*, 13, 484–490.

Chandra M., Sahay A.N., Pandey D.S., Tripathi R.P., Saxena J.K., Reddy V.J.M., Puerta M.C., Valerga P. 2004. Potential inhibitors of DNA topoisomerases II: ruthenium (II) poly-pyridyl and pyridyl-azine complexes. *Journal of Organometallic Chemistry*, 689, 2256–2267.

Chandrashekar R., Mehta K. 2000. Transglutaminase-catalysed reactions in the growth, maturation and development of parasitic nematodes. *Parasitology Today*, 16, 11–17.

Chen S.N., Howells R.E. 1981. The uptake *in vitro* of monosaccharides, disaccharides and nucleic acid precursors by adult *Dirofilaria immitis*. *Annals of Tropical Medicine and Parasitology*, 75, 329–334.

Comley J.C.W. 1985. Isoprenoid biosynthesis in filariae. *Tropical Medicine and Parasitology*, 36, Suppl., 10–14.

Comley J.C.W., Jaffe J.J. 1981. Isoprenoid biosynthesis in adult *Brugia pahangi* and *Dirofilaria immitis*. *Journal of Parasitology*, 67, 609–616.

Comley J.C.W., Jaffe J.J. 1983. The conversion of exogenous retinol and related compounds into retinyl phosphate mannose by adult *Brugia pahangi in vitro*. *Biochemical Journal*, 214, 367–376.

Comley J.C.W., Jaffe J.J., Chrin L.R. 1982. Glycosyl transferase activity in homogenates of adult *Dirofilaria immitis*. *Molecular and Biochemical Parasitology*, 5, 19–31.

Comley J.C.W., Jaffe J.J., Chrin L.R., Smith R.B. 1981. Synthesis of ubiquinone 9 by adult *Brugia pahangi* and *Dirofilaria immitis*: evidence against its involvement in the oxidation of 5-methyltetrahydrofolate. *Molecular and Biochemical Parasitology*, 2, 271–283.

Comley J.C.W., Lancaster A.R. 1983. Filarial HMG-CoA reductase activity and the effect of mevinolin inhibition on filarial isoprenoid biosynthesis. *Abstract of a paper presented at the American Society of Tropical Medicine and Hygiene, 4–8 December, San Antonio, Texas*.

Cookson E., Blaxter M.L., Selkirk M.E. 1992. Identification of the major soluble cuticular glycoprotein of lymphatic filarial nematode parasites (gp29) as a secretory homolog of glutathione peroxidase. *Proceedings of National Academy of Sciences of the United States of America*, 89, 5837–5841.

Cookson E., Tang L., Selkirk M.E. 1993. Conservation of primary sequence of gp29, the major soluble cuticular glycoprotein, in three species of lymphatic filariae. *Molecular and Biochemical Parasitology*, 58, 155–159.

Cooper A.J. 1983. Biochemistry of sulphur-containing amino acids. *Annual Review of Biochemistry*, 52, 187–222.

Cox G.N. 1992. Molecular and biochemical aspects of nematode collagens. *Journal of Parasitology*, 78, 1–15.

Croll N.A. 1975. Indole alkylamines in the coordination of nematode behavioural activities. *Canadian Journal of Zoology*, 53, 894–903.

Crossgrove K., Laudet V., Maina C.V. 2002. *Dirofilaria immitis* encodes Di-nhr-7, a putative orthologue of the *Drosophila* ecdysone-regulated E78 gene. *Molecular and Biochemical Parasitology*, 119, 169–177.

Cusack S. 1997. Aminoacyl-tRNA synthetases. *Current Opinion in Structural Biology*, 7, 881–889.

Da'dara A.A., Mett H., Walter R.D. 1998. MGBG analogues as potential inhibitors of S-adenosylmethionine decarboxylase of *Onchocerca volvulus*. *Molecular and Biochemical Parasitology*, 97, 13–19.

Dissanayake S. 2000. Upregulation of a Raf kinase and a DP-1 family transcription factor in epidermal growth factor (EGF) stimulated filarial parasites. *International Journal for Parasitology*, 30, 1089–1097.

Dissanayake S., Xu M., Piessens W.F. 1992. Filarial parasites contain a ras protein of the TC4/Ran/Spil family. *Molecular and Biochemical Parasitology*, 56, 259–268.

Driscoll M., Dean E., Reilly E., Bergholz E., Chalfie M. 1989. Genetic and molecular analysis of a *Caenorhabditis elegans* β-tubulin that conveys benzimidazole sensitivity. *Journal of Cell Biology*, 109, 2993–3003.

Dunn T.S., Raines P.S., Barrett J., Butterworth P.E. 1988. Carbohydrate metabolism in *Onchocerca gutturosa* and *Onchocerca lienalis* (Nematoda: Filarioidea). *International Journal for Parasitology*, 18, 21–26.

Elkhalifa M.Y., Ghalib H.W., Dafa'Alla T., Williams J.F. 1991. Suppression of human lymphocyte responses to specific and nonspecific stimuli in human onchocerciasis. *Clinical and Experimental Immunology*, 86, 433–439.

Fairbairn D. 1958. Trehalose and glucose in helminths and other invertebrates. *Canadian Journal of Zoology*, 36, 787–795.

Foster K.E., Burland T.G., Gull K. 1987. A mutant β-tubulin confers resistant to the action of benzimidazole-carbamate microtubule inhibitors both *in vivo* and *in vitro*. *European Journal of Biochemistry*, 163, 449–455.

Franke E.D., Riberu W., Wiady I. 1987. *In vitro* cultivation of third stage larvae of *Wuchereria bancrofti* to the fourth stage. *American Journal of Tropical Medicine and Hygiene*, 37, 370–375.

Frayha G.J., Smyth J.D. 1983. Lipid metabolism in parasitic helminths. *Advances in Parasitology*, 22, 309–387.

Fuhrman J.A. 1995. Filarial chitinases. *Parasitology Today*, 11, 259–261.

Fuhrman J.A., Lane W.S., Smith R.F., Piessens W.F., Perler F.B. 1992. Transmission-blocking antibodies recognize microfilarial chitinase in brugian lymphatic filariasis. *Proceedings of National Academy of Sciences of the United States of America*, 89, 1548–1552.

Gainutsos G., Bennett J.L. 1977. The regional distribution of dopamine and norepinephrine in *Schistosoma mansoni* and *Fasciola hepatica*. *Comparative Biochemistry and Physiology*, 58, 157–159.

Geary T.G., Nulf S.C., Alexander-Bowman S.J., Mahmoud M., Prichard R.K., Klein R.D. 1998. Cloning and characterization of cDNAs encoding β-tubulin from *Dirofilaria immitis* and *Onchocerca volvulus*. *Journal of Parasitology*, 84, 356–360.

Ginger C.D. 1991. Filarial worms: Targets for drugs. *Parasitology Today*, 10, 262–264.

Goldings E.A. 1986. Regulation of B cell tolerance by macrophage-derived mediators: Antagonistic effects of prostaglandin E2 and interleukin 1. *Journal of Immunology*, 136, 817–822.

Gomez-Bautista M., Barrett J. 1988. Cysteine metabolism in the cestode *Hymenolepis diminuta*. *Parasitology*, 97, 149–159.

Gomez-Escobar N., Gregory W.F., Maizels R.M. 2000. Identification of tgh-2, a filarial nematode homolog of *Caenorhabditis elegans* daf-7 and human transforming growth factor beta, expressed in microfilarial and adult stage of *Brugia malayi*. *Infection and Immunity*, 68, 6402–6410.

Gomez-Escobar N., Lewis E., Maizels R.M. 1998. A novel member of the transforming growth factor-beta (TGF-beta) superfam-

ily from the filarial nematodes *Brugia malayi* and *Brugia pahangi*. *Experimental Parasitology*, 88, 200–209.

Gooday G.W., Brydon L.J., Chappell L.H. 1988. Chitinase in female *Onchocerca gibsoni* and its inhibition by allosamidin. *Molecular and Biochemical Parasitology*, 29, 223–225.

Goodwin J.S., Ceuppens J. 1983. Regulation of the immune response by prostaglandins. *Journal of Clinical Immunology*, 3, 295–315.

Griffith O.W. 1987. Mammalian sulphur amino acid metabolism: an overview. *Methods in Enzymology*, 143, 366–376.

Guenette S., Prichard R.K., Klein R.D., Matlashewski G. 1991. Characterization of a beta-tubulin gene and beta-tubulin gene products of *Brugia pahangi*. *Molecular and Biochemical Parasitology*, 44, 153–164.

Gutierrez-Pena E.J., Knab J., Buttner D.W. 1996. Neutrophil granule proteins: evidence for the participation in the host reaction to skin microfilariae of *Onchocerca volvulus* after diethylcarbamazine administration. *Parasitology*, 113, 403–414.

Hack M.G., Gussin A.E., Lowe M.E. 1962. Comparative lipid biochemistry-I. Phosphatides of invertebrates (Porifera to Chordata). *Comparative Biochemistry and Physiology*, 5, 217–221.

Hanauske-Abel H.M. 1991. Prolyl 4-hydroxylase, a target enzyme for the drug development: Design of suppressive agents and *in vitro* effects of inhibitors and proinhibitors. *Journal of Hepatology*, 13, Suppl. 3, S8–S16.

Harnett W., Deehan M.R., Houston K.M., Harnett M.M. 1999. Immunomodulatory properties of a phosphorylcholine-containing secreted filarial glycoprotein. *Parasite Immunology*, 21, 601–608.

Harnett W., Harnett M.M. 2001. Modulation of the host immune system by phosphorylcholine-containing filarial glycoproteins secreted by parasitic filarial nematodes. *Biochimica et Biophysica Acta*, 1539, 7–15.

Hart R.J., Lee R.M. 1966. Cholinesterase activities of various nematode parasites and their inhibition by the organophosphate anthelmintic haloxon. *Experimental Parasitology*, 18, 332–337.

Hartmann S., Kyewski B., Sonnenburg B., Lucius R. 1997. A filarial cysteine protease inhibitor down regulates T cell proliferation and enhances interleukin-10 production. *European Journal of Immunology*, 27, 2253–2260.

Hartmann S., Lucius R. 2003. Modulation of host immune responses by nematode cystatins. *International Journal for Parasitology*, 33, 1291–1302.

Hayashi H., Oya H. 1978. Comparative study on the alternative electron transport pathway in *Clonorchis sinensis*, *Dirofilaria immitis* and *Angiostrongylus cantonensis*. *IVth International Congress of Parasitology*, *19–26 Warsaw*, *Poland*, 73–74.

Helm R., Selkirk M.E., Bradley J.E., Burns R.G., Hamilton A.J., Croft J., Maizels R.M. 1989. Localization and immunogenicity of tubulin in the filarial nematode *Brugia malayi* and *Brugia pahangi*. *Parasite Immunology*, 11, 479–502.

Hutchison W.F., Turner A.C. 1979. Glycerol metabolism in the adult dog heartworm, *Dirofilaria immitis*. *Comparative Biochemistry and Physiology*, *B*, *Comparative Biochemistry*, 64, 399–401.

Hutchison W.F., Turner A.C., Grayson D.P., White H.B. 1976. Lipid analysis of the adult dog heartworm, *Dirofilaria immitis*. *Comparative Biochemistry and Physiology*, *B*, *Comparative Biochemistry*, 53, 495–497.

Isaac R.E., Muimo R., MacGregor A.N. 1990. N-acetylation of serotonin, octopamine and dopamine by adult *Brugia pahangi*. *Science*, 216, 1012–1014.

Jaffe J.J. 1972. Dihydrofolate reductase in parasitic protozoa and helminths. In: *Comparative biochemistry of parasites* (Ed. H. Van den Bossche). Academic Press, New York, 219–233.

Jaffe J.J. 1980a. Folate related metabolism. In: *Report of the Vth Meeting of the Scientific Working Group on Filariasis*. WHO, Geneva, 19–20.

Jaffe J.J. 1980b. Filarial folate-related metabolism as a potential target for selective inhibitors. In: *Host-invader interplay* (Ed. H. Van den Bossche). Elsevier/North-Holland, Amsterdam, 605–614.

Jaffe J.J., Chrin L.R. 1979a. 10-Formyltetrahydrofolate synthetase activity in normal and *Brugia pahangi*-infected *Aedes aegypti*. *Journal of Parasitology*, 65, 226–232.

Jaffe J.J., Chrin L.R. 1979b. *De novo* synthesis of methionine in normal and *Brugia pahangi*-infected *Aedes aegypti*. *Journal of Parasitology*, 65, 550–554.

Jaffe J.J., Chrin L.R. 1980. Folate metabolism in filariae: enzymes associated with 5,10-methylenetetrahydrofolate. *Journal of Parasitology*, 66, 53–58.

Jaffe J.J., Chrin L.R. 1981. Involvement of tetrahydrofolate cofactors in *de novo* purine ribonucleotide synthesis by adult *Brugia pahangi* and *Dirofilaria immitis*. *Molecular and Biochemical Parasitology*, 2, 259–270.

Jaffe J.J., Chrin L.R., Smith R.B. 1980. Folate metabolism in filariae. Enzymes associated with 5,10-methenyltetrahydrofolate and 10-formyltetrahydrofolate. *Journal of Parasitology*, 66, 428–433.

Jaffe J.J., Doremus H.M. 1970. Metabolic patterns of *Dirofilaria immitis* microfilariae *in vitro*. *Journal of Parasitology*, 56, 254–260.

Jaffe J.J., McCormack J.J., Meymarian E. 1972. Comparative properties of schistosomal and filarial dihydrofolate reductases. *Biochemical Pharmacology*, 21, 719–731.

Jaffe J.J., McCormack J.J., Meymarian E., Doremus H.M. 1977. Comparative activity and properties of lactate dehydrogenase, xanthine dehydrogenase and dihydrofolate reductase in normal and *Brugia pahangi*-infected *Aedes aegypti*. *Journal of Parasitology*, 63, 547–553.

Jamnadas V., Thomas J. 1979. Metriphonate and organophosphate poisoning. *Central African Journal of Medicine*, 25, 130.

Jones W.D., Rothwell T.L.W., Dineen J.K., Griffith D.A. 1974. Studies on the role of histamine and 5-hydroxytryptamine in immunity against the nematode, *Trichostrongylus columbriformis* II. Amine levels in the intestine of infected guinea pig. *International Archives of Allergy and Applied Immunology*, 46, 14–27.

Kaiser L., Lamb V.L., Tithof P.K., Gage D.A., Chamberlin B.A., Watson J.T., Williams J.F. 1992. *Dirofilaria immitis*: do filarial cyclooxygenase products depress endothelium dependent relaxation in the *in vitro* rat aorta? *Experimental Parasitology*, 75, 159–167.

Kanesa-thasan N., Douglas J.G., Kazura J.W. 1991. Diethylcarbamazine inhibits endothelial and microfilarial prostanoid metabolism *in vitro*. *Molecular and Biochemical Parasitology*, 49, 11–19.

Katiyar D., Tiwari V.K., Tripathi R.P., Reddy V.M., Bhattacharya S.M., Saxena J.k. 2003. Synthesis and antifilarial evaluation of 7-O-acetamidyl-4-alkyl-2H-1-benzopyran-2-ones. *Arzneimittelforschung*, 53, 857– 862.

Kivirikko K.I., Mallyla R., Pihlajaniemi T. 1989. Protein hydroxylation: Prolyl 4-hydroxylase, an enzyme with four cosubstrates and a multifunctional subunit. *Federation of American Societies for Experimental Biology Journal*, 3, 1609–1617.

Köhler P. 1991. The pathways of energy generation in filarial parasites. *Parasitology Today*, 7, 21–25.

Kozak E.M., Tate S.S. 1982. Glutathione-degrading enzymes of the microvillus membranes. *Journal of Biological Chemistry*, 257, 6322–6327.

Krohne-Ehrich G., Schirmer R.H., Untucht-Grau R. 1977. Glutathione reductase from human erythrocytes. Isolation of the enzyme and sequence analysis of the redox-active peptide. *European Journal of Biochemistry*, 80, 65–71.

Kurelec B., Rijavec M. 1966. Amino acid pool of the liver fluke (*Fasciola hepatica* L.). *Comparative Biochemistry and Physiology*, 19, 525–531.

Lacey E. 1988. The role of cytoskeletal protein, tubulin, in the mode of action and mechanism of drug resistance to benzimidazoles. *International Journal for Parasitology*, 18, 885–936.

Lacey E., Pritchard R.K. 1986. Interactions of benzimidazoles (BZ) with tubulin from BZ-sensitive and BZ-resistant isolates of *Haemonchus contortus*. *Molecular and Biochemical Parasitology*, 19, 171–181.

Lazdins J., Kron M. 1999. New molecular targets for filariasis drug discovery. *Parasitology Today*, 15, 305–306.

Lee R.M., Hodsden M.R. 1963. Cholinesterase activity in *Haemonchus contortus* and its inhibition by organophosphorus anthelmintics. *Biochemical Pharmacology*, 12, 1241–1252.

Lewis J., Wu C., Levine J., Berg H. 1980. Levamisole-resistant mutants of the nematode *Caenorhabditis elegans* appear to lack pharmacological acetylcholine receptors. *Neuroscience*, 5, 967–989.

Liebau E., Schonberger O.L., Walter R.D., Henkle-Duhrsen K. 1994a. Molecular cloning and expression of a cDNA encoding glutathione S-transferase from *Ascaris suum*. *Molecular and Biochemical Parasitology*, 63, 167–170.

Liebau E., Walter R.D., Henkle-Duhrsen K. 1994b. Isolation, sequence and expression of an *Onchocerca volvulus* glutathione S-transferase cDNA. *Molecular and Biochemical Parasitology*, 63, 305–309.

Liebau E., Walter R.D., Henkle-Duhrsen K. 1994c. *Onchocerca volvulus*: Isolation and sequence of a second glutathione S-transferase cDNA. *Experimental Parasitology*, 79, 68–71.

Liebau E., Wildenburg G., Brophy P.M., Walter R.D., Henkle-Duhrsen K. 1994d. A novel type of glutathione S-transferase in *Onchocerca volvulus*. *Infection and Immunity*, 62, 4762–4767.

Liu L.X., Buhlmann J.E., Weller P.F. 1992. Release of prostaglandin E2 by microfilariae of *Wuchereria bancrofti* and *Brugia malayi*. *American Journal of Tropical Medicine and Hygiene*, 46, 520–523.

Liu L.X., Serhan C.N., Weller P.F. 1990. Intravascular filarial parasites elaborate cyclooxygenase-derived eicosanoids. *Journal of Experimental Medicine*, 172, 993–996.

Liu L.X., Weller P.F. 1989. *Brugia malayi*: microfilarial polyunsaturated fatty acid composition and synthesis. *Experimental Parasitology*, 69, 198–203.

Liu L.X., Weller P.F. 1992. Intravascular filarial parasites inhibit platelet aggregation: role of parasite-derived prostanoids. *Journal of Clinical Investigation*, 89, 1113–1120.

Lok J.B., Morris R.A., Sani B.P., Shealy Y.F., Donnelly J.J. 1990. Synthetic and naturally occurring retinoids inhibit third- to fourth-stage larval development by *Onchocerca lienalis in vitro*. *Tropical and Medical Parasitology*, 41, 169–173.

Lomaestro B.M., Malone M. 1995. Glutathione in health and disease: Pharmacotherapeutic issues. *Annals of Pharmacotherapy*, 29, 1263–1273.

Longworth D.L., Foster D.W., Dvorak A.M., Weller P.F. 1985. Incorporation of arachidonic acid by microfilariae of *Brugia malayi*. *Journal of Infectious Diseases*, 152, 1317–1323.

Longworth D.L., King D.C., Weller P.F. 1987. Rapid uptake and esterification of arachidonic acid and other fatty acids by microfilariae of *Brugia malayi*. *Molecular and Biochemical Parasitology*, 23, 275–284.

Lüersen K., Müller S., Hussein A., Liebau E., Walter R.D. 2000. The γ-glutamylcysteine synthetase of *Onchocerca volvulus*. *Molecular and Biochemical Parasitology*, 111, 243–251.

Lustigman S., Brotman B., Huima T., Castelhano A.L., Singh R.N., Mehta K., Prince A.M. 1995. Transglutaminase-catalysed reaction is important for molting of *Onchocerca volvulus* third stage larvae. *Antimicrobial Agents and Chemotherapy*, 39, 1913–1919.

Lustigman S., Brotman B., Huima T., Prince A.M., McKerrow J.H. 1992. Molecular cloning and characterization of onchocystatin, a cysteine protease inhibitor of *Onchocerca volvulus*. *Journal of Biological Chemistry*, 267, 17339–17346.

Mackenzie C.D., Kron M.A. 1985. Diethylcarbamazine: a review of its action in onchocerciasis, lymphatic filariasis and inflammation. *Tropical Diseases Bulletin*, 82, 1–37.

Mahalanabis D., Jalan K.N., Maitra T.K., Agarwal S.R. 1976. Vitamin A absorption in ascariasis. *American Journal of Clinical Nutrition*, 29, 1372–1375.

Maizels R.M., Bundy D.A.P, Selkirk M.E., Smith D.F., Anderson R.M. 1993. Immunological modulation and evasion by helminthic parasites in human populations. *Nature*, 365, 797–804.

Maizels R.M., Gregory W.F., Kwan-Lim G.E., Selkirk M.E. 1989. Filarial surface antigens: the major 29-kilodalton glycoprotein and a novel 17–200 kilodalton complex from adult *Brugia malayi* parasites. *Molecular and Biochemical Parasitology*, 32, 213–227.

Manoury B., Gregory W.F., Maizels R.M., Watts C. 2001. Bm-CPI-2, a cystatin homolog secreted by the filarial parasite *Brugia malayi*, inhibits class II MHC-restricted antigen processing. *Current Biology*, 11, 447–451.

Martin R.J. 1996. An electrophysiological preparation of *Ascaris suum* pharyngeal muscle reveals a glutamate-gated chloride channel sensitive to the avermectin analogue, milbemycin D. *Parasitology*, 112, 247–252.

McCarthy J.S., Wieseman M., Tropea J., Kaslow D., Abraham D., Lustigman S., Tuan R., Guderian R.H., Nutman T.B. 2002. *Onchocerca volvulus* glycolytic enzyme fructose-1,6-bisphosphate aldolase as a target for a protective immune response in humans. *Infection and Immunity*, 70, 851–858.

McManus D.P. 1986. Intermediary metabolism in parasitic helminths. *International Journal for Parasitology*, 17, 79–95.

Medina-De la Garza C.E., Brattig N.W., Tischendorf F.W., Jarrett J.M. 1990. Serum-dependent interaction of granulocytes with *Onchocerca volvulus* microfilariae in generalised and chronic hyperreactive onchocerciasis and its modulation by diethylcarbamazine. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 84, 701–706.

Mehta K., Rao U.R., Vickery A.C., Birckbichler P.J. 1990. Significance of transglutaminase-catalyzed reactions in growth and development of filarial parasite, *Brugia malayi*. *Biochemical and Biophysical Research Communications*, 173, 1051–1057.

Mehta K., Rao U.R., Vickery A.C., Fesus L. 1992. Identification of a novel transglutaminase from the filarial parasite *Brugia malayi* and its role in growth and development. *Molecular and Biochemical Parasitology*, 53, 1–15.

Meister A. 1983. Selective modification of glutathione metabolism. *Science*, 220, 472–477.

Mendis A.H., Rose M.E., Rees H.H., Goodwin T.W. 1983. Ecdysteroids in adults of the nematode *Dirofilaria immitis*. *Molecular and Biochemical Parasitology*, 9, 209–226.

Mendis A.H., Townson S. 1985. Evidence for the occurrence of the respiratory electron transport in adult *Brugia pahangi* and *Dipetalonema viteae*. *Molecular and Biochemical Parasitology*, 14, 337–354.

Middleton K.R., Saz H.J. 1979. Comparative utilization of pyruvate by *Brugia pahangi*, *Dipetalonema viteae* and *Litomosoides carinii*. *Journal of Parasitology*, 65, 1–7.

Müller S., Lüchow A., McCann P.P., Walter R.D. 1991. Effect of bis(benzyl)polyamine derivatives on polyamine transport and survival of *Brugia pahangi*. *Parasitology Research*, 77, 612–615.

Müller S., Walter R.D., Fairlamb A.H. 1995. Differential susceptibility of filarial and human erythrocyte glutathione reductase to inhibition by the trivalent organic arsenical melarsen oxide. *Molecular and Biochemical Parasitology*, 71, 211–219.

Müller S., Wittich R.M., Walter R.D. 1988. The polyamine metabolism of filarial worms as chemotherapeutic target. *Advances in Experimental Medicine and Biology*, 250, 737–743.

Needleman P., Turk J., Jakschik B.A., Morrison A.R., Lefkowith J.B. 1986. Arachidonic acid metabolism. *Annual Review of Biochemistry*, 55, 69–102.

Nelson N.F., Saz H.J. 1984. Effects of amoscanate on the utilisation of glucose by *Brugia pahangi* and *Litomosoides carinii*. *Journal of Parasitology*, 70, 194–196.

Oppenheimer L., Wellner V.P., Griffith O.W., Meister A. 1979. Glutathione synthetase: Purification from rat kidney and mapping of the substrate binding sites. *Journal of Biological Chemistry*, 254, 5184–5190.

Orlowski M., Meister A. 1973. γ-Glutamyl cyclotransferase. Distribution, isozymic forms, and specificity. *Journal of Biological Chemistry*, 248, 2836–2844.

Pastrana D.V., Raghavan N., FitzGerald P., Eisinger S.W., Metz C., Bucala R., Schleimer R.P., Bickel C., Scott A.L. 1998. Filarial nematode parasites secrete a homologue of the human cytokine macrophage migration inhibitory factor. *Infection and Immunity*, 66, 5955–5963.

Pegg A.E., Kameji T., Shirahata A., Stanley B., Madhubala R., Pajunen A. 1988. Regulation of mammalian S-adenosylmethionine decarboxylase. *Advances in Enzyme Regulation*, 27, 43–55.

Pemberton D.J., Franks C.J., Walker R.J., Holden-Dye L. 2001. Characterization of glutamate-gated chloride channels in the pharynx of wild type and mutant *Caenorhabditis elegans* delineates the role of the subunit GluCl-alpha 2 in the function of the native receptor. *Molecular Pharmacology*, 59, 1037–1043.

Pfaff A.W., Schulz-Key H., Soboslay P.T., Taylor D.W., MacLennan K., Hoffmann W.H. 2002. *Litomosoides sigmodontis* cystatin acts as an immunomodulator during experimental filariasis. *International Journal for Parasitology*, 32, 171–178.

Plummer J.L., Smith B.R., Sies S., Bend J.R. 1981. Chemical depletion of glutathione *in vivo*. *Methods in Enzymology*, 77, 50–59.

Powell J.W., Stables J.N., Watt R.A. 1986a. An investigation of the glucose metabolism of *Brugia pahangi* and *Dipetalonema viteae* by nuclear magnetic resonance spectroscopy. *Molecular and Biochemical Parasitology*, 18, 171–182.

Powell J.W., Stables J.N., Watt R.A. 1986b. An NMR study on the effect of glucose availability on carbohydrate metabolism in *Dipetalonema viteae* and *Brugia pahangi*. *Molecular and Biochemical Parasitology*, 19, 265–271.

Precious W.Y., Barrett J. 1989. Xenobiotic metabolism in helminths. *Parasitology Today*, 5, 156–160.

Prichard R.K. 1973. The fumarate reductase reaction of *Haemonchus contortus* and the mode of action of some anthelmintics. *International Journal for Parasitology*, 3, 409–417.

Raghavan N., Freedman D.O., Fitzgerald P.C., Unnasch T.R., Ottesen E.A., Nutman T.B. 1994. Cloning and characterization of a potentially protective chitinase-like recombinant antigen from *Wuchereria bancrofti*. *Infection and Immunity*, 62, 1901–1908.

Ramp T., Köhler P. 1984. Glucose and pyruvate catabolism in *Litomosoides carinii*. *Parasitology*, 89, 229–244.

Rao U.R., Mehta K., Subrahmanyam D., Vickery A.C. 1991. *Brugia malayi* and *Acanthocheilonema viteae*: antifilarial activity of transglutaminase inhibitors *in vitro*. *Antimicrobial Agents and Chemotherapy*, 35, 2219–2224.

Rathaur S. 1980. Study of intermediary metabolism in microfilarial and adult stages of bovine filarial parasite *Setaria cervi*. PhD Thesis, Banaras Hindu University, Varanasi, India.

Rathaur S., Anwar N., Ghatak S. 1980. Biochemical composition of microfilarial and adult stages of *Setaria cervi*. *Zeitschrift für Parasitenkunde*, 62, 85–93.

Rathaur S., Kaushal N.A., Ghatak S. 1985. Biogenic amines and their metabolites in microfilariae and adults of *Setaria cervi*. *Indian Journal of Parasitology*, 9, 203–205.

Rew R.S., Saz H.J. 1977. The carbohydrate metabolism of *Brugia pahangi* microfilariae. *Journal of Parasitology*, 63, 123–129.

Ross A.C. 1993a. Cellular metabolism and activation of retinoids: roles of cellular retinoid-binding proteins. *Federation of American Societies for Experimental Biology Journal*, 7, 317–327.

Ross A.C. 1993b. Overview of retinoid metabolism. *Journal of Nutrition*, 123, 346–350.

Sakuda S., Isogai A., Matsumoto S., Suzuki A. 1987. Search for microbial insect growth regulators II. Allosamidin, a novel insect chitinase inhibitor. *Journal of Antibiotics* (*Tokyo*), 40, 296–300.

Salinas G., Braun G., Taylor D.W. 1994. Molecular characterization and localization of an *Onchocerca volvulus* pi-class glutathione S-transferase. *Molecular and Biochemical Parasitology*, 66, 1–9.

Sani B.P. 1990. Parasite retinoid-binding proteins. *Methods in Enzymology*, 189, 348–356.

Sani B.P., Comley J.C.W. 1985. Role of retinoids and their binding proteins in filarial parasites and host tissues. *Tropical Medicine and Parasitology*, 36, Suppl. 1, 20–23.

Sani B.P., Vaid A. 1988. Specific interaction of ivermectin with retinol-binding protein from filarial parasites. *Biochemical Journal*, 249, 929–932.

Sani B.P., Vaid A., Comley J.C.W., Montgomery J.A. 1985. Novel retinoid-binding proteins from filarial parasites. *Biochemical Journal*, 232, 577–583.

Saxena J.K., Bose S.K., Sen R., Chatterjee R.K., Sen A.B., Ghatak S. 1977. *Litomosoides carinii*: Biogenic amines in microfilariae and adults. *Experimental Parasitology*, 43, 239–243.

Saz H.J. 1981. Biochemical aspects of filarial parasites. *Trends in Biochemical Sciences*, 6, 117–119.

Saz H.J., Dunbar G.A. 1975. The effect of stibophen on phosphofructokinases and aldolases of adult filariids. *Journal of Parasitology*, 61, 794–801.

Schad V., Phipps R.P. 1989. Prostaglandin E2-dependent induction of B cell unresponsiveness. Role of surface Ig and Fc receptors. *Journal of Immunology*, 143, 2127–2132.

Schirmer R.H., Schollhammer T., Eisenbrand G., Krauth-Siegel R.L. 1987. Oxidative stress as a defence mechanism against parasitic infections. *Free Radical Research Communications*, 3, 3–12.

Schonemeyer A., Lucius R., Sonnenburg B., Brattig N., Sabat R., Schilling K., Bradley J., Hartmann S. 2001. Modulation of tumor T cell responses and macrophage function by onchocystatin, a secreted protein of the filarial nematode *Onchocerca volvulus*. *Journal of Immunology*, 167, 3207–3215.

Seelig G.F., Meister A. 1982. Cystamine-sepharose: A probe for the active site of γ-glutamylcysteine synthetase. *Journal of Biological Chemistry*, 257, 5092–5096.

Shahabuddin M., Toyoshima T., Aikawa M., Kaslow D.C. 1993. Transmission-blocking activity of a chitinase inhibitor and activation of malarial parasite chitinase by mosquito protease. *Proceedings of National Academy of Sciences of the United States of America*, 90, 4266–4270.

Sharma V., Tekwani B.L., Saxena J.K., Gupta S., Katiyar J.C., Chatterjee R.K., Ghatak S., Shukla O.P. 1991. Polyamine metabolism in some helminth parasites. *Experimental Parasitology*, 72, 15–23.

Singh D.L., Rathaur S., Misra S., Chatterjee R.K., Ghatak S., Sen A.B. 1985. Studies on causation of adverse reactions in microfilariaemic host following diethylcarbamazine therapy (*Di-*

petalonema viteae in *Mastomys natalensis*). *Tropical Medicine and Parasitology*, 36, 21–24.

Singh R.N., Chandrashekar R., Mehta K. 1995. Purification and partial characterization of a transglutaminase from dog filarial parasite, *Dirofilaria immitis*. *International Journal of Biochemistry and Cell Biology*, 27, 1285–1291.

Singh R.P., Saxena J.K., Ghatak S., Shukla O.P., Wittich R.M., Walter R.D. 1989. Polyamine metabolism in *Setaria cervi*. *Parasitology Research*, 75, 311–315.

Singh S.N., Srivastava A.K., Chatterjee R.K. 1996. Gamma-glutamyl transpeptidase in adult *Setaria cervi* and *Acanthocheilonema viteae* and the effect of inhibitors. *Journal of Parasitic Diseases*, 20, 163–166.

Srivastava A.K., Jaffe J.J. 1985. Phosphatidylcholine synthesis in adult *Dirofilaria immitis* females. *International Journal for Parasitology*, 15, 27–31.

Srivastava A.K., Jaffe J.J. 1986. Phosphatidylserine synthesis in adult *Dirofilaria immitis* females. *International Journal for Parasitology*, 16, 9–11.

Srivastava A.K., Jaffe J.J. 1987. Phosphatidylinositol, phosphatidylglycerol and cardiolipin synthesis in adult *Dirofilaria immitis* females. *International Journal for Parasitology*, 17, 917–920.

Srivastava A.K., Jaffe J.J., Lambert R.A. 1985. Phosphatidylethanolamine synthesis in adult *Dirofilaria immitis* females. *International Journal for Parasitology*, 15, 429–433.

Srivastava A.K., Walter R.D., Jaffe J.J. 1987. Synthetic pathways of glycerophospholipids in adult *Brugia pahangi* and *Brugia patei*. *International Journal for Parasitology*, 17, 1321–1328.

Srivastava D.K., Roy T.K., Shukla O.P. 1980. Polyamines of helminths. *Indian Journal of Parasitology*, 4, 187–189.

Srivastava V.M.L., Chatterjee R.K., Sen A.B., Ghatak S., Krishna Murti C.R. 1970. Glycolysis in *Litomosoides carinii*, the filarial parasite of cotton rat. *Experimental Parasitology*, 28, 176–185.

Srivastava V.M.L., Ghatak S. 1971. Glycolytic and carbondioxide metabolising enzymes in *Chandlerella hawkingi*. *Indian Journal of Biochemistry and Biophysics*, 8, 108–111.

Srivastava V.M.L., Saz H.J., deBruyn B. 1988. Comparisons of glucose and amino acid use in adults and microfilariae of *Brugia pahangi*. *Parasitology Research*, 75, 1–6.

Storey D.M. 1982. Vitamin A deficiency and the development of *Litomosoides carinii* (Nematoda, Filarioidea) in cotton rats. *Zeitschrift für Parasitenkunde*, 67, 309–315.

Sturchler D., Hanck A., Weiser H., Marn U., Weiss N. 1985. Retinol deficiency and *Dipetalonema viteae* infection in the hamster. *Journal of Helminthology*, 59, 201–210.

Sturchler D., Wyss F., Hanck A. 1981. Retinol, onchocerciasis and *Onchocerca volvulus*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 75, 617.

Subrahmanyam D. 1967. Glyceral ether phospholipids and plasmalogens of filarial parasite *Litomosoides carinii*. *Canadian Journal of Biochemistry*, 45, 1195–1197.

Subrahmanyam D. 1983. Problems in filariasis: current approaches in development of antifilarials. In: *Tropical parasitoses and parasitic zoonoses* (Ed. J.D. Dunsmore). *Proceedings of the 10th International Conference of World Association for the Advancement of Veterinary Parasitology*, *Perth*, *Australia*, 107–123.

Subrahmanyam D. 1987. Antifilarials and their mode of action. Ciba Foundation Symposium, 127, 246–264.

Svensson F., Kockum I., Persson L. 1993. Diethylglyoxal bis(guanylhydrazone), a potent inhibitor of mammalian S-adenosylmethionine decarboxylase. Effects on cell proliferation and polyamine metabolism in L1210 leukemia cells. *Molecular and Cellular Biochemistry*, 124, 141–147.

Tabor C.W., Tabor H. 1984. Polyamines. *Annual Review of Biochemistry*, 53, 749–790.

Tang L., Prichard R.K. 1989. Characterization of tubulin from *Brugia malayi* and *Brugia pahangi*. *Molecular and Biochemical Parasitology*, 32, 145–152.

Tang L., Smith V.P., Gounaris K., Selkirk M.E. 1996. *Brugia pahangi*: the cuticular glutathione peroxidase (gp29) protects heterologous membranes from lipid peroxidation. *Experimental Parasitology*, 82, 329–332.

Tate S.S., Meister A. 1985. γ-Glutamyl transpeptidase from kidney. *Methods in Enzymology*, 113, 400–419.

Tiwari V.K., Tewari N., Katiyar D., Tripathi R.P., Arora K., Gupta S., Ahmad R., Srivastava A.K., Khan M.A., Murthy P.K., Walter R.D. 2003. Synthesis and antifilarial evaluation of N^1,N^n-xylofuranosylated diaminoalkanes. *Bioorganic and Medicinal Chemistry*, 11, 1789–1800.

Tripathi R.P., Rastogi S.K., Kundu B., Saxena J.K., Reddy V.J.M., Srivastava S., Chandra S., Bhaduri A.P. 2001. Identification of inhibitors of DNA topoisomerases II from a synthetic library of glycoconjugates. *Combinatorial Chemistry and High Throughput Screening*, 4, 237–244.

Tripp C., Frank R.S., Selkirk M.E., Tang L., Grieve M.M., Frank G.R., Grieve R.B. 1998. *Dirofilaria immitis*: molecular cloning and expression of a cDNA encoding a selenium-independent secreted glutathione peroxidase. *Experimental Parasitology*, 88, 43–50.

Turner A.C., Hutchison W.F. 1979. Lipid synthesis in the adult dog heartworm, *Dirofilaria immitis*. *Comparative Biochemistry and Physiology*, *B*, *Comparative Biochemistry*, 64, 403–405.

Ueda T., Sawada T. 1968. Fatty acid and sugar compositions of helminths. *Japanese Journal of Experimental Medicine*, 38, 145–147.

Unnasch T.R., Bradley J., Beauchamp J., Tuan R., Kennedy M.W. 1999. Characterization of a putative nuclear receptor from *Onchocerca volvulus*. *Molecular and Biochemical Parasitology*, 104, 259–269.

Van den Bossche H., de Nollin S. 1973. Effects of mebendazole on the absorption of low molecular weight nutrients by *Ascaris suum*. *International Journal for Parasitology*, 3, 401–407.

Walker J., Barrett J. 1991a. Cystathionine β-synthase and γ-cystathionase in helminths. *Parasitology Research*, 77, 709–713.

Walker J., Barrett J. 1991b. Pyridoxal 5'-phosphate dependent enzymes in the nematode *Nippostrongylus brasiliensis*. *International Journal for Parasitology*, 21, 641–649.

Walker J., Barrett J. 1997. Parasite sulphur amino acid metabolism. *International Journal for Parasitology*, 27, 883–897.

Walter R.D. 1988. Polyamine metabolism of filarial and allied parasites. *Parasitology Today*, 4, 18–20.

Walter R.D., Albiez E.J. 1981. Inhibition of NADP-linked enzyme from *Onchocerca volvulus* and *Dirofilaria immitis* by suramin. *Molecular and Biochemical Parasitology*, 4, 53–60.

Walter R.D., Ossikovski E., Albiez E.J. 1985. Dolichol kinase from *Onchocerca volvulus* and *Ascaris suum*. *Molecular and Biochemical Parasitology*, 14, 211–217.

Walter R.D., Schulz-Key H. 1980. *Onchocerca volvulus*: effect of suramin on lactate dehydrogenase and malate dehydrogenase. *Tropenmedizin und Parasitologie*, 31, 55–58.

Walter R.D., Van den Bossche H. 1980. Actions of suramin. In: *Report of the Fifth Meeting of the Scientific Working Group on Filariasis*. WHO, Geneva.

Wang E.J., Saz H.J. 1974. Comparative biochemical studies of *Litomosoides carinii*, *Dipetalonema viteae* and *Brugia pahangi* adults. *Journal of Parasitology*, 60, 316–321.

Warbrick E.V., Barker G.C., Rees H.H., Howells R.E. 1993. The effect of invertebrate hormones and potential hormone inhibitors on the third larval moult of the filarial nematode, *Dirofilaria immitis*, *in vitro*. *Parasitology*, 107, 459–463.

Werf P.A.D., Griffith O.W., Meister A. 1975. 5-Oxo-L-prolinase (L-pyroglutamate hydrolase) purification and properties. *Journal of Biological Chemistry*, 250, 6686–6692.

Weston K., Youchem J., Greenwald I. 1989. A *Caenorhabditis elegans* cDNA that encodes a product resembling the rat glutathione S-transferase P subunit. *Nucleic Acids Research*, 17, 2138.

WHO 1997a. Lymphatic filariasis: Reasons for hope, Document No. WHO/CTD/FIL/97.4, Geneva.

WHO 1997b. Lymphatic filariasis elimination. Report of a meeting of the principals for the further enhancement of public/private partnership. WHO, Geneva.

WHO 2000. Onchocerciasis (river blindness) revised. February 2000, Fact Sheet No. 95, 1–4.

Willis A.L. 1987. CRC handbook of eicosanoids. In: *Prostaglandins and related lipids*. Vols 1 and 2. CRC Press Inc., Boca Raton.

Wilson W.R., Tuan R.S., Shepley K.J., Freedman D.O., Greene B.M., Awadzi K., Unnasch T.R. 1994. The *Onchocerca volvulus* homologue of the multifunctional polypeptide protein disulfide isomerase. *Molecular and Biochemical Parasitology*, 68, 103–117.

Wittich R.M., Kilian H.D., Walter R.D. 1987. Polyamine metabolism in filarial worms. *Molecular and Biochemical Parasitology*, 24, 155–162.

Wolf G. 1991. The intracellular vitamin A-binding proteins: and overview of their functions. *Nutrition Reviews*, 49, 1–12.

Wolff K.M., Scott A.L. 1995. *Brugia malayi*: Retinoic acid uptake and localization. *Experimental Parasitology*, 80, 282–290.

Worthington D.J., Rosemeyer M.A. 1974. Human glutathione reductase: purification of the crystalline enzyme from erythrocytes. *European Journal of Biochemistry*, 48, 167–177.

Yates D.M., Portillo V., Wolstenholme A.J. 2003. The avermectin receptors of *Haemonchus contortus* and *Caenorhabditis elegans*. *International Journal for Parasitology*, 33, 1183–1193.

Zahner H., Sani B.P., Shealy Y.F., Nitschmann A. 1989. Antifilarial activities of synthetic and natural retinoids *in vitro*. *Tropical Medicine and Parasitology*, 40, 322–326.

Zang X., Maizels R.M. 2001. Serine protease inhibitors from nematodes and the arms race between host and pathogen. *Trends in Biochemical Sciences*, 26, 191–197.

Zang X., Yazdanbaksh M., Jiang H., Kanost M.R., Maizels R.M. 1999. A novel serpin expressed by blood-borne microfilariae of the parasitic nematode *Brugia pahangi* inhibits human neutrophil serine proteinases. *Blood*, 94, 1418–1428.

Zhang L.P., Maiorino M., Roveri A., Ursini F. 1989. Phospholipid hydroperoxide glutathione peroxidase: specific activity of tissue of rats of different age and comparison with other glutathione peroxidases. *Biochimica et Biophysica Acta*, 1006, 140–143.

(Accepted December 10, 2004)