Review of Literature
Prevention, treatment and newer strategies are important steps for combating malaria. Malaria parasite infection especially by *P. falciparum* deserves particular attention because of the variety of severe disease syndromes that it causes. The malaria parasite, like all organisms, must acquire nutrients from the environment and convert them to other molecules or energy (catabolism) used to maintain the homeostasis of the parasite, and in growth and reproduction of the parasite (anabolism). Both anabolic and catabolic processes are catalyzed by enzymes. The malaria parasite exhibits a rapid growth and multiplication rate during many stages of its life cycle. This necessitates that the parasite acquires various macromolecules and other biochemicals from the host and metabolize them for the maintenance of cellular structure and function in order to survive and reproduce. Obviously, the parasite's metabolism will be intertwined with that of the host's because of the intimate relationship between the host and parasite. These host-parasite interactions are further complicated by the complex life cycle of the parasite involving vertebrate and invertebrate hosts as well as different locations within both these hosts.

The unique life cycle and resulting microenvironments of the parasite has led to the evolution of metabolic pathways that differ from the human host. A better understanding of the important biochemical pathways along the *Plasmodium* life cycle (particularly blood stage) may lead to the design and development of novel therapeutic strategies that exploit the uniqueness of the parasite. For example, many antimalarials are known to affect the food vacuole, which is a special organelle for the digestion of host hemoglobin (Hb). Of particular interest are the pyrimidine *de novo*, mitochondrial electron transport systems (Kita *et al.*, 2002), haemoglobin degradation pathway operating in the food vacuole, the presence of a peculiar electron transport system and acrystate mitochondrial physiology, and the redox metabolism of *Plasmodium*. Below is a brief discussion of selected aspects of parasite metabolism that could be exploited as promising targets for antimalarial drug --with an emphasis on the unique features of the parasite--according to the classes and functions of biomolecules:
Carbohydrates and Energy production

Many reports indicate that glycolysis is the main source of ATP and NADH in the erythrocytic stage of malaria parasites with very little or no contribution from mitochondrial involvement (Fry et al., 1990). The metabolic steps involved in the conversion of glucose to lactate (glycolysis) are essentially the same as that found in other organisms. All of the enzyme activities have been identified in Plasmodium and some of the genes cloned. It has been reported that glucose consumption in the infected erythrocyte is 50-100 fold higher than that of the uninfected erythrocytes.

Most (~ 85%) of the glucose is converted to lactate and the high lactate dehydrogenase (LDH) activity is believed to function in the regeneration of NAD⁺ from NADH that is produced earlier in the glycolytic pathway by glyceraldehyde-3-phosphate dehydrogenase. However, some of the glycolytic intermediates may be diverted for synthetic purposes. For example, enzymes of the pentose phosphate pathway have been identified. This pathway probably provides some of the ribose sugars needed for nucleotide metabolism and provides for the regeneration of reduced NADPH to be used in biosynthesis or defence against reactive oxygen intermediates. Similarly, the further metabolism of pyruvate may provide intermediates in several biosynthetic pathways. Aerobic metabolism involves the further catabolism of pyruvate to carbon dioxide and hydrogen atoms via the tricarboxylic acid (TCA) cycle. However, the blood-stages of mammalian malaria parasites do not exhibit a complete TCA cycle. An explanation for this inefficiency is the abundance of glucose in the mammalian blood stream. In contrast, the parasite does appear to exhibit a TCA cycle in the glucose-poor environment of the mosquito host.

The TCA cycle and oxidative phosphorylation are generally carried out in the mitochondria of eukaryotes. These processes are generally assumed to be non-functional in the blood-stage parasite as evidenced by the acristae mitochondria. However, recently a functional electron transport chain and oxidative phosphorylation have been demonstrated in the blood-stage parasite (Uyemura et al., 2000). In addition, the parasite mitochondrion does have a membrane potential and cytochrome oxidase is present. The antimalarial drug atovaquone has been shown to inhibit electron transport and to collapse the mitochondrial membrane potential in malaria parasite. One possible function of the mitochondrion during the blood stage is for
pyrimidine synthesis. Triose phosphate isomerase (TIM) is found to be a good target enzyme in this pathway because of a large difference in the structure of the host and parasite enzyme (Velanker et al., 1997). LDH, the last enzyme of the pathway has been found to be less sensitive by its own product inhibition. In the crystal structure of the parasite LDH, a distinctive cleft has been discovered. Preferential accommodation of bulky hydrophobic drugs (like Gossylic nitrate) is found to accumulate in this cleft and selectively inhibit the parasite LDH (Vander Jagt et al., 1981).

Energy Metabolism of Malaria parasites: (http://sites.huji.ac.il/malaria/, 2004)

Parasites are able to use their mitochondria for production of ATP. By means of an ATP/ADP translocator they supply ATP to their host erythrocyte. The major product of the energy metabolism, lactic acid, translocates across the parasite membrane and the host cell membrane in the acid form, probably by simple diffusion. Oxidizing radicals produced by the parasite enhance the hexose monophosphate shunt of the host cell, thus increasing the levels of purine nucleosides that enter into the parasite thereby providing it with ribose-phosphate.

Fatty acids and Lipids

Lipids are a major component of membranes. The rapidly growing parasite requires large amounts of lipids for this increase in parasite surface area and volume of internal membranes. This huge demand for lipids makes lipid metabolism an attractive target for antimalarial drugs, and several potential drugs targeting lipid metabolism have been identified (Mitamura and Palacpac, 2003). Membrane lipids are composed of a glycerol backbone, which has a polar head group and two long chain fatty acids. Historically, the parasite has been considered to be incapable of synthesizing fatty acids de novo and restricted to obtaining preformed fatty acids and lipids from the host. However, several enzymes associated with the type II fatty acid synthesis (FAS) pathway have been identified in Plasmodium and appear to be located in the apicoplast. This type II pathway is found in plants and prokaryotes, whereas the type I FAS is found in yeast and metazoa, including humans, the vertebrate host for the malaria parasite. Several parasite enzymes involved in lipid synthesis from glycerides and fatty acids, as well as enzymes involved in the
remodeling of lipid polar head groups have been identified (Mitamura and Palacpac, 2003). An enzyme capable of activating fatty acids (necessary for incorporation into lipids) has been localized to membranous structures found within the cytoplasm of the infected erythrocyte (Matesanz et al., 1999). A FAS pathway was recently discovered in *P. falciparum*. Studies have shown that Triclosan, a phenyl ether drug inhibits both type II FAS and enoyl-ACP reductase in *P. falciparum* (Surolia et al., 2001).

### The Apicoplast—A vestigial chloroplast-like organelle

A non-photosynthetic plastid has been described in the Apicomplexa (Wilson, 2002). This plastid is most likely of red alga origin and has a long evolutionary history within the Apicomplexa. Possible functions associated with the apicoplast are biosynthesis of 1) fatty acids, 2) isoprenoid precursors, and 3) heme. *Plasmodium* homologs of enzymes involved in type II fatty acid synthesis have apicoplast-targeting sequences and are sensitive to known inhibitors of type II FAS. Similarly, the synthesis of isoprenoids in *Plasmodium* also appears to involve enzymatic pathways that are found in bacteria and plastids and is distinct from the synthetic pathway found in eukaryotes. Both of these pathways are particularly attractive drug targets since the human host synthesizes fatty acids and isoprenoids via different pathways utilizing different enzymes. Some of the enzymes in heme biosynthesis appear to be targeted to the apicoplast, whereas others appear to be targeted to the mitochondrion.

### Proteins and Amino acids

Proteins are responsible for cellular structure and function through their roles as enzymes or structural proteins. The blood-stage parasite obtains amino acids for protein synthesis from three sources: 1) degradation of ingested Hb, 2) uptake of free amino acids from the plasma (or cells) of the host, and 3) *de novo* synthesis. The most abundant source of amino acids is the ordered degradation of Hb. The parasite digests up to 65% of the total host Hb into amino acids. However, most of these amino acids are effluxed from the infected erythrocyte and only 16% of the digested Hb is incorporated into parasite proteins (Krugliak, 2002).
Several amino acids are taken up by infected erythrocytes at accelerated rates (Ginsburg, 1994) and in vitro culture studies indicate that *P. falciparum* requires seven exogenously supplied amino acids: isoleucine, methionine, cysteine, glutamate, glutamine, proline and tyrosine (Divo *et al.*, 1985). The parasite is also able to fix carbon dioxide and thereby synthesize alanine, aspartate and glutamate. However, the amino acids formed via carbon dioxide fixation and some of the exogenously added amino acids are not readily incorporated into proteins. Many of these amino acids (through transamination reactions) can interact with pathways involved in energy production and possibly serve as fuel sources. In addition, some amino acids serve as precursors or components of biosynthetic or other metabolic pathways (eg, glutamate metabolism or methionine metabolism). Of particular note is the proposal that glutamate dehydrogenase provides the reduced NADPH needed for glutathione reductase (Krauth-Siegel *et al.*, 1996) which presumably functions in redox metabolism. Interestingly, different rRNA molecules are expressed during the vertebrate and invertebrate stages of the parasite's life cycle (McCutchan *et al.*, 1995). The functional significance of stage specific ribosomes is not known.

**Nucleotides and Nucleic acids**

DNA and RNA are polymers of nucleotides, which in turn consist of a ribose sugar group linked to either a purine (adenine and guanine) or a pyrimidine (cytosine, uracil, and thymine) base. These bases can either be obtained via *de novo* synthesis or from the environment by the 'salvage' pathway. The malaria parasite obtains preformed purines by the salvage pathway and synthesizes pyrimidines *de novo*. Since the host can obtain both types of bases by either pathway, it may be possible to exploit the parasite's limited capability in nucleotide metabolism.

**Pyrimidine de novo and Respiratory Electron Transport Chain coupled pathway**

Unlike human host cells, malaria parasites are unable to scavenge preformed pyrimidines (Subbayya *et al.*, 1997) and must synthesize them *de novo* from bicarbonate and glutamine. One step of pyrimidine synthesis involves an electron transport in which dihydroorotate dehydrogenase (DHODH) transfers electrons to an electron transport chain involving ubiquinone, cytochrome and molecular oxygen.
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(Gutteridge et al., 1979). This activity is probably located in the mitochondria and accounts for the microaerophilic requirements of the parasite. Pyrimidine synthesis also requires folates as cofactors. DHODH catalyses the rate limiting step of UMP formation in the pyrimidine biosynthetic pathway. It catalyzes the oxidation of dihydro orotate to orotate utilizing the flavin cofactor (FMN). In the second step, the enzyme catalyzes the reoxidation of FMNH$_2$ by using respiratory chain type quinones (coenzyme Q). All the cytoplasmic forms of dehydrogenases oxidize FMNH$_2$ through NAD$^+$ or fumarate, while membrane or mitochondrial forms require respiratory quinones. Since the erythrocytic stages of the *Plasmodium* lack a number of enzymes of the TCA cycle, the electrons from reduced quinones generated from DHOD reaction, are funnelled to the electron transport chain of the parasite respiratory pathway. However, complete information about the intermediate electron acceptors of the respiratory pathway in the parasite is unknown. In the absence of a completely functional TCA cycle in parasite, ATP production is dependent upon the glycolytic and HMP shunt pathway. The question, therefore, arises as to the physiological function of the mitochondrial respiratory chain in the *Plasmodium* parasite. One important function is presumed to be the formation of membrane and chemical potential via electron transfer linked proton transport across the respiratory chain. The importance of the pyrimidine biosynthesis is crucial for its survival. A number of inhibitors of this pathway like 5-fluoroorotate (Baldwin et al., 2002) (drug inhibitor of dihydroorotate dehydrogenase), atovaquone (Srivastava et al., 1997) (napthoquinone inhibitor of cytochrome bc$_1$ complex and dissipater of mitochondrial membrane potential) and antifolates like pyrimethamine and proguanil (Ridley, 2002) have been synthesized. All these above compounds are validated drugs of these complex biochemical pathways.

The electrons that are released by the oxidation of reduced coenzyme Q (obtained from DHOD reaction in pyrimidine biosynthesis pathway) are transferred to the parasite mitochondrial respiratory chain. Such electron transfer is coupled to the proton transport and a large membrane potential is generated across the mitochondria (Uyemura et al., 2000). This system is a possible target of primaquine and atovaquone by two distinct mechanisms (http://sites.huji.ac.il/malaria/). Being an important rate limiting enzyme of the pyrimidine biosynthesis, DHODH is thus not only a regulator
of nucleic acid biosynthesis, but also has a direct link to the generation of membrane potential through the formation of reduced quinones. Hence the hybrid pyrimidine and respiratory chain system is an attractive target for malaria chemotherapy, but yet to be fully validated.

**Purine salvage pathway**

An essential step in the life cycle of malaria parasite is cellular replication of the parasite in human erythrocyte. RNA and DNA biosynthesis require large quantities of purines. In the absence of *de novo* purine biosynthesis pathway, a large number of purines are salvaged from the infected erythrocytes and host environment. Hypoxanthine, obtained from the host plasma is the major purine precursor for the salvage pathway where hypoxanthine guanine phosphoribosyl transferase (HGPRT) is the key enzyme (Shi *et al.*, 1999). Purine salvaging is also funneled through purine nucleoside phosphorylase (PNP) (Kicska *et al.*, 2002) and adenosine deaminase (ADA). Adenosine in the host plasma can be converted to hypoxanthine following deamination and dephosphorylation. Through a series of reactions the hypoxanthine is converted into ATP and GTP (or deoxy-ATP and GTP) and incorporated in RNA (or DNA). Immucilins are powerful transition state analogue inhibitors of HGPRT and PNP where as Coformycin is a powerful inhibitor of ADA. Adenylosuccinate lyase (ASL) and adenylosuccinate synthetase (ASS) appear to be unique to the parasite and is absent in the mature human erythrocytes. These two enzymes are responsible for formation of AMP in the parasite while formation of guanosine monophosphate (GMP) and inosine monophosphate (IMP) involve the presence of IMP dehydrogenase (IMPDH) and GMP synthase (GMPS). Sredinin and Hadacdin are known to inhibit IMPDH and ASS, respectively.

**Vitamins and Co-factors**

Many biochemical processes require cofactors, which do not directly participate in growth processes; vitamins are usually required in small amounts and are usually recycled. Pantothenate appears to be the only vitamin not supplied by the erythrocytes (Divo *et al.*, 1985) and is probably needed for the formation of acyl-Coenzyme A, which is needed in lipid biosynthesis.
Folate and its derivatives are important cofactors in the synthesis of nucleotides and amino acids and especially for the transfer of methyl groups. Especially important is the role of the dihydrofolate cycle in de novo pyrimidine synthesis. Dihydrofolate (FH$_2$) is reduced to tetrahydrofolate (FH$_4$) by dihydrofolate reductase (DHFR). Several antimalarials, such as pyrimethamine and cycloguanil, preferentially inhibit parasite DHFR. The FH$_4$ is methylated by serine hydroxymethyltransferase and the resulting methylene-FH$_4$ functions as a methyl donor. For example, thymidylate synthase catalyzes the formation of dTMP from dUMP by transferring the methyl group from methylene-FH$_4$. During this reaction the methylene-FH$_4$ is converted back to dihydrofolate (FH$_2$), which is then recycled. Increased folates are needed to accommodate the demand for pyrimidines, which are associated with DNA replication. The parasite cannot utilize preformed folate and must synthesize FH$_2$ from GTP, para-aminobenzoic acid and glutamate. Sulfadoxine and other sulfâ drugs inhibit the de novo synthesis of FH$_2$. Fansidar, a combination of sulfadoxine and pyrimethamine, inhibits folate metabolism.

**Uptake and Permeability**

The malaria parasite is a rapidly growing organism that exhibits a high metabolic rate. The infected RBC exhibits a substantial increase in its permeability to low molecular weight solutes as compared to the uninfected RBC (Ginsburg, 1994). Some of this increase represents higher rates of the endogenous RBC transporters and reflects the high anabolic metabolism of the parasite. However, new permeation pathways, which are not found in the uninfected RBC, are observed on the RBC membrane after infection. Much of this increase in permeability can be attributed to single type of permeation pathway with characteristics quite distinct from those of the host RBC (Kirk et al., 1999). A large number of transporters are present on the parasite plasma membrane and mitochondria. Transporters of macromolecule, like oxidized glutathione (GSSG), nucleosides, or relatively smaller molecules like glucose (Woodrow et al., 1999), amino acids, lactate, ADP-ATP and ions like Na$^+$, K$^+$, H$^+$, carry out diverse transport activities linked to metabolism. Lactate is extruded by the symporter to generate proton gradient membrane potential. These gradients serve to drive GSSG and H$^+$ outside and glucose, proteins, peptides, nucleosides, Na$^+$
and K⁺ inside the parasite organelle. Specific host enzymes—like δ-aminolevulinate dehydrase (ALAD), δ-aminolevulinate synthase (ALAS), coproporphyrinogen oxidase and ferrochetalase of host RBC are shown to be transported into the human parasite *P. falciparum* and mouse parasite *P. berghei* (Bonday et al., 1997; Varadharajan et al., 2002; Bonday et al., 2000). These enzymes are utilized by the parasite *de novo* synthesis. These enzymes and the related transporters are important targets for developing unique drugs.

**Detoxification of Heme and ROI**

Erythrocyte Hb degradation pathway is a major pathway in the parasite for its own protein synthesis. *P. falciparum*, the most virulent human malaria parasite, transports erythrocytic hemoglobin to an acidic food vacuole. During intraerythrocytic development and proliferation, constant degradation of host Hb inside the parasite food vacuole occurs through a sequentially ordered process where Hb is catabolized into heme and globin by aspartic, cysteine and metalloproteases (Hill et al., 1994; Shenai et al., 2000; Eggleson et al., 1999). These proteases are also called Plasmepsin I and II, falcipain and falcilysin respectively.

Heme is subsequently detoxified by the parasite through a specific mechanism known as heme polymerization. Free heme is toxic due to its ability to destabilize and lyse membranes, as well as inhibiting the activity of several enzymes. Three, and possibly four, mechanisms by which heme is detoxified have been identified: sequestration of the free heme into hemozoin, or the malarial pigment; a degradation facilitated by H₂O₂ within the food vacuole; a glutathione-dependent degradation which occurs in the parasite's cytoplasm; and possibly a heme oxygenase which has been found in *P. berghei* (rodent parasite) and *P. knowlesi* (simian parasite), but not *P. falciparum*. The polymerized heme commonly referred to as hemozoin or malaria pigment is accumulated in food vacuole of the intraerythrocytic parasite as insoluble black brown color (Gluzman et al., 1994). The degraded globin part is utilized by parasite for its own protein synthesis. A portion of the free heme may be degraded into non-toxic metabolites.

Proteases of each of these mechanistic classes are preferential chemotherapeutic targets. Falcipain-II is inhibited by cysteine protease inhibitors.
Peptido-mimetic inhibitors of plasmepsin show anti-parasitic activity. E-64 and leupeptin are specific peptide inhibitors of cysteine proteases that kill the parasite in nano molar concentration (Hill et al., 1994; Shenai et al., 2000). Hb degradation and heme polymerization pathway are very unique to all parasites and offers potential biochemical targets. Artemisinin is found to be taken up by the parasite in its food vacuole compartment and forms covalent adducts with heme. Additionally, antimalarial effect of artemisinins also results because of peroxidation of many malarial proteins especially proteases (Pandey et al., 1999). Chloroquine and other 4-aminoquinolines exert their effect by inhibiting pigment formation, as well as by preventing polymerization of toxic heme released during Hb degradation in Plasmodium digestive food vacuole (Ginsburg et al., 1999), and thereby prevent the detoxification of heme. The free heme destabilizes the food vacuolar membrane and other membranes and leads to the death of the parasite. It is shown that quinolones first bind heme in the food vacuole and then the drug-heme complex attaches the elongation sites of hemozoin with the accumulation of unpolymerized heme that kills the parasite (Sullivan et al., 1998). Mefloquine, quinine and quinidine also inhibit heme polymerization (Sullivan et al., 1996 a, b).

Redox Metabolism and Glutathione metabolism in Plasmodium falciparum-infected erythrocytes

A bi-product of metabolism and respiration are reactive oxygen intermediates (ROI) such as superoxide, hydroxyl radical and H₂O₂. The biochemical origin of the oxidative stress that the parasite impinges on its host cell occurs during the proteolytic oxidation of host cell Hb inside the acid food vacuole of the parasite. The release of the heme results in the iron bound to it being oxidized from ferrous state (Fe²⁺) to ferric state (Fe³⁺); electrons liberated thus promote the formation of ROI, which can damage lipids, proteins and nucleic acids and therefore, need to be oxidized to oxygen and water. Redox systems like GSH/GSSG, NADPH/NADP and the enzymes like catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) play a major role in preventing oxidative stress by detoxifying ROIs and maintaining the redox balance of the parasite and host parasite interaction. H₂O₂ can also be exported into the parasite cytoplasm where it is detoxified by catalase and
GPx. Some of the $\text{H}_2\text{O}_2$ produced as a result of the $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$ conversion may also be used for the peroxidative degradation of heme.

Intracellular parasites such as the *P. falciparum* require a highly efficient thiol metabolism to protect themselves from intracellular ROS and their derivatives (Docampo and Moreno, 1984). This implies that they are highly susceptible to oxidative stress and this sensitivity is a promising target for drug action (Hunt and Stocker, 1990; Schirmer et al., 1987). The role of oxidative stress as an important mechanism for the destruction of parasites and tumor cells is particularly well illustrated by many congenital and acquired factors that generate oxidative stress in human erythrocytes and offer partial protection against malaria (Hunt and Stocker, 1990). Central to defense against intracellular oxidative stress in humans is the glutathione redox cycle, which involves the enzymes human GR and GPx. Parasite enzymes involved in redox metabolism have been identified. GSSG is recycled by GR and the reducing equivalents of NADPH are probably generated through the pentose phosphate cycle. Glutamate dehydrogenase is another potential source of NADPH. It has also been proposed that the parasite uses host catalase and SOD within the food vacuole. Interestingly, the malaria parasite may supply the host erythrocyte with glutathione, which could participate in protecting the host cell from oxidative damage (Atamna and Ginsburg, 1997).

In the intact, trophozoite-infected erythrocyte, concentration of GSH is lower and that of GSSG is higher compared to normal erythrocytes. Normal erythrocytes and the parasite compartment display high GSH/GSSG ratios of 72.6 and 64.1 respectively, indicating adequate antioxidant defence. This ratio drops to 6 in the host cell compartment. Both $\gamma$-glutamyl-cysteine and GSH can penetrate through the new pathways from the extracellular space into the host cytosol, but the parasite membrane is impermeable to these peptides. Exogenous $\gamma$-Glu-Cys is not converted into GSH in the host cell, arguing that GSH synthase may not be functional due to depletion of Mg$^{2+}$. The parasite has a huge capacity for *de novo* synthesis of GSH, much of which is expelled in the form of GSSG; part of this is reduced by the host cell GR.
TREATMENT AND PROPHYLAXIS

The aims of antimalarial drug use vary with the epidemiologic situation. The choice of specific drugs and drug regimens is determined by the *Plasmodium* species causing the infection, drug sensitivities of the infecting parasites, and by the economic realities of the particular situation. Antimalarial drugs act selectively on different stages of the parasite life cycle. Blood schizonticides is the traditional term for drugs that destroy asexual parasites (trophozoites) in RBCs; these drugs are effective in treating symptomatic patients. In fact, none of the antimalarial drugs is effective against meronts (schizonts), so this term is slightly misleading. Tissue schizonticides act on the exoerythrocytic stages to prevent relapses of *P. vivax* and *P. ovale* infections. Some antimalarials prevent transmission: gametocides destroy the sexual stages, and sporontocides inhibit the development of oocytes on the gut wall of the mosquito. Blood schizonticides alone eradicate *P. falciparum* and *P. malariae* infections, but for complete (radical) cure of *vivax* and *ovale* malaria, a tissue schizonticide is necessary as well. Certain drugs act in a prophylactic capacity by preventing infection or by abrogating clinical symptoms. Casual prophylactics act on hepatic stages of malaria parasites. Suppressive (clinical) prophylactics prevent clinical symptoms by destroying malaria parasites as they initially invade RBCs; all blood schizonticides can be considered suppressive prophylactics. Some antimalarials exert multiple effects; however, no single antimalarial encompasses all these actions.

ANTIMALARIAL PHARMACOLOGY

**Quinine:** Quinine has been used for more than three centuries and until the 1930’s it was the only effective agent for the treatment of malaria. It is one of the four main alkaloids found in the bark of the cinchona tree and is the only drug which over a long period of time has remained largely effective for treating the disease. It is active against mature asexual erythrocytic stages of all forms of human malaria, except exoerythrocytic forms. It is now used only for treating severe *falciparum* malaria because of the undesirable side effects, and parasite resistance (WHO, 1987).

**Atebrin (Mepacrine):** This drug is a 9-amino-acridine developed in the early 1930’s. It was used as a prophylactic on a large scale during the 2nd world war and was then considered a safe drug. It is no longer used due to many undesirable side effects.
**Chloroquine:** It was first used in the 1940s shortly after the Second World War and was effective in curing all forms of malaria; with few side effects when taken in the dose prescribed for malaria and it was low in cost. A very effective 4-amino-quinoline both for treatment and prophylaxis, this drug is marketed as a diphosphate or sulphate salt and for IM or IV injection as the dihydrochloride. Chloroquine has a marked, rapid blood schizonticidal activity against susceptible strains of malaria parasites; it is most active against *P. vivax*, *P. ovale* and *P. malariae*, and is effective against immature, but not mature, gametocytes of *P. falciparum*. Unfortunately most strains of *falciparum* malaria are now resistant to chloroquine, and more recently chloroquine resistant *vivax* malaria has also been reported.

**Primaquine:** This drug has primarily been used against gametocytes and hypnozoites. It has been suggested that the drug works by inhibiting the electron transport chain of the parasite. There is no evidence that gametocyte resistance exists, but if the drug is used against schizonts, then resistance is rapidly attained (Merhli and Peters, 1976). The surviving parasites had increased numbers of mitochondria suggesting that resistance mechanism involves production of extra organelles to compensate for the damage caused by the drug.

**Sulfonamides:** These drugs work by inhibiting para-aminobenzoic acid (pABA), which is needed in an intermediary step of FH₄ synthesis. Little is known about this side of parasite metabolism, or the exact mechanisms of resistance - though resistance is clearly stable, transmissible, and prolific (WHO, 1987), and seems to be present in all stages of the parasite metabolism.

**Proguanil and pyrimethamine (antifols):** Proguanil was first synthesized in 1946. This drug falls into the biguanide class of antimalarials and is close in structure to pyrimethamine. Both the drugs are folate antagonists and destroy the malaria parasite by binding to the enzyme dihydrofolate reductase. As with sulfonides, resistance occurs in all stages of the lifecycle. They are still used as prophylactics in some countries. As with sulfonamides, resistance occurs in all stages of the lifecycle.

**Mefloquine (Lariam, 4-quinoline-carbinolamines):** First introduced in 1971, this quinoline methanol derivative is related structurally to quinine. The compound was effective against malaria when first introduced and because of its long half-life was a good prophylactic, but widespread resistance has now developed, and this together
with undesirable side effects including several cases of acute brain syndrome have resulted in a decline in its use. Because of its relationship to quinine the two drugs must not be used together.

**Artemesin:** Artemesin (qinghaosu) is a sesquiterpene lactone extracted in 1972 from a medicinal herb (Artemesia annua), sweet wormwood. The parent compound and two artemesin derivatives are currently in use: *artemether*, most commonly administered by intramuscular injection, and *artesunate*, which can be administered orally, by IV infusion, or by rectal suppository. Another derivative, *arteether*, is in the late stages of development. This class of drugs has stage-specific antimalarial effects. Late-stage ring parasites and trophozoites are more susceptible than schizonts (meronts) or small rings. They are also gametocidal and thus may reduce transmission. These drugs may act by becoming free radicals in an iron-catalysed reaction; these reactive species could then react with and damage specific membrane associated proteins. These drugs are extremely useful in settings where *P. falciparum* has developed multidrug resistance. Because recrudescence is common and resistance is more likely to develop when they are used alone, combination chemotherapy with longer half-life drugs (mefloquine) is the current approach in areas where multidrug resistant *falciparum* parasites predominate.

**Malarone:** This new drug combination of proguanil and atovaquone was released in Australia in 1998. This combination causes a synergistic effect and is at present a very effective antimalarial treatment. The drug combination has undergone several large clinical trials and has been found to be 95% effective in otherwise drug resistant *falciparum* malaria. A fixed dose combination of atovaquone and proguanil hydrochloride (Malarone™) is now approved for both treatment and prophylaxis of malaria. It has been claimed to be largely free from undesirable side effects but it should be noted that proguanil is an antifolate. This is not likely to be a problem with a single treatment course of the drug but some caution should be exercised when using it for prophylaxis. At present it is a very expensive drug.

**Maloprim:** Maloprim is a combination of dapsone and pyrimethamine. Resistance to this drug is now widespread and its use is no longer recommended.
Fansidar: This is a combination drug containing sulfadoxine and pyrimethamine. It acts by interfering with folate metabolism. Resistance to fansidar is now widespread and serious side effects have been reported. It is no longer recommended.

Amodiaquine: This drug is marketed as the dihydrochloride dihydrate. The efficacy, side effects, recommendations and precautions for amodiaquine are essentially the same as those for chloroquine, with a few exceptions. It has been used as an alternative to chloroquine in the first line treatment of uncomplicated *P. falciparum* malaria in areas with a high level of chloroquine resistance. However, *P. falciparum* in these areas is almost always resistant to amodiaquine as well.

Halofantrin (Halfan): Halofantrin hydrochloride is a 9-phenanthrene-methanol; the drug exists as a racemic mixture and both stereoisomers have equal antimalarial activity. It is an effective antimalarial introduced in the 1980’s but due to its short half-life of 1 to 2 days, it is not suitable for use as prophylactic. Unfortunately resistant forms are increasingly being reported and there is some concern about side effects including neuropsychiatric disturbances, abdominal pain, diarrhoea, pruritus, and skin rash. It is contraindicated during pregnancy and is not advised in women who are breastfeeding.

Other than the above-mentioned drugs, many new drugs have been tested for their potential antimalarial effects. Research into newer antimalarials being scanty, such attempts might throw up some candidates; however, these drugs are yet to find a place in standard antimalarial regimen, and are still undergoing trials.

Arteether: Arteether, a blood schizontocidal antimalarial, has been developed from the plant *Artemisia annua* at the Central Drug Research Institute, Lucknow, India and is effective in treatment of uncomplicated and complicated cases of malaria caused by *P. falciparum*. It is marketed by Themis Chemicals Ltd. under the trade name E-Mal.

Bulaquin: It is an anti relapse antimalarial, developed at the Central Drug Research Institute, Lucknow, India, and is safer in comparison to primaquine. It is marketed by Nicholas Piramal India Ltd. as a combination therapy along with chloroquine under the trade name Aablaquin (http://www.cdriindia.org)

Clindamycin: It acts by inhibiting the protein synthesis by binding to the 50s subunit of ribosomes. It can be used for drug resistant malaria along with quinine. Adverse effects include pseudomembrane colitis and skin rashes.
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**Fluoroquinolones**: Both ciprofloxacin and norfloxacin have been found to have antimalarial activity both *in vitro* and *in vivo*. However, results are not consistent.

**Azithromycin**: Azithromycin is found to have antimalarial activity and has been found to be useful as a causal prophylactic agent. It was found to be effective as a prophylactic agent against chloroquine resistant *P. falciparum* infection.

**Atovaquone**: It is a synthetic hydroxy-naphthoquinone developed in the early 1980s, and has been found to be useful against the *Plasmodia*. It is a highly lipophilic molecule that supposedly interferes with the mitochondrial electron transport and thereby ATP and pyrimidine biosynthesis and in *Plasmodia*, it is found to target cytochrome bc1 complex. It may cause rash, fever, vomiting, diarrhoea and headache. Safety in pregnancy, lactation, children, and elderly is yet to be established.

**Pyronaridine**: Structurally, it resembles amodiaquine and has been found to be highly effective against chloroquine resistant strains in China.

**Piperaquine**: Its activity is similar to that of chloroquine. Further studies are needed.

**WR-288, 605**: It is 7.4 times more active than primaquine as a tissue schizonticidal drug. It has lesser toxicity, good oral bioavailability and longer half-life.

As the probability of the evolution of a mutant resistant to one compound is very low, resistance to a combination is extremely unlikely to emerge. So, to prevent the advent of resistance to the artemisinins, these drugs are being combined with other, synthetic compounds. And as artemisinin works quickly and is removed from the body quickly, the other compounds should be those that last for longer in the body, mopping up any parasites that have escaped. Of these, artemether–lumefantrine has proved equally effective to artesunate–mefloquine and better tolerated. It is now at the centre of the *WHO Roll Back Malaria Initiative*. Dihydroartemisinin–piperaquine is a very exciting newcomer from China but is still in development phase.

**DRUG RESISTANCE IN MALARIA**

Drug resistant malaria represents a major global public health concern today. Drug resistant strains of *P. falciparum*, the most deadly form of malaria, are a major problem in the tropical and subtropical areas of the world, where the burden of malaria morbidity and mortality is highest. It started with the spread of chloroquine resistance across the continent during the 1980s, especially in East Africa. The effects
of drug (chloroquine) resistance are related to the malaria endemic situation. There is also a complex interaction between chemotherapy and immunity (Bjorkman, 1988). The drug pressure on the existing *Plasmodium* population is strong and the positive selection for resistant parasites is high. Resistance *in vivo* has been reported to all antimalarial drugs except artemisin and its derivatives (WHO Geneva, 1995). The problem of drug resistance can be attributed primarily to increased selection pressures on *P. falciparum* in particular, due to indiscriminate and incomplete drug use for self-treatment (Fig. 3). Several mechanisms can account for changes in drug sensitivity in the malaria parasites, for example, physiological adaptations due to non genetic changes, selection of previously existing drug resistant cells from a mixed population under drug pressure, spontaneous mutation, mutation of extranuclear genes, or the existence of plasmid-like factors. Several African countries have now switched their first line drug to sulfadoxine/pyrimethamine. Importantly, wherever the sulfadoxine/pyrimethamine has been used on a larger scale, resistance has developed and spread rapidly. The following major problem is that alternative treatments are more costly and/or often associated with more adverse side effects. The epidemiology of antimalarial drug resistance has been described (Bjorkman and Phillips-Howard, 1990; White, 1992; Wongsrichanalai *et al*., 2002) and also its implications in sub-Saharan Africa (Bjorkman, 1991; Marsh, 1998). Generally, the identification of gene mutations associated with drug resistance will offer better tools to look at pharmacoresistance dynamics more closely in relation to different drug treatment strategies, i.e. drug exposure. Selection and possibly induction of resistance has been partly studied in continuous *in vitro* cultures and in rodent malaria. Repetitive exposure to sub-inhibitory drug concentrations has generally been the optimal condition for the development of resistance. However, more in-depth studies on pharmacodynamics, drug resistance and viability of resistant mutants are warranted.
BIOCHEMISTRY OF FILARIAL PARASITES

Filariasis has been a scourge of civilisation for thousands of years, being first depicted on the pharaonic murals of Egypt and in the ancient medical texts of China, India, Japan and Persia. Elephantiasis and hydrocoele were first associated with parasitic filarial worms and their mosquito vectors in the late 19th century by French, English and Australian physicians working with patients from Cuba, Brazil, China and India. Filariasis occurs primarily in poor communities, and exacerbates poverty by physically incapacitating people and thus preventing them from having a normal working life. The fight against filariasis is also a fight against poverty.

Filariasis exerts a heavy social burden, particularly since chronic complications are considered shameful and are often hidden. For men, the genital damage is a severe handicap leading to physical limitations, poor self-image and social stigmatisation. Marriage, usually an essential source of security, is often impossible for men and women with chronic complications.

A review of the available literature on the biochemistry of filarial parasites reveals the fascinating mosaic of biochemical reactions employed by the organisms for their survival and adaptations to different hosts, different tissues with different structure and chemical composition, defence parameters, and pH and redox potentials.

Carbohydrate Metabolism

Filarial parasites have been shown to have active glycogenic and glycolytic pathways and a somewhat subdued citric acid cycle, and most of the enzymes of these pathways have been identified (Ramp and Kohler, 1984; Barrett et al., 1986). This is in contrast to mammals where energy metabolism has active Krebs cycle and the cytochrome system. However, it has been shown that *O. volvulus* and *B. pahangi* can also survive by glutaminolysis (McKenzie et al., 1989). Adult *L. carinii* is aerobic and forms lactate and acetate as major end products of carbohydrate utilization. The adults of *B. pahangi* and *D. viteae* are homolactate fermenters and convert glucose to lactate and traces of acetate. In contrast to adults, mf of *L. carinii*, *B. pahangi* and *D. viteae* clearly exhibit an aerobic carbohydrate catabolism and lose mobility under anaerobic conditions. They metabolise glucose to lactate, acetate and carbon dioxide, requiring oxygen for motility, but apparently not for survival (Rew and Saz, 1977).
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. 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* LDH, MDH (malate dehydrogenase) and malic enzyme (Walter and Albiez, 1981). But 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. DEC has been shown to alter glucose uptake and inhibit phosphoenolpyruvate carboxykinase, fumarate reductase and succinate dehydrogenase (Subrahmanyam, 1987). Benzimidazoles inhibit fumarate reductase (Prichard, 1973) and glucose transport (Vanden 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 effect carbohydrate metabolism by decreasing utilization of glucose together with a shift to a more nearly homo lactate fermentation (Rew and Saz, 1977). The arsenicals have been shown to affect glucose uptake (Subrahmanyam, 1987). 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); allosamidin is known to be a competitive inhibitor of the enzyme chitinase (Sakuda et al., 1987).

**Lipid Metabolism**

Investigations of lipid metabolism in helminths are few and are mainly restricted to analyses of lipid composition and studies of lipid synthesis (McManus, 1986). Analysis for lipid composition has been done in *D. immitis, S. cervi* and *C. hawkingi* and was found to be in the range from 1.3% to 8% (Hutchison et al., 1976; Ansari et al., 1973; Rathaur et al., 1980). Little is known about catabolism of lipids except that *B. pahangi* adults are unable to oxidize exogenous palmitic acid (Middleton and Saz, 1979). *D. immitis* has been found to possess all the major classes of neutral lipids-triacylglycerols, diacylglycerols, sterols, sterol esters, hydrocarbons
and traces of free fatty acids (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). Comley et al. (1981) reported the presence of triacylglycerols, sterols, free fatty acids and hydrocarbon fractions in B. pahangi.

All the major phospholipid classes have been identified in filarial worms, phosphatidylcholine being the predominating one. Studies have been done on adult D. immitis, adult B. pahangi, adult L. carinii and adult S. cervi (Hack et al., 1962; Comley and Jaffe, 1981; Subrahmanyam, 1967; Ansari et al., 1973). 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). The worms contain a range of saturated and unsaturated fatty acids from C_{12} to C_{26}, including branched chain and odd numbered fatty acids, the most abundant being C_{18}. The incorporation of mevalonate (Comley and Jaffe, 1981) into free fatty acids suggests the presence of fatty acid chain lengthening system.

The pathways of glycerophospholipid syntheses in adult B. pahangi and B. patei have been examined by radioisotopic incorporation and demonstration of the enzymatic steps. Radiolabelling studies have shown that L-U-^{14}C-glycerol was rapidly incorporated into glycerophospholipids of B. pahangi and B. patei, respectively (Srivastava et al., 1987). No work seems to have been done on the lipid catabolism by filarial parasites.

**Retinol binding Proteins**

The occurrence and uptake of retinol and retinoic acid and formation of retinol from β-carotene has been demonstrated in several helminth parasites (Sani and Comley, 1985). Parasitic nematodes appear to require retinoids (vitamin A and its metabolites) for a variety of their metabolic and developmental demands, such as growth, differentiation, embryogenesis, glycoprotein synthesis and as antioxidants (Sani, 1990). Specific retinol binding proteins have been detected in parasite and inhibitors of retinol binding or retinol analog could have possible chemotherapeutic significance (Sani and Comley, 1985). 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). Also, ivermectin, a potent and widely used antiparasitic drug competes efficiently with retinol for retinol binding sites on PREP, 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 that 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 were observed between the host and parasite binding proteins could be employed advantageously in the selective control of parasitic growth (Sani et al., 1985).

**Protein/Amino acid Metabolism**

There has been little work done on the amino acid metabolism of the filarial parasites; amino acid compositions have been studied for *S. cervi*, *C. hawkingi* and *A. galli* and *D. immitis* (Rathaur et al., 1980; Jaskoski and Ozuk, 1977). Protein constitutes over 63 and 57% of the dry weight of adult and mf of *S. cervi* (Rathaur et al., 1980), most of amino acids are present in bound form and only traces of free amino acid could be detected in these filarial parasites. Amino acids are essential for maintaining the viability of helminth worms. Free amino acids have been demonstrated in free pool of parasitic helminths (Arme and Whyte, 1975), which are altered during *in vitro* incubation of worms. Parasitic helminths are able to pick up amino acids from their surroundings through their tegument (Ash and Read, 1975). The functional importance of free amino acid pool has been indicated in intracellular osmoregulation and protein synthesis (Kurelec and Rijavec, 1966). Interconversion of amino acids by extracts of Schistosomes was first noticed by Garson and Williams (1957) who stated that transamination occurs between α-KG and alanine/arginine/aspartate and glycine. However, according to Huang et al. (1962) transamination occurs between α-KG and first three amino acids listed above. Transamination has also been observed in the intact worms (Seneft, 1963).
The incorporation of amino acids into proteins has been demonstrated in a number of filarial worms such as adults of *L. carinii* (Akinwande and Akinrimisi, 1980), adults of *S. cervi* (Anwar et al., 1978) and mf of *D. immitis* (Jaffe and Doremus, 1970). Serine-hydroxymethyl transferase, an enzyme responsible for conversion of serine into glycine and vice versa has also been demonstrated in adult *D. immitis* and *B. pahangi* (Jaffe and Chrín, 1981).

The filariae such as *D. immitis* and *B. pahangi* require methionine from exogenous source as methionine synthetase and betaine; homocysteine transmethylase are not present in these parasites (Jaffe, 1980 a, b; Jaffe and Chrín, 1979, 1981). Glutamate dehydrogenase has been demonstrated in mf of *D. immitis* (Langer and Jiampermpoon, 1970). Transaminase activity has not been detected in *W. bancrofti* microfilariae (Govindwar et al., 1974). According to Jaffe and Doremus (1970) active protein synthesis in *D. immitis* is consistent with the viability and high metabolic activity under experimental conditions. In case of *L. carinii* glutamine provides an input of carbon into TCA cycle intermediates may stimulate the rate of pyruvate oxidation via acetyl CoA and TCA cycle (Davies and Kohler, 1990).

Sulphur amino acid metabolism of filarial parasites is yet another attractive target for antifilarial chemotherapy. *D. immitis* and *B. pahangi* possess three enzymes of methionine cycle viz. methionine adenosyltransferase, S-adenosylmethionine methyltransferases and S-adenosylhomocysteine hydrolase for the conversion of methionine to homocysteine, but apparently lack the final enzymes - Methionine synthetase and/or betaine: homocysteine transmethylase for the conversion of homocysteine to methionine (Jaffe, 1980b). All the enzymes involved in the methionine to cysteine pathway have been identified in the filarial nematodes *B. pahangi* and *D. immitis* (Jaffe, 1980b). 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).

**Nucleic acid Metabolism**

Not many reports are available on nucleic acid and nucleotide composition and metabolism of filariids. Genome of filarial parasites, *D. immitis* and *B. pahangi*
are composed of 10-20% of repetitive DNA and 80-90% unique copy DNA and complexity of unique copy DNA is $10^8$ bp. Genome size is very close to *Caenorhabditis elegans*, a free living soil nematode and about ¼ of human. Another parameter for identification of genome is the molar ratio of AT and GC base pairs known as GC content is extremely useful in analyzing phylogenetic relationship (Rajan, 1990). Rothstein *et al.* (1988) demonstrated that GC content of *B. malayi* DNA is 28%; this is lowest molar GC content for a metazoan organism.

In case of adults and microfilariae of *D. immitis* and *B. malayi* uptake of radiolabelled purine and pyrimidine bases and nucleosides (adenine, adenosine, uracil, uridine, hypoxanthine and guanine) have been reported (Jaffe and Doremus, 1970; Jaffe and Chrin, 1981). However, thymine, thymidine or cytosine are not being taken up. The incorporation of labelled glycine into DNA and RNA and $^{32}$Pi into RNA has also been demonstrated in adults of *L. carinii* (Akinwande and Akinrimisi, 1980). Filarial worms have both *de novo* and salvage pathways for nucleotide synthesis. Synthetic pathways are usually under tight metabolic control and in the presence of exogenous product or in non-growing stages their activity may be suppressed (Barrett, 1983). DNA topoisomerase II, an essential enzyme that displays an important role in DNA replication, repair and transcription has recently been identified as a target for the development of antifilarial compounds (Tripathi *et al.*, 2001).

**Folate Metabolism**

The role of folic acids in growth and reproduction of nematodes has been demonstrated in axenic cultures. Folate derivatives are concerned with the transport and interconversion of one-carbon units for synthetic reactions. Several enzymes of the folate pathways have been identified in filarial parasites (Jaffe, 1980a). The presence of an array of enzymes involved in the interconversion of folate analogues 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). An analysis of the different steps of folate metabolism in the parasites reveals certain basic differences from those operating in vertebrates. The enzyme 5,10-methylenetetrahydrofolate (FH$_4$) reductase catalyses the irreversible formation of 5-methyl FH$_4$ from 5,10-methylene
FH₄ in almost all vertebrate tissues. However, in filarial parasites, this enzyme, a flavoprotein, operates preferentially in the reverse direction, favoring the formation of 5,10-methylene FH₄. The parasites possess 10-formyl FH₄ dehydrogenase which catalyzes the deformylation of 10-formyl FH₄ and thus regulates the endogenous concentrations of FH₄ cofactors. This enzyme is more active in *B. pahangi* and *D. immitis* than in mammalian liver. Another enzyme of interest is serine hydroxymethyl transferase (glycine hydroxymethyl transferase), which catalyzes the synthesis of 5,10-methylene FH₄ from FH₄ and requires pyridoxal phosphate. There was a virtual block in the development of infective larvae of *L. carinii* to the adult stage in pyridoxine-deficient rodents, possibly because of interference with folate metabolism (Subrahmanyam, 1987). Suramin inhibits the dihydrofolate reductase of *O. volvulus* and NADP-dependent N¹⁰-formyl FH₄ dehydrogenase of *B. pahangi*. DEC has been shown to inhibit a number of folate metabolism enzymes, but 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 is still controversial (Jaffe and Chrin, 1980).

**Polyamine Metabolism**

A number of biogenic amines have been identified in filarial parasites. Histamine, 5-hydroxytryptamine, norepinephrine and dopamine have been detected in both mf and adults of *L. carinii* and *S. cervi*. Biogenic amine content of mf has been found higher than adults (Saxena *et al.*, 1977). Dopamine is the major monoamine of *S. cervi* followed by norepinephrine and 5-hydroxytryptamine, monoamine oxidase activity has also been detected in both mf and adults of *S. cervi* (Agarwal *et al.*, 1990). Among polyamines, low level of putrescine but much higher level of spermidine and spermine has been detected in *O. volvulus, D. immitis, B. patei* and *L. carinii* (Wittich *et al.*, 1987). Uptake and interconversion of labelled polyamines have been observed in *O. volvulus* and *D. immitis*. N-acetylated polyamines are also present in very low amount in filarial worms. Ornithine-decarboxylase and arginine decarboxylase has not been detected in any of the filarial species (Wittich *et al.*, 1987). Key enzymes of polyamine biosynthesis have not been demonstrated in filariae.
Endosymbiont Bacteria

Another novel approach to filariasis control is to target the endosymbiont bacteria, which live within filariids, and appear to be essential to healthy growth and development of the parasite. The results obtained to date are very promising. Tetracycline, doxycycline and rifamycin have been shown to inhibit the motility, viability and release of microfilariae and impede their development (Hoerauf et al., 1999, 2000; Taylor and Hoerauf, 1999; Taylor, 2000; Taylor et al., 2000).

Glutathione metabolism

Glutathione is a major chemotherapeutic target in filarial species as it has been proposed to constitute the antioxidant system responsible for the long term existence of filarial worms in mammalian host by protecting them from the reactive oxygen species produced by the normal metabolism and the immune cells of the host (Brophy and Pritchard, 1992; Callahan et al., 1988). GSH, either alone or in combination with enzymes like GPXs, glutathione-S-transferase (GSTs), Glutathione reductase (GR) (Lomaestro and Malone, 1995; Zhang et al., 1989; Carlberg and Mannervik, 1985; Schirmer et al., 1987), protects the filarial worms from oxidative damage. The inhibition of enzymes involved in GSH synthesis and metabolism thus deprives the parasite of its major defence against oxidative stress and makes them unable to survive.

ANTIFILARIAL PHARMACOLOGY

The mainstay of filarial control is chemotherapy, and this is likely to remain so for the foreseeable future. The treatment of filariasis consists of chemotherapy directed against the adult worms (macrofilaricidal) and against the microfilariae (microfilaricidal) combined with symptomatic treatment to relieve the damage caused by the body's immunological reaction to dead and dying worms. Control of filariasis remains disappointing due to lack of appropriate one-shot chemotherapeutic agents capable of eliminating adult parasites (Ottesen and Ramchandran, 1995).

Diethylcarbamazine (1-diethylcarbamoyl-4-methylpiperazine; DEC): Also known as Heterazan, Banocide and Notezine, acts on neuromuscular system, cuticular surface, carbohydrate and folate metabolism and host-immune factors. At present
DEC is the only drug extensively used for the treatment of lymphatic filariasis. Although DEC is one of the oldest drugs in the treatment of microfilaraemic subjects, its precise mode of action is still not clear. Piperazines, to which class DEC belongs, cause hyperpolarization of the muscle membranes of nematodes with concomitant flaccid paralysis. This drug is rather tiresome to use, as it has to be given in multiple doses over days or weeks. If a full course of treatment is given DEC, is definitely macrofilaricidal. But the main problem associated with the drug is the side effects associated with its constant use—including nausea, dizziness and febrile episodes (Fan, 1992). Although DEC is non-toxic and can be safely administered in combination with food items such as common salt but allergic reactions may occur due to the release of antigens from the large number of microfilariae that die on administration of drug.

Ivermectin (IVM): Ivermectin acts on neuromuscular system and host immune factors. It promotes γ-amino butyric acid (GABA) release and its binding to postsynaptic receptors, thereby affecting GABA-mediated neurotransmission. It is a better substitute to DEC and requires only a single dose treatment but it fails to eliminate adult worms (Campbell, 1993). Ivermectin is also known as Mectizan (22, 23-dihydroavermectin B1, a macrocyclic lactone produced by an actinomycete, Streptomyces avermitilis). It is active at low dosage against a wide variety of nematode and arthropod parasites (Campbell et. al., 1983). Ivermectin is relatively safe to higher animals including humans because it does not pass readily through the blood-brain barrier.

Isothiocyanates and their derivatives: These act on cuticular surface, carbohydrate metabolism, cyclic AMP phosphodiesterase, 5'-nucleotidase and aminoacyl-tRNA synthetases. These are both microfilaricidal and macrofilaricidal and affect the energy metabolism of the parasites. CGP 20376, a 5-methoxyl-6-dithiocarbamic-S-(2-carboxy-ethyl) ester derivative of benzothiazole, had complete adulticidal and microfilaricidal activities against the parasite. However, as the compound or its metabolites caused hepatotoxicity, its clinical use in the present formulation is not recommended. (Mak et al., 1991)

Organic Arsenicals and Antimonials: Organic arsenicals (Friedheim, 1962) and antimonials (Rogers, 1920) act on neuromuscular system, carbohydrate metabolism
Biochemistry of Filarial Worms and Application to Chemotherapy

intestinal epithelium and glutathione metabolism. These were amongst the earliest agents used to combat filariasis. Mel-W is a water-soluble form of arsenic compound, Mel-B, which has been used to treat human filariasis. It has high activity against female adult worms with little effect on mf. However, arsenicals on account of their toxicity for certain percentage of patients have practically no place in the chemotherapy of filariasis. Various antimonials like stibsol, neostibosan, MSbB and neostam were evaluated for their adulticidal activity against different filarial parasites in man and animals. As with arsenicals, the use of antimonials has been limited because of their toxicity.

**Suramin (Antrypol):** Suramin acts on carbohydrate and folate metabolism, protein kinases and intestinal epithelium. It is macrofilaricidal and is principally used in the treatment of onchocerciasis (Hawking, 1978). Suramin markedly affects a number of enzymes. Examples are enzymes of the glycolytic pathway; it inhibits the LDH and MDH activities of both *D. immitis* and *O. volvulus* (Walter and Albiez, 1981). An inhibition of this reoxidation will reduce the energy supply. Other targets for suramin might be the enzymes of phosphorylation-dephosphorylation reactions. Suramin inhibits the dihydrofolate reductase of *O. volvulus* and the NADP-dependent 10-formyl FH4 dehydrogenase of *B. pahangi* (Jaffe, 1980a, b).

**Benzimidazoles:** Thiabendazole, benomyl, flubendazole, mebendazole etc, disrupt microtubule assembly (Vanden Bossche et al., 1982), cause fumarate reductase inhibition (Prichard, 1973) and inhibition of glucose transport (Vanden Bossche and de Nollin, 1973). These possess a high order of activity against intestinal helminths and to some extent against tissue dwelling filariae but were found to be poorly absorbed through the gastrointestinal tract and showed teratogenicity (Townsend and Wise, 1990). Nevertheless, in the absence of any totally safe macrofilaricide, albendazole is being field-tried presently (Ottesen et al., 1999).

**Coumarin (Benzopyrone) derivative: 5-6 Benzopyrone,** has been reported to reduce lymphoedema when administered along with DEC (Casley-Smith et al., 1993). This combination was beneficial since the undesirable side reactions are minimised probably due to inhibition of interleukin-6 production that increases in response to death of microfilariae by DEC (Turner et al., 1994). Reduction of lymphodema by benzopyrone may be due to protective activity against oxidative damage of the tissue.
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as they hinder superoxide generation by inhibition of NADPH oxidase activity (Paya et al., 1993). Superoxide radicals amplify the inflammatory process, increase vascular permeability and adhesion of polynuclear lymphocytes to endothelium and stimulate platelet aggregation. Six synthetic 2H-1-benzopyran-2-one (coumarin) derivatives were evaluated for filaricidal activity against *L. carinii* and *A. viteae* infections in cotton rats (*Sigmodon hispidus*) and *M. coucha* respectively. Thus detection of filaricidal activity in benzopyrones, so far known for anti-inflammatory activity, provides a new lead for the development of superior filaricidal agents for combating filariasis (Tripathi et al., 2000).

**Combination Therapy:** A combination of diethylcarbamazine and ivermectin has shown to be very effective in providing rapid and long-term clearance of microfilariae (Moulia-Pelat et al., 1995, 1996). Nicolas et al. (1997) found that this combination was much more effective than either drug alone for clearing circulating filarial antigen in both amicrofilaraemic and microfilaraemic subjects. Ismail et al. (1998) studied the effects of albendazole, DEC and ivermectin alone and in combination and concluded that, although all were well-tolerated and effective microfilaricides, a single dose of a combination of albendazole and of ivermectin was the most effective. The most effective was a combination of albendazole with DEC. Not all researchers agree on the efficacy of albendazole alone, or on the benefits of using it in combination with ivermectin. Addiss et al. (1997) found that treating children with a combination of ivermectin and albendazole seemed to be more effective than treatment with ivermectin alone in clearing microfilariae, but albendazole alone had no significant effect. Quite obviously, there needs to be further careful research of this kind to clearly establish the benefits of using albendazole as an antifilarial.
GLUTATHIONE METABOLISM: AN OVERVIEW

All aerobic living organisms are exposed to reactive oxygen species (ROS), which confer modifications of nucleic acids, thiol-containing proteins and membrane lipids (Sies, 1986). Apart from the occurrence of oxidative stress as a normal by-product of the aerobic metabolism, parasites are exposed to ROS generated by activated eosinophils, neutrophils and monocytes of the host organism. A major component of the host defense against invading parasites is the respiratory burst of host effector cells, which involves the release of superoxide anions, hydrogen peroxide etc. In order to prevent cellular damage due to ROS, the organism possesses complex and effective antioxidative systems. The enzymatic systems are SOD, catalase and GPx. In addition there are non-enzymatic antioxidants like vitamin C and E and Glutathione (GSH).

**GSH biosynthesis and enzymes of the γ-glutamyl cycle**

GSH is the principal nonprotein tripeptide thiol found virtually in all animal cells. The tripeptide consists of glutamic acid, cysteine, and glycine covalently joined end-to-end (Fig. 4).

![Structure of Glutathione](image)

**Fig 4: Structure of Glutathione**

GSH has two characteristic structural features: a γ-glutamyl linkage and a sulphydryl (-SH) group. These moieties of the tripeptide facilitate its participation in an impressive number and variety of functions. The sulphydryl group, which gives the molecule its electron-donating character, comes from the cysteine residue. The γ-glutamyl linkage promotes intracellular stability. The peptide bond linking the amino-terminal glutamate and the cysteine residue of GSH is through the γ-carboxyl group of glutamate rather than the conventional α-carboxyl group. This unusual arrangement
resists degradation by most intracellular peptidases and is subject to hydrolysis by only one known enzyme, \(\gamma\)-glutamyltranspeptidase (GGT), which is on the external surfaces of certain cell types. GSH is present inside cells mainly in its reduced (electron-rich, antioxidant) form. In the healthy cell GSSG, the oxidized (electron-poor) form, rarely exceeds 10% of total cell glutathione (Kosower and Kosower, 1978). Intracellular GSH status appears to be a sensitive indicator of the cell's overall health, and of its ability to resist toxic challenge. Experimental GSH depletion can trigger suicide of the cell by a process known as apoptosis (Duke et al., 1996, Slater et al., 1995).

GSH biosynthesis is mediated consecutively by two mechanistically related ligases, **Glutamate cysteine ligase** (GCL; EC 6.3.2.2), with the availability of cysteine usually being the rate-limiting factor, and glutathione synthetase (GS; EC 6.3.2.3). (Fig. 5, Reactions 1 and 2)

\[
\text{L-Glutamate} + \text{L-Cysteine} + \text{ATP} \rightarrow \text{L-} \gamma\text{-Glutamyl-L-cysteine} + \text{ADP} + \text{Pi}
\]

\[
\text{L-} \gamma\text{-Glutamyl-L-Cysteine} + \text{Glycine} + \text{ATP} \rightarrow \text{GSH} + \text{ADP} + \text{Pi}
\]

The intracellular concentration of GSH, which provides protection against oxidative damage directly and by the action of GPx, is controlled by the subsequent reactions of GCL and GS as well as by the **Glutathione reductase** (glutathione: NADP\(^+\) oxidoreductase, GR, EC 1.8.1.7), which recycles GSSG back into its reduced and active form.

GSH is a sulfhydryl (-SH) antioxidant, antitoxin, and enzyme cofactor. It is ubiquitous in animals, plants, and microorganisms, and being water-soluble is found mainly in the cell cytosol and other aqueous phases of the living system. (Kosower and Kosower, 1978; Meister, 1976; Kidd, 1991; Lomaestro and Malone, 1995). Glutathione often attains millimolar levels inside cells, which makes it one of the most highly concentrated intracellular antioxidants.

The GSSG/GSH ratio may be a sensitive indicator of oxidative stress. GSH has potent electron-donating capacity, as indicated by the high negative redox potential of the GSH/GSSG "redox couple" (Lewin, 1976). Its high redox potential renders GSH both a potent antioxidant per se and a convenient cofactor for enzymatic reactions that require readily available electron pairs, the so-called "reducing equivalents." (Kehrter and Lund, 1994) The reducing power of GSH is a measure of its
Glutathione Metabolism: An Overview

free-radical scavenging, electron-donating, and sulfhydryl-donating capacity. Reducing power is also the key to the multiple actions of GSH at the molecular, cellular, and tissue levels, and to its effectiveness as a systemic antitoxin (Meister, 1994a). GSH status is homeostatically controlled, being continually self-adjusting with respect to the balance between GSH synthesis (by GSH synthetase enzymes), recycling from GSSG (by GR), and its utilization (by peroxidases, transferases, transhydrogenases, and transpeptidases).

The build-up of GSH acts to feedback-inhibit GCL, thereby helping to ensure homeostatic control over GSH synthesis. Intracellular GSH is exported from most cells, but it is not significantly taken up by cells under normal conditions. Once outside of the cell, the γ-glutamyl bond of GSH may be cleaved by the membrane bound γ-glutamyl transpeptidase (γ-GT) whose active site is on the outside of some cells/organs. The product of the reaction is a γ-glutamyl enzyme, which can accept an amino acid to form γ-glutamyl amino acid. After transport, the γ-glutamyl amino acid is cleaved by γ-glutamyl cyclotransferase (Reaction 4) to yield free amino acid and 5-oxoproline, which is ring opened by 5-oxoprolinase (5-OPase, Reaction 5) to give glutamate. Build-up of 5-oxoproline can have adverse consequences due to metabolic acidosis. Glutamate can be reused in the first step of GSH synthesis. Therefore, 5-OPase links the synthesis and the degradation pathways of GSH in the γ-glutamyl cycle. The biosynthetic enzymes, together with these latter 3 enzymes, form the γ-glutamyl cycle summarized in Fig. 5. The salvage pathway of GSH synthesis refers to the GSH synthesis from the cysteine and glycine released from cystinylglycine by the action of dipeptidase. Glutamate is usually rich in cells and can be readily obtained from diet and transamination reactions from other amino acids, while cysteine is generated from the essential amino acid methionine, from the degradation of dietary protein, or from turnover of endogenous proteins.

The GSH pool is drawn on for 3 major applications: (a) as cofactor for the GSH-S-transferases in the detoxicative pathways (Reaction 7); (b) as substrate for the γ-glutamyl transpeptidases, enzymes which are located on the outer cell surface and which transfer the glutamine moiety from GSH to other amino acids for subsequent uptake into the cell (Reaction 3); and (c) for direct free-radical scavenging and as an antioxidant enzyme cofactor (Reaction 12). The GSH transferases are a large group of
isozymes that conjugate GSH with fat-soluble substances as the major feature of liver detoxification (Meister and Larsson, 1995; Meister 1994b; Anderson, 1997).

![Diagram of GSH metabolism]

**Fig 5: Overall summary of GSH metabolism:**

*Reaction 1. Glutamate cysteine ligase; Reaction 2. GSH synthetase; Reaction 3 and 3a. γ-glutamyl transpeptidase; Reaction 4. γ-glutamyl cyclotransferase; Reaction 5. 5-Oxoprolinase; Reaction 6 and 6a. dipeptidase; Reaction 7. GSH S-transferases; Reaction 8. N-acetylase; Reaction 9. GSH Peroxidase; Reaction 10. Transhydrogenase; Reaction 11. GSSG reductase; Reaction 12. Oxidation of GSH by O₂; Reaction of free radicals with GSH*

GSH is an essential cofactor for antioxidant enzymes, namely the GSH peroxidases (GPx) and the more recently described phospholipid hydroperoxide GPx (Zhang, 1989). The GPx serve to detoxify peroxides (hydrogen peroxide, other peroxides) in the water-phase, by reacting them with GSH (Reaction 9); the latter
enzymes use GSH to detoxify peroxides generated in the cell membranes and other lipophilic cell phases (Cathecart, 1985). This is one instance of the water-soluble GSH providing electrons to help reduce oxidized biomolecules located away from the water phase. Enzymes collectively known as GSH transhydrogenases use GSH as a cofactor (Reaction 10) to reconvert dehydroascorbate to ascorbate, ribonucleotides to deoxyribonucleotides, and for a variety of \(-S-S- \leftrightarrow -SH\) inter- conversions.

After GSH has been oxidized to GSSG, the recycling of GSSG to GSH is accomplished mainly by the enzyme GR (Reaction 11). This enzyme uses the coenzyme NADPH as its source of electrons. Therefore, NADPH, coming mainly from the pentose phosphate shunt, is the predominant source of GSH reducing power.

Through its significant reducing power, GSH also makes major contributions to the recycling of other antioxidants that have become oxidized. This could be the basis by which GSH helps to conserve lipid-phase antioxidants such as alphatocopherol (vitamin E), and perhaps also the carotenoids. Meister and his group used BSO to inhibit GSH synthesis in rodents, and concluded from their findings that GSH almost certainly plays such a role in vivo (Meister, 1994a, 1994b, Anderson, 1997, Meister, 1995).

Earlier studies have shown that parasites and hosts have markedly different requirements for GSH (Hussein and Walter, 1996). Thus, modulation of its levels can be used as a target for chemotherapy to parasitic infections. The design and synthesis of specific inhibitors of these physiologically important enzymes are of critical importance for the development of therapeutic agents as well as for use as mechanistic and physiological probes in GSH metabolism (Meister and Anderson, 1983).

With regard to the essentiality of GSH for the survival of the whole organism, substantial information is available from studies on hereditary GSH depletion in the human, and from experimental depletion and repletion of GSH in animal models and cell cultures (Meister and Larsson, 1995, Beutler, 1989). Inherited deficiency of the enzyme GCL has been described in two human siblings. They exhibited generalized GSH deficiency, hemolytic anemia, spinocerebellar degeneration, peripheral neuropathy, myopathy, and aminoaciduria, and severe neurological complications as they moved into their fourth decade of life (Meister and Larsson, 1995). Their red cell
GSH was less than 3% of normal, their muscle GSH less than 25%, and their white cell GSH less than 50% normal. One of them may have been hypersensitive to antibiotics, having developed psychosis after a single dose of sulfonamide for a urinary tract infection. Deficiency in GSH synthetase, the second enzyme of GSH synthesis, also is associated with hemolytic tendency and defective central nervous system function. This condition is complicated by the metabolic consequences of an excess of 5-oxoproline, formed as a "spillover" from the accumulation of gamma-glutamylcysteine after its normal synthesis by the first enzyme and its lack of conversion to GSH by the second enzyme (Meister and Larsson, 1995; Beutler, 1989).

GLUTATHIONE METABOLISM AND PLASMODIA PARASITES

Malaria-infected human erythrocytes are under a substantial degree of oxidative stress, which appears to promote growth and differentiation of *P. falciparum* (Ginsburg and Atamna, 1994). Plasmodia species have not only to overcome the oxidant attack of phagocytes that is supposed to be critical during the short period between dissemination and re-invasion of the host cell (Nnalue and Friedman, 1988; Mohan *et al.*, 1993; Jensen and de Waa, 1988; Malhotra *et al.*, 1988). The primary habitat of the parasite, the red blood cell, is a pro-oxidant environment. Moreover, the parasite itself generates a kind of oxidative burst within the erythrocyte, as evident, e.g., from the generation of hydroxy alkenals (Buffinton *et al.*, 1988; Schwarzer *et al.*, 1996) and met-Hb (Atamna and Ginsburg, 1993). Malaria parasites impose an oxidative stress upon their host cell mainly due to the metabolic process of oxy-haemoglobin digestion (Atamna and Ginsburg, 1993). Hemozoin, a byproduct of Hb digestion by the parasite that is also released into the circulation, there triggers an additional oxidative response by phagocytes (Schwarzer *et al.*, 1996; Green, *et al.*, 1996). Being themselves sensitive to oxidant killing (Mehrotra, 1996), *Plasmodia* species depend on an effective antioxidant system. In this context GSH appears to play a dominant role. Although the intracellular parasite leads to formation of elevated levels of ROS, both the infected host cell and the parasite are still able to survive. However, their redox balance is compromised and it was shown that the *Plasmodium*-infected erythrocytes are more vulnerable than normal RBCs towards
reactive oxygen intermediates derived from activated macrophages or experimental systems (Clark and Hunt, 1984; Wozencraft, 1986).

The potential importance of the host cell glutathione system for the survival of blood stage *Plasmodia* forms, has been discussed for decades (Ginsburg and Atamna, 1994). The key role of GSH in important processes during the intra-erythrocytic life of the malaria parasite greatly increases interest in studying the *Plasmodium* enzymes involved in its biosynthesis, in view of their possible use as targets for the design of new effective drugs. Basically inferred from epidemiologic observations that glucose-6-phosphate dehydrogenase deficiency and genetically altered Hbs prevail in areas where malaria is endemic, the pro-oxidant status of the affected erythrocytes, due to either impaired glutathione reduction or increased consumption, is considered to create an uncomfortable environment for the parasites (Kosower and Kosower, 1970; Yuthavong *et al.*, 1990; Miller *et al.*, 1984; Senok *et al.*, 1998).

On the basis of early reports (Roth, 1986), malaria parasites were thought to depend on their host for the supply of GSH required to maintain the redox status of the cell and to protect it against oxidant stress (Penninckx and Elskens, 1993). However, recent studies (Atamna and Ginsburg, 1997) on GSH metabolism and transport show that *Plasmodia* species have their own GSH metabolism, which is largely independent from that of the host cell (Atamna and Ginsburg, 1997; Ayi *et al.*, 1998). Neither the GSH precursor γ-glutamyl-cysteine nor GSH itself can penetrate from the hosting erythrocyte into the infecting trophozoite (Atamna and Ginsburg, 1997) indicating that the parasite’s GSH must be derived from *de novo* synthesis. An efficient synthesis of GSH could also be demonstrated by isolated parasites (Ayi *et al.*, 1998). Also, the regeneration of GSH from GSSG was 10–20 times faster in the parasite than in nonparasitized erythrocytes (Ayi *et al.*, 1998). GSH turnover appears to be higher in infected than in normal RBCs (Atamna and Ginsburg, 1997). It has also been shown (Dubois *et al.*, 1995) that chloroquine resistance in *P. berghei* is accompanied by an increase in GSH content, and that GSH depletion, induced by treatment with a specific inhibitor of GSH biosynthesis, generates a partial reversion of this resistance, while normal RBCs are not affected. Taken together, these findings suggest the presence in the parasite of an endogenous biosynthetic pathway that can be modulated in response to cytotoxic drugs.
According to their function for oxygen transport, erythrocytes are exposed to ROS and thus are equipped with high levels of enzymatic and non-enzymatic antioxidants like SOD, catalase and the enzymes of the GSH redox cycle: GPx and GR (Beutler and Dale, 1989; Hunt and Stocker, 1990). In order to evade oxidative damage not only the host cell but also the parasites possess endogenous antioxidative mechanisms. Recently, several enzymatic antioxidants have been identified within the malaria parasites. The genes of an endogenous iron containing SOD as well as GR and GPx have been isolated from the parasites (Fairfield et al., 1983; Arias and Walter, 1998; Becuwe et al., 1996; Farber et al., 1996; Gamain et al., 1996). Further, high levels of the non-enzymatic antioxidants ascorbate, tocopherol and GSH have been detected in Plasmodia (Stocker et al., 1985, 1986a, 1986b). This strongly suggests that the parasites possess the enzymes of the GSH synthesis as a part of a functional γ-glutamyl cycle. Although the Plasmodium-infected erythrocyte is well equipped with antioxidant systems, the balance between the formation of ROS and their removal appears to be fragile. This is further supported by results from red blood cell disorders like sickle cell anaemia, thalassemia and glucose-6-phosphate dehydrogenase deficiency, which provide partial protection against malaria. Interestingly, all these conditions have one metabolic key in common, as they expose the parasite to the increased toxic effects of ROS (Flint et al., 1986; Golenser and Chevion, 1989; Nagel, 1990; Hunt and Stocker, 1990; Becker et al., 1994).

The rapid emergence of multidrug-resistant *P. falciparum* is a worldwide concern (Wernsdorfer, 1994). Although major research efforts have been made to understand the mechanisms of drug resistance in malaria parasites, a definitive explanation remains elusive. Recently, evidence has been reported linking GSH pools to drug resistance in Plasmodia (Ginsburg et al., 1999; Meierjohann et al., 2002). A marked increase in both GSH levels and GSH-related enzymes activity was reported in some strains of *P. berghei* and *P. falciparum* lines resistant to chloroquine as compared to sensitive ones (Dubois et al., 1995; Meierjohann et al., 2002; Srivastava et al., 1999). Ginsburg et al. (1998) reported that the toxic heme molecule, produced during Hb catabolism (Fitch et al., 1982), is degraded by GSH. In addition, Famin et al. (1999) showed that chloroquine, amodiaquine, and halofantrine inhibit GSH degradation of membrane bound heme in a competitive way, but mefloquine and
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quinine do not. The significance of these observations remains to be investigated, since mefloquine and quinine block the degradation of heme in aqueous solution by GSH (Famin et al., 1999). In that context, increased levels of GSH in the parasite may prevent heme-induced damage and lead to resistance to these drugs. There is ample evidence that parasite survival and virulence depends on endogenous antioxidant defence systems, which may resemble the host antioxidant defence or not. Depending on the degree of relatedness of parasite and host systems, the enzymes involved in parasitic antioxidant defence may be considered as potential drug targets. Therefore, the disturbance of the antioxidative defence system of the host-parasite unit might be a potential target for the development of new antimalarial drugs.

GLUTATHIONE METABOLISM AND FILARIAL WORMS

GSH has been identified as an important part of the antioxidant system of filarial worms to protect them from the oxidative stress created by the host. The enzymes involved in its de novo synthesis, replenishment and utilization can thus be harnessed as important targets. In the case of filarial worms, which survive for many years in the host, the question has been raised, how they manage this long-term persistence in spite of the host immune response, especially as filarial worms are reported to be sensitive towards oxidative stress (Ferrante et al., 1986). On the other hand, there is some forthcoming evidence that filarial worms have developed defence systems of enzymatic and non-enzymatic antioxidants against the host induced oxidative damage (Callahan et al., 1988). Three functional SODs have been identified in filarial parasites (Henkle et al., 1991, 1993; Henkle-Dührsen et al., 1995; James et al., 1994; Ou et al., 1995). One of the Cu/Zn SODs from *O. volvulus* possesses a signal peptide and strong evidence has recently been provided that this SOD is secreted by the parasite to detoxify superoxide anions produced by the host immune response (Henkle-Dührsen et al., 1997). Other antioxidants like GPx and catalase appear to be either not functional with hydrogen peroxide or their existence is questionable (Tang et al., 1995). However, recently a thioredoxin dependent peroxidase was identified in helminths but its function in the detoxification of H₂O₂ in these organisms is not clarified to date (McGonigle et al., 1997). Therefore, GSH represents one of the major antioxidants of the parasites. GSH has been analyzed in
many filarial species viz. *A. viteae*, *L. carinii* and *S. cervi* (Singh et al., 1997b; Gupta et al., 2002). It has been shown that interference with the filarial GSH metabolism has curative effect on filarial infections in mice (Bhargava et al., 1983). Treatment of *L. carinii*-infected mice with arsenicals and subsequent analysis of glutathione levels of parasite and host tissues suggested that these compounds profoundly affect GSH levels of the filaria (Bhargava et al., 1983). Further analysis showed that one of the targets of the trivalent arsenicals might be GR of the filarial parasite (Müller et al., 1995). However, arsenicals are not suitable as macrofilaricidals because of severe side effects and therefore, new antifilarial drugs need to be identified.

The significance of GSH and related metabolism to filariae has been indicated by the finding that sustained severe reduction in endogenous GSH pools within adult filariae accelerated a drop in microfilarial output and eventually led to the death of the parasites. The finding that filariae in vitro are also able to salvage exogenous GSH, unlike most, if not all, mammalian cell populations, points to a promising target for selective inhibition. In view of published findings (Meister and Anderson, 1983; Bhargava et al., 1983) suggesting that maintenance of a characteristic and relatively high intracellular ratio of reduced to oxidized glutathione (GSH/GSSG) is vital to adult filariae, it seems worthwhile to develop drugs that could selectively deplete or distort GSH stores in these parasites. Promising targets for novel selective disruptors of GSH metabolism and/or functions in filariae might be identified when this area of filarial biochemistry is thoroughly investigated. Although filariae appear to have relatively high GSH content, the comparative importance of de novo and salvage pathways in the maintenance of GSH and GSSG pools and factors affecting the turnover of these pools have not yet been indicated. A majority if not all, the normal mammalian tissues have a large excess of GSH compared to filarial parasites that invade them have levels close to that required for survival. Therefore, depletion of filarial intracellular GSH levels by antifilarial agents may be more harmful to parasites than to the normal tissues of the host organisms.

**GLUTAMATE CYSTEINE LIGASE**

The first step of GSH biosynthesis is generally regarded as rate limiting (Plummer et al., 1981) and catalyzed by Glutamate cysteine ligase (GCL, earlier
known as γ-glutamylcysteine synthetase EC 6.3.2.2), which is regulated physiologically by feedback competitive inhibition by GSH, the availability of cysteine and by various endogenous and exogenous compounds (DeLeve and Kaplowitz, 1991; Richman and Meister, 1975; Anderson, 1998). The GCL enzyme is composed of a heavy (Mr~73 000) and a light (Mr~30 000) subunit, which are encoded for by different genes and dissociate under reducing conditions (Yan and Meister, 1990; Huang et al., 1993a). The two subunits of GCS are differentially regulated (Lu, 1999; Cai et al., 1997; Huang et al., 1998; Lu et al., 1999a, 1999b). The heavy subunit exhibits all of the catalytic activity of the isolated enzyme as well as feedback inhibition by GSH (Seelig et al, 1984). The light subunit is enzymatically inactive, but plays an important regulatory function by lowering the Km of GCL for glutamate and raising the Ki for GSH (Huang et al, 1993a, Huang et al, 1993b). Hormones such as insulin and hydrocortisone and rapid liver growth after two-thirds partial hepatectomy induced the expression of the heavy subunit without any influence on the light subunit (Cai et al., 1995, 1997; Huang et al., 1998). In contrast, agents that induce oxidative stress such as DEM, BSO with profound GSH depletion, and TBH induced the expression of both subunits (Cai et al., 1997). Recently in vivo treatment of rats with thioacetamide was described, which induced the expression of both GCS subunits, while intragastric ethanol infusion induced the expression of the heavy subunit only (Lu et al., 1999 a,b). Since GCL is a major determinant of the overall GSH synthesis capacity, regulation of GCL subunits has been a topic of extensive research (Lu, 1999).

Inhibition of GSH synthesis by buthionine-sulfoximine (BSO), a selective inhibitor of GCL, has been shown to decrease GSH levels in various organisms and tissues, namely in mice and rats, tumors, Trypanosoma brucei and Ascaris suum (Griffith and Meister, 1979, Arrick et al., 1981, Lee et al., 1987, Martensson et al., 1990, Hussein and Walter, 1996). Treatment of mice infected with T. brucei with BSO has already proved to have a curative effect on mice (Arrick et al., 1981). Although BSO is currently in clinical trial for malignant melanoma and ovarian cancer as an adjuvant to melphalan (Schröder, et al., 1996; Bailey et al., 1994; O'Dwyer et al., 1996; Bailey et al., 1997), it is not sufficiently potent to allow complete GCL inhibition to be easily maintained in vivo. In addition, BSO is rapidly
excreted in the urine, necessitating administration of large amounts of drug (Bailey et al., 1997; Gallo et al., 1995; Malaker et al., 1994). Inactivation of GCL by disulfide compounds was first described for cystamine by Griffith et al. (1977). They reported the covalent nature of the inactivation and its reversibility by reducing agents like dithiothreitol. These findings suggested that the inactivation took place by disulfide interchange, presumably with a cysteine residue, in or near the glutamate-binding site. This was further corroborated by studies that GCL is very potently inhibited by cystamine (Lebo and Kredich, 1978; Beamer et al., 1980). This observation and other data indicate that interaction between the enzyme and cystamine leads to the formation of a mixed disulfide between cysteamine and an enzyme sulfhydryl group (Griffith et al., 1977; Beamer et al., 1980)

GCL has been purified from a variety of sources (Meister and Anderson, 1983; Meister, 1974; Yan and Meister, 1990). In case of filariids this enzyme has been characterized in *O. volvulus* (Lüersen et al., 2000a) and detected in *S. cervi* (Tiwari et al., 2003). Recent studies have shown the presence of this enzymatic activity in *P. falciparum* (Atamna and Ginsburg, 1997), the agent of the most severe form of human malaria. Interestingly, *P. falciparum*, which infects the host’s erythrocytes, essentially produces all the GSH present in infected cells, while *de novo* synthesis of GSH by the host cell is impaired (Atamna and Ginsburg, 1997). In addition, GSH-mediated degradation is one route through which malaria parasites react to the toxic effect of heme generation by host Hb degradation (Atamna and Ginsburg, 1995; Ginsburg et al., 1998). The *P. falciparum* and *P. berghei* ggs homologues have been cloned (Birago et al., 1999; Lüersen et al., 1998). Recently the chloroquine resistance in *P. berghei* was correlated with the intracellular GSH levels and significantly sensitised the resistant parasites to chloroquine (Dubois et al., 1995).

**GLUTATHIONE REDUCTASE**

The flavoenzyme glutathione reductase (Glutathione: NADP+ oxidoreductase, GR, EC 1.8.1.7) is the central enzyme of antioxidative defence and catalyses the regeneration of the reducing tripeptide glutathione (GSH) from its disulfide GSSG.

\[
NADPH + H^+ + GSSG \rightarrow NADP^+ + 2 \text{GSH}
\]
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GR creates an intracellular GSH/GSSG ratio of 20 to 1000 depending on the respective metabolic conditions (Schirmer and Schulz, 1987). A high GSH/GSSG ratio is essential for protection against oxidative stress. (Carlberg and Mannervik, 1985) Due to its central role in cellular redox metabolism, inhibition of the GR from the parasites represents an important approach to antiparasitic drug development. If the enzyme from host and parasite differ in their susceptibility to inhibitors, it will of practical relevance. The wide distribution of reduced glutathione (Jocelyn, 1972) reflects the general importance of GR.

The enzyme has been purified from many different sources, such as human erythrocytes, (Worthington and Rosemeyer, 1975, Krohne-Ehrich et al., 1977) porcine erythrocytes, (Boggaram et al., 1979) rat liver, (Carlberg and Mannervik, 1975; Carlberg et al., 1981) calf liver, (Carlberg and Mannervik, 1981) gerbil liver, (Trang et al., 1983) and sheep brain (Acan and Tezcan, 1989). Human GR is a well-characterized protein; the catalytic cycle, as well as the binding mode of drugs and other ligands have been studied in great detail (Schirmer et al., 1995; Williams, 1992; Schirmer et al., 1989; Karplus and Schulz, 1987; Savvides and Karplus, 1996; Nordhoff et al., 1993). P. falciparum GR (PfGR) has recently come into focus as a drug target. The enzyme was purified from parasitized RBCs but only in μg quantities (Krauth-Siegel et al., 1996). To promote structural and functional studies on this protein, the gene of PfGR was isolated (Farber et al., 1996). Recombinant PfGR (rPfGR) has been expressed, purified and characterized, in particular as a target of methylene blue. This antimalarial compound, the first chemotherapeutic agent to be successfully used in humans (Vennerstrom et al., 1995), is known to interfere with the glutathione metabolism of parasitized erythrocytes (Kelner and Alexander, 1985). P. falciparum possesses a complete glutathione system (Farber et al., 1996; Lüersen et al., 2000b; Meierjohann et al., 2002) and the parasite GR has long been discussed as a potential drug target molecule (Schirmer et al., 1995). It is assumed that both human erythrocyte and P. falciparum GR play important roles for the intraerythrocytic growth of parasites, protecting it from oxidative stress. (Schirmer et al., 1995; Becker et al., 1990; Schonleben-Janas et al., 1996; Becker et al., 1999; Farber et al., 1998; Bohme et al., 2000)
The central role of GR in maintenance of the thiol redox state and in antioxidative defence has to be evaluated in more detail in order to establish the essential function of this enzyme for the survival of the filarial parasite. Findings that active outward transport of oxidized glutathione (GSSG) by filariae increases significantly when (a) GR is inhibited or (b) when the worms are subjected to any sort of oxidative stress suggest the presence of a special mechanism for protection against GSSG build-up. A toxic potential of elevated intracellular GSSG that is of major importance to filariae is the ability of this disulfide to inhibit a number of glycolytic enzymes. The *O. volvulus* GR (OvGR) gene was cloned and sequenced by Müller *et al.* (1997). The GRs from two cattle filariae (*S. digitata* and *O. gutturosa*) have been isolated and their properties have been compared to those of human erythrocyte GR. Although their physical and kinetic properties were very similar, the studies on the inhibition of the enzymes by the trivalent melaminophenyl arsenical melarsen oxide revealed that human GR is less susceptible to inhibition by the arsenical than the filarial enzymes (Müller *et al.*, 1995). Moreover, the mechanism of inhibition was found to be different in host and filarial enzymes, competitive inhibition was found in the case of human enzyme where as the filarial GRs were inhibited in two stages: an immediate partial inactivation followed by a time-dependent stage with saturable pseudo-first-order kinetics. These differences between host and parasite enzyme might reflect differences in the primary and secondary structure of the proteins that might be exploitable for the design of new specific macrofilaricidal drugs (Müller *et al.*, 1995). As reported previously, partially purified *L. carinii* GR is much more susceptible towards inhibition with melarsen oxide than the enzyme isolated from human erythrocytes (Bhargava *et al.*, 1983). At present there is no safe and effective macrofilaricidal drug available that can be used in combination with the microfilaricide ivermectin. Although annual doses of ivermectin are effective in killing the microfilariae, there is a pressing need for the development of better and safer drugs that are effective against adult worms. It is well known that organic arsenicals have macrofilaricidal effects, and some evidence suggests glutathione metabolism of filarial worms might be the drug target (Bhargava *et al.*, 1983; Krohne-Ehrich *et al.*, 1977; Worthington and Rosemeyer, 1974), but these have been
discarded because of their toxic effect to mammals. Other compounds targeting filarial GR thus required.

Inhibition of GR has also been proposed for anti cancer and antiparasitic chemotherapy (Schirmer et al., 1989, 1995). GR inhibitors like carmustine (BCNU), which belongs to the nitrosoureas and act with potent alkylating or carbamoylating ability, are clinically used as antitumor drugs and also show antimalarial activity against P. falciparum in vitro (Eisenbrand, 1996; Schallreuter and Wood, 1991; Zhang et al., 1988a, 1988b; Vanhoefer et al., 1997). Apart from the alkylating nitrosoureas, GR and other oxidoreductases are targets for redox cycling agents like menadione and related napthoquinones. These redox cyclers also tend to arylate thiols, which increases their efficacy (Bellamo et al., 1987; Gant et al., 1988; Henry and Wallace, 1995). Nitrofurans, drugs that are broadly used as antimicrobials, have been screened against various experimental filarial infections and have shown considerable variation in the efficacies between drug and filarial species as summarised by Zahner and Schaeres (1983). Further this class of redox cyclers is known to inhibit GR (Grinblat et al., 1989) and to be effective against T. cruzi and T. congolense infections and it has been suggested that they interfere with the trypanothione metabolism of the parasites, where they act as ‘subversive substrates’ or ‘turncoat inhibitors’ of trypanothione reductase (Krauth-Siegel and Schoneck, 1995; Cenas et al., 1994; Henderson et al., 1988). Nitrofuran sensitivity of filarial parasites implies that they metabolise these compounds by a similar route to that in trypanosomatids. However, side effects of nitfurtimox are severe and there is concern about mutagenicity (Ohnishsi et al., 1980). Nevertheless a number of other compounds function by a similar mechanism and so it may be possible to retain activity but reduce toxicity. Considering the potential of these classes of drugs in tumour therapy and some parasite infections, they appear to be attractive chemical leads for the design of specific chemotherapeutics against malaria and filariasis.