Annu. Rev. Pharmacol. Toxicol. 1995. 35:93–127

MOLECULAR MECHANISMS AND THERAPEUTIC APPROACHES TO THE TREATMENT OF AFRICAN TRYPANOSOMIASIS

C. C. Wang
Department of Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, CA 94143-0446

KEY WORDS: African trypanosomes, mechanisms of drug action, targets for chemotherapy

ABSTRACT

There are only a handful of drugs available today for treating African trypanosomiasis, most of which were discovered more than forty years ago. These drugs are plagued by various problems, ranging from oral inabsorption, acute toxicities, short durations of action, and low efficacies to the emergence of trypanosomal resistance. Mechanisms of antitrypanosomal action of these drugs are mostly unknown, except for eflornithine, which is a suicide inhibitor of ornithine decarboxylase. On the other hand, the African trypanosomes are among the most extensively studied parasitic protozoa to date. Many of their intriguing biological features have been well documented and can be viewed as attractive targets for antitrypanosomal chemotherapy. These features include the glycosomal functions and protein import, the *trans*-splicing of mRNAs, the machineries for controlled protein degradations, the polyamine metabolism, the trypanothione metabolism, the purine salvage enzymes, and the glycolipid anchor for the surface glycoproteins.

INTRODUCTION

African trypanosomiasis, transmitted by the tsetse flies of the genus *Glossina* in Africa, causes sleeping sickness in humans and nagana in cattle. It also

0362-1642/95/0415-0093$05.00

affects a variety of other domestic livestock and game animals. The African trypanosomes are flagellated parasitic protozoa belonging to the order kinetoplastidae and the family trypanosomatidae (1). One of the major species, *Trypanosoma brucei,* is further divided into three virtually biochemically identical subspecies: *T. brucei brucei* primarily infects cattle, goats, and sheep; *Trypanosoma brucei gambiense* is distributed in West and Central Africa and causes Western African sleeping sickness; and *Trypanosoma brucei rhodesiense* in Eastern and Southern Africa is the cause of Eastern African sleeping sickness. Two other major species, *Trypanosoma congolense* and *Trypanosoma vivax*, infect cattle, sheep, and goats. These two species, together with *T. brucei brucei,* constitute a main obstacle to the cattle industry in Africa (2). On the human side, an estimated 50 million people living in some 200 foci in Africa today are in danger of acquiring the sleeping sickness (3). About 25,000 new cases of sleeping sickness are reported annually, which is considered an underestimate of the real situation because of the difficulties in diagnosis and inaccessibility of affected areas. The early stage of sleeping sickness is characterized by fever, headache, and joint pains, followed by neurological symptoms, endocrinal disorders, coma, and eventually death.

The trypanosomes enter their mammalian host via a bloodmeal by the infected tsetse fly. They are transformed from the metacyclic forms in the tsetse salivary gland to bloodstream forms in the mammalian blood, where they begin to multiply by asexual binary fission once every six to eight hours (4). Glycolysis is the sole source of energy (5), which eventually leads to hypoglycemia of the infected mammalian host. Some of the trypanosomes invade the central nervous system at the advanced stage of infection, presumably attracted by the glucose-enriched environment in the brain, and cause lesions in the brain and sleeping sickness. The trypanosomes will then change their shapes from the long, slender, actively dividing to the intermediate and finally to the short, stumpy, dormant state (162). Upon entering the midgut of a tsetse fly, the trypanosomes undergo a unique process of differentiation, which, within a matter of 48 hours, will complete the process of mitochondrial biogenesis to acquire a fully functional tricarboxylic acid (TCA) cycle, an electron-transport chain, and oxidative phosphorylation (6). The trypanosomes begin to rely more on mitochondrial metabolism for energy and have their morphology altered to a somewhat more extended, less flexible shape—the procyclic (insect) form. From there, they are further transformed into the epimastigote form, migrating into the salivary gland of the infected tsetse fly, and differentiated once more into the dormant metacyclic form, thus completing the life cycle (7).

While existing in the metacyclic form, each trypanosome is coated with a uniform population of one glycoprotein species on the outer surface of its plasma membrane. The glycoprotein, known as the variant surface glycoprotein (VSG), remains on the membrane surfaces while the trypanosomes are

transformed into the actively dividing bloodstream forms in the mammalian host (8). But soon afterwards, the bloodstream trypanosomes begin a series of successive expressions of immunologically distinct VSGs on the membrane accompanied by successive waves of parasitemia. This phenomenon, known as antigenic variation, is attributed to the spontaneous switch of VSGs at a rate of 10^{-4} to 10^{-6} per cell division (9) through random transpositions of distinctive VSG genes to a specific VSG gene expression site (8). The end result from these antigenic variations is a highly effective evasion of host immune responses by the parasites. Thus, little protective immunity can be developed by the infected host. Chemotherapy appears to be the only realistic means to control the disease. Unfortunately, due to its confinement within the continent of Africa, the problem has been largely neglected by the pharmaceutical research institutions in the West. Only about a handful of antitrypanosomal drugs have been discovered, developed, and made available for disease treatment during this century. These drugs are, however, inadequate in controlling the disease (see below). The principal method of controlling African trypanosomiasis today is still by avoiding the tsetse-infested areas and by setting up tsetse fly traps.

EXISTING ANTITRYPANOSOMAL DRUGS AND THEIR MECHANISMS OF ACTION

There are currently four drugs available for treating African trypanosomiasis in humans: suramin, pentamidine, melarsoprol, and eflornithine (3). Treatment of the disease in cattle, sheep, and goat is currently dependent upon three other compounds: homidium, diminazene, and isometamidium (10) (see Figure 1). Some of these drugs are also applied to treating other types of trypanosomiasis such as the infections of *Trypanosoma evansi* in camels and the *Trypanosoma equiperdum* infection in horses (10). A bis-quarternary compound quinapyramine (11) and an organoarsenite melarsenoxide cysteamine (Mel Cy) (12) were recently introduced for treatment of *T. evansi* infections. However, since *T. evansi* and *T. equiperdum* are transmitted sexually without going through tsetse fly, infections by these two organisms are not confined in Africa; thus they are beyond the scope of this review.

With the exception of eflornithine, which was introduced for treatment of West African sleeping sickness in 1990 (3), all of the antitrypanosomal drugs presented in Figure 1 were discovered more than forty years ago. Judging from their chemical structures, many of these compounds are either highly negatively charged (suramin) or highly positively charged (the phenanthridiniums) under physiological conditions. Thus drug absorption tends to be a problem. They must be administered by intravenous or intramuscular injections (3). In spite of the many studies accumulated in the past decades, the mechanisms of

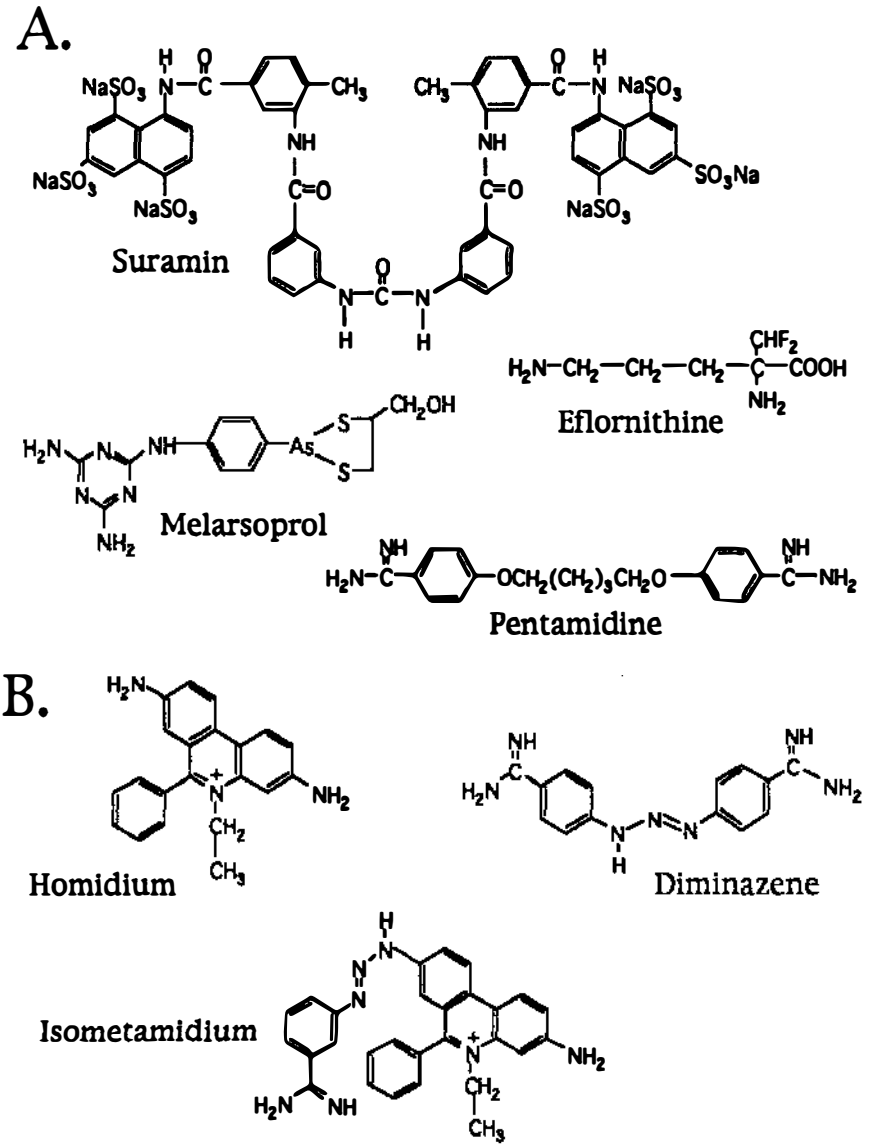


Figure 1 Drugs currently available for the treatment of African trypanosomiasis. (*A*) Drugs for African sleeping sickness in humans. (*B*) Drugs for African trypanosomiasis in cattle, sheep, and goat.

their antitrypanosomal actions are not well understood, except for those of eflornithine. We know that eflornithine acts on trypanosome by inhibiting its ornithine decarboxylase (13), because of the fact that the compound was originally designed and synthesized as a potential suicide inhibitor of ornithine decarboxylase (14).

Suramin

Introduced in 1922, suramin is a sulfonated naphthylamine polyanionic molecule carrying six negative charges at physiological pH and having a molecular weight of 1429. It is usually administered to patients in five intravenous injections at a dosage of 20 mg/kg body weight once every 5 to 7 days (3). It is generally considered the drug of choice for treating the early stages of human African trypanosomiasis, especially the *T. brucei rhodesiense* infections. In view of its chemical structure, there is little wonder why suramin is ineffective against the late stages of the disease; it should not be able to cross the blood brain barrier! In fact, suramin is not expected to pass through a regular lipid-bilayer membrane structure. Two close analogues of suramin, Trypan Blue (molecular weight 961) and Trypan Red (molecular weight 1003) are both smaller than suramin. Their names were derived from the fact that both compounds were used as effective antitrypanosomal drugs in the early twentieth century. Both compounds are now routinely used as biological stains for indicating viability of cells, because they cannot pass through intact biological membranes. Thus, in order for suramin, Trypan Blue, and Trypan Red to demonstrate antitrypanosomal activity, they must first enter the trypanosome cells. The ability of the trypanosomes to take up these drugs may provide the basis for the therapeutic value of suramin as an antitrypanosomal agent without being toxic to the mammalian host.

Suramin has been found to bind to albumin (15) and light-density lipoprotein (LDL) in serum (16). Fairlamb & Bowman (17) have shown that in the presence of serum proteins, suramin is taken up by trypanosomes at a rate that is 18-fold higher than could be explained by fluid endocytosis alone. Thus suramin most likely binds to a variety of serum proteins that enter the trypanosomes primarily by receptor-mediated endocytosis of some of the serum proteins. Since mammalian cells are also capable of performing receptor-mediated endocytosis of serum proteins, the preferential toxicity on trypanosomes exhibited by suramin must mean that either trypanosomes possess more vigorous endocytotic activities on serum proteins in general, or certain suramin-serum protein complexes are preferentially taken up by trypanosomes. Fairlamb & Bowman (18) found that in the suramin-treated infected rats where the plasma suramin reaches a level of 100 μM, the suramin taken up by *T. brucei* amounts to about 0.5 nmol/mg protein. Assuming a cellular protein concentration of about 200 mg/ml in *T. brucei* (19), the intracellular suramin concentration is calculated to be about 100 μM, which is equivalent to the exogenous concentration.

Suramin is an inhibitor of many different dehydrogenases and kinases from mammalian, bacterial, and fungal sources (15). This multiaction comes as no surprise because suramin is a member of the sulfonated aromatic compounds

known to bind to the active sites of dehydrogenases and kinases (20). Some of the derivatives, such as Cibacron Blue, have been routinely employed in affinity column chromatographies for purifying dehydrogenases and kinases. In *T. brucei* suramin has been found to inhibit dihydrofolate reductase (21) and thymidine kinase (22). In addition, it is also a potent inhibitor of all the glycolytic enzymes in *T. brucei:* hexokinase (HK), phosphoglucoisomerase (PGI), phosphofructokinase (PFK), triosephosphate isomerase (TIM), aldolase (ALDO), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), glycerol-3-phosphate dehydrogenase (GPDH), and glycerol kinase (GK) (23). Most remarkably, the IC_{50} values of suramin on all of these glycolytic enzymes are in the range of 10 to 100 μM, which are much lower than those on the same enzymes from mammalian sources. One possible reason for this discrepancy is the unusually high isoelectric point (pI) values for most of the glycolytic enzymes from *T. brucei,* which range from 9 to 10 (24). The basic properties of these enzyme proteins bring additional positive charges to their surfaces, which should facilitate binding by the highly negatively charged suramin and thus potentiate the inhibitory effects. This seemingly perfect theory on the mechanism of antitrypanosomal action of suramin is spoiled, however, by the fact that all the glycolytic enzymes mentioned above are confined within single membrane-bound microbody organelles, termed the glycosomes by Opperdoes & Borst (25) (see below). The glycosome has a diameter of 0.2–0.3 μm, consists primarily of the nine glycolytic enzymes at high concentrations, and is enclosed by a typical phospholipid-bilayer membrane structure (26). The glycosome will not likely be able to take up suramin by passive diffusion or endocytosis. Thus the nine glycolytic enzymes are protected from suramin via compartmentalization. This situation has been reflected by the observation that suramin does not kill the trypanosomes instantly; instead, it has a slow inhibitory action on the parasites over a matter of days (18). If any of the glycolytic enzymes become inhibited by suramin, a quick lysis of trypanosomes should occur within a matter of minutes, because glycolysis provides the only source of energy for the cells (27). Suramin does not affect trypanosomes by directly inhibiting the function of any of the glycolytic enzymes inside the glycosomes. The genes encoding these enzymes are located in the nucleus, and the enzymes are synthesized on free polysomes in cytoplasm and imported into the glycosomes posttranslationally without any proteolytic modification within 3 to 5 min (28). It is entirely possible that suramin may bind to the glycolytic enzymes in the cytoplasm within the interim few minutes and interfere with the import of these enzymes into the glycosomes. The glycolytic enzymes have an average half-life of about 48 h inside the glycosome (29). Thus, inhibition of glycosomal protein import may lead to a gradual decrease of enzyme concentrations in the glycosome and a slowing down of energy metabolism in suramin-treated trypanosomes, which was ex-

actly what was observed in some of the previous studies (17, 18). Although this hypothetical mechanism of suramin action has yet to be proven experimentally, trypanosomal resistance to suramin has not been a serious problem after 70 years of treating trypanosomiasis with it. This fact indirectly supports the theory that suramin acts on multiple targets in trypanosomes. Even the inhibition of *T. brucei* cytoplasmic dihydrofolate reductase and thymidine kinase by surmain may contribute to the antitrypanosomal action of suramin.

Pentamidine

Pentamidine, an aromatic diamidine introduced in 1937, is effective against the early stage of *T. brucei gambiense* infection (3). It is given at a dose of 4 mg/kg body weight by 7 to 10 intramuscular injections daily or on alternative days. The drug has also been used for treating antimony-resistant leishmaniasis (30) and *Pneumocystis carinii* pneumonia (31). Its mechanism of antitrypanosomal action is unknown, but Kapusnik & Mills (32) have reported a wide range of pentamidine bindings to nucleic acids. Edwards et al (33) recently cocrystallized pentamidine with a dodecanucleotide $d(CGCGAATTCGCG)_2$, and they determined the structure of the complex to a resolution of 2.1 Å. The drug was bound in the 5′-AATT minor grove region of the duplex, with amidinium groups H-bonded to adenine N_3 atoms in an interstand manner. The pentamidine molecule adopts an extended conformation, and the immediate binding site spans four base pairs. Pentamidine has long been known to bind preferentially to the minor grooves of the kinetoplast DNA (the mitochondrial DNA, see below) in *T. brucei* (34). It disrupts the kinetoplast DNA (35) and generates dyskinetoplastic cells that retain mitochondrial membranes but lack detectable kinetoplast DNA (36). Recent investigations by Shapiro & Englund (37) on *T. equiperdum* demonstrated that pentamidine (5 μM) promotes cleavage of 5% of the kinetoplast minicircle DNA to generate linearized DNA in a manner similar to that of a topoisomerase II inhibitor. But, unlike the typical topoisomerase II inhibitor etoposide, which denatures both nuclear and kinetoplast DNA–topoisomerase II cleavable complexes, pentamidine exerts no effect on the trypanosomal nuclear DNA. This specific denaturation of kinetoplast DNA–topoisomerase II cleavable complex by pentamidine is interesting, but it may not account for the antitrypanosomal action of pentamidine, because generation of dyskinetoplastic trypanosomes in infected mammalian host is not expected to cure the disease. There must be another aspect of the pentamidine action not yet discovered.

Berger et al also made an interesting observation on pentamidine (38). By exposing *T. brucei brucei* to pentamidine at a therapeutic dose of 4 mg/kg in the infected rats for 4 h, they found a 13-fold increase in lysine content and a 2.5-fold increase in arginine in the trypanosomes. The biological meaning of this drug effect remains unclear. Pentamidine was also found to inhibit the in

vitro splicing of a group I intron in the transcripts of ribosomal RNA genes from the opportunistic fungal pathogen *P. carinii* (39). The group I self-splicing intron, first observed in the Tetrahymena ribozyme, has been found in the mitochondria of many fungi (40) but has not yet been identified in metazoa, including humans (41). Whether the group I intron self-splicing also exists in the trypanosomes is unclear, but we do know that kinetoplastidae perform a unique, although somewhat similar, activity in the mitochondria, termed RNA editing (42). It involves addition and deletion of uridine residues within coding regions of the transcripts from maxicircle cryptogenes. The process is mediated by small transcripts from kinetoplast minicircle and maxicircle DNA network known as guide RNAs (gRNAs) (43). gRNAs contain the edited sequence information and provide the source of uridine residues for edited mRNA. The 5′-portions of specific gRNAs have complementary sequences to specific regions of the preedited mRNA that enable the formation of anchored duplexes to initiate the editing process at the 3′-end of the preedited region and continue in a 5′-direction (44). There are two proposed models for the mechanisms of RNA editing: the enzyme cascade (43) and the transesterification (45). The latter mechanism predicts a ligation between gRNA and pre-mRNA by an attack of the 3′-hydroxyl end of gRNA at the phosphorus atom of a standard phosphoester linkage that is identical to that for the group I and group II intron self-splicing reactions (46). Thus pentamidine may actually inhibit RNA editing in trypanosomes; this possibility should be tested experimentally.

Because RNA editing occurs only in the mitochondria of trypanosomes, it is unlikely that the potential inhibition by pentamidine could block growth of the organism in mammalian blood. There is, however, another unique RNA-slicing activity in trypanosomes; pre-mRNAs in the nucleus are all *trans*-spliced to acquire a common 39-nucleotide sequence at the 5′-ends during maturation (47). There is apparently no *cis*-splicing of mRNAs in trypanosomes. The process of *trans*-splicing is mediated by small nuclear ribonucleoprotein particles (snRNPs) different from those for *cis*-splicings in mammalian cells. Both U1 and U5 snRNPs are missing from the trypanosomal snRNPs (48). U1 is most likely replaced by the 39-nucleotide spliced-leader RNA, whose 3′-hydroxyl group forms a phosphodiester linkage with the 5′-phosphate of the mRNA toward the end of the splicing process. Whether pentamidine or any of the antitrypanosomal diamidines have an inhibitory effect on mRNA *trans*-splicing is unknown. If they do, that action alone can be lethal to the trypanosomes and may account for the mechanism of their antitrypanosomal activities. Experimental tests on this possibility are long overdue (see below).

Pentamidine-resistant *T. brucei brucei* can be readily isolated in the laboratory (38). Damper & Patton (49) observed some years ago that pentamidine is transported into the bloodstream forms of *T. brucei brucei* via a carrier-me-

diated process. The drug-resistant strains of *T. brucei brucei* collected from the field were shown to have a diminished ability to import pentamidine, which is believed to be the primary cause of pentamidine resistance. Neither the drug efflux nor the drug metabolism appeared to play a major role in pentamidine resistance.

Melarsoprol

Melarsoprol, otherwise known as the melarsen oxide [p-(4,6-diamino-S-triazinyl-2-yl) aminophenylarsenoxide]-2,3-dimercaptopropanol adduct, is a trivalent organic arsenical made available to Africa in 1949 (3). Until 1990 it was the only drug capable of treating late stage *T. brucei gambiense* and *T. brucei rhodesiense* infections. It is administered by intravenous injection at a dose of 3.6 mg/kg body weight in 3 to 4 series of 4 injections separated by at least one week. A major problem with melarsoprol is its toxic side effects. It causes reactive encephalopathy in 5–10% of the patients treated, with a fatal outcome in 1–5%. Another problem is in the development of resistance to melarsoprol in both *T. brucei gambiense* and *T. brucei rhodesiense.*

Melarsoprol's mechanism of antitrypanosomal action is not well understood. Melarsen oxide was believed to act by blocking glycolysis in trypanosomes through inhibition of the pyruvate kinase (50). The drug-treated cells rapidly lose their motility, and cell lysis follows within minutes. However, later studies indicated that the lytic effect of melarsen oxide is not the primary result of inhibiting pyruvate kinase (51), because melarsen oxide inhibited trypanosomal PFK ($K_i < 1$ μM) and fructose-2,6-bisphosphatase ($K_i = 2$ μM) much more potently than the pyruvate kinase ($K_i > 100$ μM). At the concentration of 1 μM, the drug completely inhibited the formation of fructose-2,6-bisphosphate. At higher concentrations of 3 to 10 μM, it caused cell lysis resembling that induced by the steroid detergent digitonin. However, it can be argued that since (*a*) the most powerful action of melarsen oxide is its inhibition of trypanosomal PFK, which should result in cessation of glycolysis and subsequent cell lysis (27), and (*b*) the depletion of fructose-2,6-bisphosphate following the inhibition of PFK is known to lead to inactivation of pyruvate kinase (52), melarsen oxide probably still acts on trypanosomes by blocking the glycolysis. The mechanism of action of the drug is by inhibiting trypanosomal pyruvate kinase, albeit indirectly. If this conclusion turns out to be correct, the procyclic trypanosomes, which do not depend on the function of pyruvate kinase for survival, should be much more resistant to the lytic action of melarsen oxide. There is a need to carry out such a test.

A study by Fairlamb et al (53) demonstrated that melarsen oxide or melarsoprol can form a stable adduct with a unique trypanosomatid metabolite, trypanothione [N^1,N^8-bis(glutathionyl) spermidine], which is believed to be a major cofactor involved in the thiol-disulfide redox balance in trypanosomes

(54). The melarsen-trypanothione adduct (Mel T) has a modest stability constant of 1.05×10^7 M^{-1} and inhibits glutathione reductase and the *T. brucei brucei* trypanothione reductase, a key enzyme in regulating the thiol-disulfide state of trypanothione; K_i values are 9.6 and 17.2 μM, respectively (55). These observations have led to recent discussions on a possible mechanism of action of melarsoprol that inhibits trypanosomal trypanothione reductase (see below). But, in view of the 18-fold-higher K_i value required for this action than that for inhibition of PFK, this new theory presently appears less attractive. Furthermore, Fairlamb et al (53) indicated in their study that when bloodstream *T. brucei brucei* cells were incubated in 10 μM melarsen oxide, the intracellular concentrations of reduced trypanothione, reduced glutathione, and Mel T were estimated to be 0.43 mM, 0.16 mM, and 17 μM, respectively, prior to cell lysis. When the cells were incubated with 50 or 75 μM melarsoprol for 30 min, only 0.7% and 2.6% of the intracellular reduced trypanothione were converted to Mel T, respectively. Such a slight decrease in intracellular reduced trypanothione and reduced glutathione probably could not have led to lysis of trypanosomes. In fact, Farilamb et al observed that when 50% of the melarsen oxide–treated trypanosomes became swollen and nonmobile, the bulk of the intracellular reduced trypanothione remained intact (53). The cell lysis was more likely caused by the blocked glycolysis.

Melarsen oxide is most efficient in forming adducts with a variety of dithiols (56). In addition to forming melarsoprol with 2,3-dimercaptopropanol and Mel T with trypanothione, it also binds to the coenzyme dihydrolipoate and some proteins in which cysteine residues come close together. The drug may be a nonspecific inhibitor of many different enzymes, which may explain the many toxic side effects of melarsoprol and the necessity of using melarsen adducts as applicable antitrypanosomal agents instead of melarsen oxide itself.

In view of problems in the resistance to melarsoprol among *T. brucei,* Carter & Fairlamb (57) recently conducted an interesting in vitro study and found that the trypanolytic effect of melarsen oxide can be abrogated by adenine, adenosine, and dipyridamole, an inhibitor of nucleoside transport in mammalian cells. Apparently, all the compounds compete for uptake by an unusual transporter, P2, in *T. brucei brucei.* Another transporter, P1, transports only adenosine and inosine. A cloned melarsen-resistant line of *T. brucei brucei* was found that was missing the P2-mediated transport, and the P1-mediated transport was threefold less than that in the melarsen-susceptable clone. This finding delineated the mechanism of melarsen resistance in this particular *T. brucei brucei* clone and provided an explanation of how the resistant clone survives without a functioning P2 transporter for purine salvage (the trypanosomes cannot perform de novo purine nucleotide synthesis) (58). Apparently, this clone survives on a partially functioning P1 transporter. This study by Carter & Fairlamb (57) also identified two transporters with substrate speci-

ficities different from those observed in the mammalian cells (59). These differences are undoubtedly worth pursuing further as potential targets for antitrypanosomal chemotherapy (see below). Since melarsoprol must also be taken up by the mammalian cells in order to exhibit those toxic side effects, the mechanism of its transport into the mammalian cells should be studied as well. If one of the mammalian nucleoside transporters is involved, it may lead to specific ways of reversing the toxicities of the arsenical. For instance, the mammalian nucleoside transporters also transport pyrimidine nucleosides (59). One could try to use those nucleosides to reverse the toxicity of melarsoprol without compromising its antitrypanosomal activity, because P1 and P2 transporters do not recognize pyrimidine nucleosides as substrates.

Eflornithine

The chemical name of eflornithine is DL-α-difluoromethylornithine (DFMO), which was first synthesized as a potential suicide inhibitor of ornithine decarboxylase (ODC) (14). ODC is the key enzyme in the pathway leading to biosynthesis of polyamines: putrescine, spermidine, and spermine, which are essential for proliferation of prokaryotic as well as eukaryotic cells (60). It was originally anticipated that an effective inhibition of the ODC in tumor cells would deplete the polyamines and thus arrest the tumor growth. Instead, DFMO was found to have good antitrypanosomal activity. It was registered by the US Food and Drug Administration in 1990 and the European Committee for Proprietary Medicinal Products in 1991 for treatment of early and late stage *T. brucei gambiense* infections (3). DFMO is a remarkably safe drug, but its efficacy is relatively weak and the duration of action is so short that it must be adminstered intravenously at a large dose of 400 mg/kg body weight per day in 4 equal doses every 6 h for 14 days (61, 62). It is also relatively ineffective against *T. brucei rhodesiense* infections. However, it is the first new antitrypanosomal drug applied to humans in the last 41 years, and to the delight of research scientists, it was made by rational design, though the end results were not quite expected originally.

As a fluorinated amino acid derivative, DFMO is a zwitterion under physiological conditions, making the drug poorly absorbable and rapidly excreted in the urine, which explains its moderate activity and brief duration of action. It is an irreversible inhibitor of mouse ODC. An elegant study by Poulin et al (63) indicated formation of a major covalent adduct between DFMO and the residue cysteine 360 in mouse ODC. The adduct was tentatively identified as the cyclic imine S-[2-(1-pyrroline) methyl] cysteine, which is consistent with the predicted mechanism of DFMO suicide inhibition; the cysteine 360 acts as an attacking neucleophile in the active pocket of mouse ODC. Human ODC shares 99% identity with mouse ODC (64), and it is presumed to be inhibited by DFMO in the same manner as is mouse ODC. One unique aspect shared

by mammalian ODCs lies in their in vivo instabilities. They turn over more rapidly than most other eukaryotic proteins, with an estimated in vivo half-life of about 20 min (60). Thus, upon one dose of DFMO, the irreversibly inhibited mammalian ODC protein will be degraded steadily at a high rate. As DFMO is also rapidly cleared out of the body system, newly synthesized ODC will no longer encounter DFMO and will thus remain in the active state. This is probably why DFMO is so remarkably nontoxic to the mammalian host and so ineffective as an antitumor agent (61).

DFMO depletes the polyamines putrescine and spermidine from *T. brucei brucei* both in vitro and in vivo (65). Subsequently, the DFMO-treated trypanosomes are transformed to a nondividing, short, stumpy form that is apparently incapable of changing its VSG and is eventually caught up by the host immune reaction (66). The drug effects can be reversed by putrescine in vivo (67) and in vitro (13) but not by L-ornithine in vitro (13). Apparently, DFMO acts against trypanosomes by inhibiting trypanosomal ODC. The gene encoding *T. brucei brucei* ornithine decarboxylase was cloned and sequenced, and the recombinant protein is expressed in transformed *Escherichia coli* in its native form (13, 68). The protein is a homodimer with an estimated subunit molecular weight of 45,000, which is smaller than the 53-kDa subunits in the mouse ODC homodimer (69). There is 61.5% sequence identity between *T. brucei brucei* and mouse ODC. When the homologous amino acid substitutions are discounted, the percentage of similarity reaches 90%. Although thorough characterizations of the mechanism of action of DFMO on *T. brucei brucei* ODC have not yet been performed, the high degree of sequence similarity and the fact that there is also a cysteine 360 residue in *T. brucei brucei* ODC (128) suggest that DFMO may act on the latter by the same mechanism as that on mouse ODC.

When the K_i values of DFMO were estimated against the ODCs of *T. brucei brucei* and mouse, they turned out to be 220 μM and 39 μM, respectively, suggesting that *T. brucei brucei* ODC may be even a little less susceptible to DFMO. The really important question then is, Why is DFMO antitrypanosomal but innocuous to the mammalian host? The only major difference between the two enzyme proteins is that the mouse ODC has an extra 36–amino acid peptide at its C terminus contributing to the higher subunit molecular weight (13). This C-terminal peptide is rich in proline, glutamic acid, serine, threonine, and aspartic acid and flanked by lysine, arginine, and histidine. Rogers et al (70) designated this type of peptide sequence the PEST sequence, which has been identified among many eukaryotic proteins that are known to turn over rapidly in vivo. Since the PEST sequence in mouse ODC is missing from *T. brucei brucei* ODC, a simple postulation would be that the latter may be a very stable protein in vivo. Phillips et al (13) went on to test this hypothesis and found

that *T. brucei brucei* ODC is indeed most stable in procyclic trypanosomes. Subsequent evidence suggested that the ODC in bloodstream trypanosomes is also highly stable (7).

Our understanding of the protein degradation machineries in trypanosomes has progressed since these studies were published. The information accumulated to this point is already sufficient for explaining the mechanism of antitrypanosomal action of DFMO. Due to the lack of in vivo turnover of ODC in the trypanosomes, DFMO can effectively inhibit the enzyme activity and deplete the intracellular polyamines on a long-lasting basis to bring the cells into a dormant state subject to the host immune reaction. The only possible salvation from this situation is perhaps by taking up exogenous polyamines to bypass the inhibition of ODC. The trypanosomes are capable of taking up polyamines (67). But the polyamine level in mammalian plasma is extremely low due to the presence of very high levels of polyamine oxidase (72). Thus, no adequate polyamine uptake can be depended upon to circumvent the consequence of ODC inhibition. This low polyamine level in the living environment may also help to explain why, among the many species of parasitic protozoa, the African trypanosomes are the only ones susceptible to DFMO treatment. They are the only parasites staying in the bloodstream of mammalian hosts, where the level of polyamines is low. The others, which reside in tissues or rapidly proliferating cells, are surrounded by high levels of polyamines and will not be affected by losing their ODC activities.

Since trypanothione is a conjugate of glutathione and spermidine, DFMO-treated trypanosomes were shown to suffer a 40 to 60% decrease in the content of trypanothione and monoglutathionyl spermidine, presumably due to a depletion of spermidine (73). The reduction in the level of trypanothione was also postulated as the mechanism of DFMO action. However, with this significant loss of trypanothione, the DFMO-treated cells should have become highly susceptable to oxidative stresses and lyse readily; instead, they only turned short-stumpy and dormant (65). No sign of enhanced sensitivities toward oxidative stresses occurred. The DFMO-treated bloodstream trypanosomes were still able to differentiate into the procyclic forms in vitro (74), thus suggesting that other than the inability to proliferate, few other abnormalities are associated with the DFMO-treated cells. The experimental results also suggest that the level of trypanothione in trypanosomes can be lowered by 60% without causing a redox imbalance in the cells.

Since the clinical application of DFMO, there has not yet been any report on the occurrence of drug resistance among the trypanosomes. But many isolates of *T. brucei rhodesiense*, which had never been exposed to DFMO previously, were found to exhibit innate resistance to the drug (75). Reasons behind this phenomenon are unclear at present. In laboratory environments, *T.*

brucei brucei mutants resistant to DFMO were isolated independently on two occasions (76, 77). Both mutants turned out to be deficient in the uptake of DFMO.

Homidium, Diminazene, and Isometamidium

Homidium, diminazene, and isometamidium are the three drugs currently available for treating trypanosomiasis in cattle, sheep, and goat in Africa (10). Homidium, otherwise known as ethidium, is a phenanthridinium compound routinely used for staining nucleic acids in research laboratories because of its fluorescenting and nucleic acid–intercalating properties. Its antitrypanosomal activity was first demonstrated 50 years ago (78). Diminazene (berenil) is an analogue of pentamidine that was orginally introduced in 1955 as a trypanocide and a babesiacide (79). Isometamidium resembles a conjugate between homidium and diminazene (80) and thus possesses the properties of both compounds.

Homidium is a mutagen with many nondiscriminating biochemical reactivities (81). The mechanism of its antitrypanosomal action is not well understood. Homidium has been shown to interfere with the glycosomal functions (82), the function of an unusual AMP binding protein (82), trypanothione metabolism (83), and replication of kinetoplast minicircles (2% of total minicircles linearized by 1 μM homidium) in the trypanosomes (37). In every case, the uniqueness of the target is always cited as the supporting evidence that homidium must act on the trypanosomes by inhibiting the functions of that target. But one could argue that inhibition of those not-so-unique targets in trypanosomes can also lead to antitrypanosomal action, as long as the targets are important for the survival of trypanosomes. A careful quantitative analysis should thus be performed to identify the utmost target, which can be inhibited by homidium at its lowest concentration. Homidium was extensively used in the 1960s and 1970s, but its usefulness has been greatly reduced owing to widespread trypanosomal resistance (84). The mechanism of resistance is unknown.

Diminazene acts, as expected, very much like pentamidine. It binds to the minor groove of DNA with a higher affinity to 5′-AATT-3′ than to 5′-TTAA-3′ (85). The attachment to specific sites in DNA occurs via electrostatic and hydrogen-bond forces (86). It also inhibits the kinetoplast topoisomerase II in trypanosomes, resulting in cleavage of 2% of the minicircle DNAs in the presence of 1 μM diminazene (37). This diamidine may also interfere with RNA editing and RNA *trans*-splicing in trypanosome, as discussed above. Long-term use of diminazene has not resulted in widespread development of drug resistance in the field (87), but quinapyramine and melarsomine appear able to induce cross-resistance to diminazene, both in the laboratories and in

the field (88, 89). Preliminary studies suggest that the mechanism of resistance to diminazene is most likely the diminished uptake of the drug by resistant trypanosomes (90).

Isometamidium shares cross-resistance with homidium (91) and exhibits acute toxicity to mammals that is not observed with either homidium or diminazene (92). The acute toxic effects of isometamidium in mice can be reduced by atropine, suggesting that isometamidium may inhibit acetylcholinesterase (93). The mechanism of antitrypanosomal action of isometamidium has not been well studied, but it presumably shares some of the activities found in both homidium and diminazene. In an in vitro study, isometamidium at 1 μM was capable of promoting linearization of 6% of the total minicircle DNA from *T. equiperdum* (37). The underlying mechanism of resistance to isometamidium was recently found to be associated with reduced accumulation of the drug in trypanosomes (94).

POTENTIAL TARGETS FOR FUTURE ANTITRYPANOSOMAL CHEMOTHERAPY

The current status of antitrypanosomal chemotherapy is gloomy indeed. Among only a handful of available drugs, none has the correct chemical structure for easy absorption and prolonged duration of action. Many have very severe toxicities, and some are even bonafide mutagens. After decades of applications, resistance is developing against most of the drugs. Meanwhile, the only new drug, DFMO, has rather weak efficacies and a very narrow spectrum of action. The mechanisms of action of these drugs are generally poorly understood, resulting in a decrease in opportunities for future drug discoveries.

Viewed from another angle, however, the African trypanosomes are perhaps one of the most intriguing families of eukaryotic microorganisms presently known to biologists. Many unique and distinctive features are associated with the development and metabolism of these organisms. Some aspects of them have been thoroughly investigated, adding to our understanding of the biology of trypanosomes as well as the evolution of the ancient eukaryotes. We have already touched upon some of these interesting topics in our discussions above, namely, RNA editing, *trans*-splicing of mRNA, the kinetoplast DNAs, antigenic variations, trypanothione, and glycosome. Many of these features could be turned into targets for antitrypanosomal chemotherapeutic investigations, provided that the following two requirements can be met: (*a*) It is essential for the survival of trypanosome, and (*b*) it is unique enough that a counterpart either does not exist or is sufficiently different in the mammalian host to allow for selective inhibition.

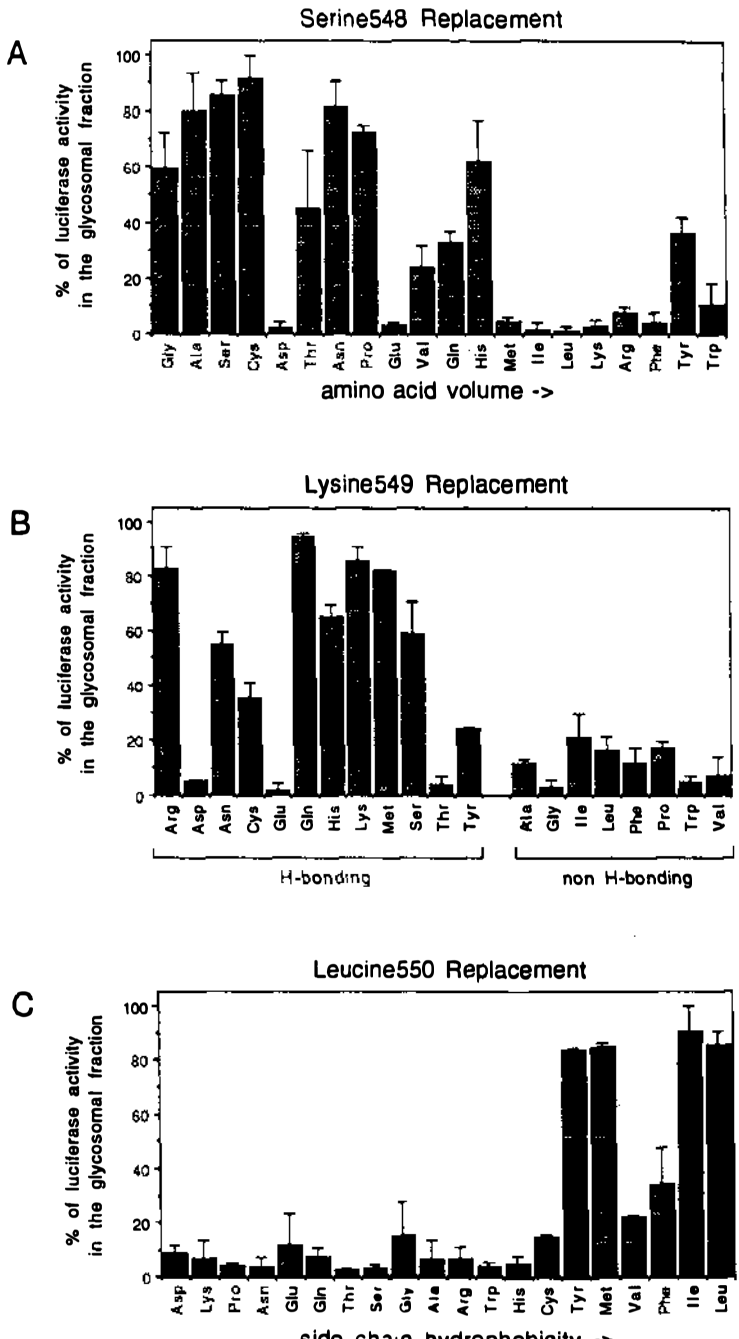
Glycosome

The compartmentalization of glycolytic enzymes in glycosome is generally assumed to result in a glycolytic rate in bloodstream trypanosomes that is 50 times higher than that in mammalian cells (95), because the enzymes are in a state of high concentration (340 mg protein/ml), and the steady concentrations of glycolytic intermediates are in the millimolar range (96). The high rate of glycolysis appears necessary to compensate for its poor yield of energy; only two ATP molecules can be generated from each glucose molecule by this process (97), in contrast to the 36 ATP molecules generated by mitochondrial oxidative phosphorylation. The trypanosomes need to be replicated every 6 to 8 h in mammalian blood (4), and the VSGs must be changed at high enough frequencies to evade host immune response (8). Thus, inhibition of any one of the glycolytic enzymes inside the glycosome should block the chain of glycolytic activity and kill the bloodstream trypanosomes efficiently (27). Most of the glycolytic enzymes in the glycosome have distinctively high pI values between 9 and 10 (24). The extra basic amino acids in these enzyme proteins were first suspected to play the role of targeting signals in importing the proteins into glycosome, but subsequent studies have ruled out this possibility (98). The basic nature of these glycosomal proteins is probably required for packing them into high densities inside the glycosome and facilitating bindings of sugar phosphates to the enzymes in an intraglycosomal environment where the pH was estimated to be neutral (CC Wang & JA Thissen, unpublished data). The genes encoding several of the glycosomal enzymes have been cloned from *T. brucei brucei.* They include genes for PGK (99), ALDO (100), a 56-kDa PGK (101), GAPDH (102), PGI (103), TIM (104), and phosphoenol-pyruvate carboxykinase (PEPCK) (105, 106), which is an enzyme expressed primarily in the procyclic form of *T. brucei brucei* (107). The derived protein sequences of these enzymes all bear decent identities (~50%) to those from the mammalian sources. The three-dimensional structure of *T. brucei brucei* glycosomal TIM was determined at 2.4 Å resolution a few years ago and found to be very similar to that of the mammalian enzyme (108). More recently, the three-dimensional structure of *T. brucei brucei* glycosomal GAPDH was determined by molecular replacement at 3.2 Å with a collected X-ray data set using the Laue method (109). A comparison with the structure of homologous human muscle enzyme suggested that in the NAD^+ binding region, amino acid differences that occur between the two enzymes could provide opportunities for designing selective inhibitors by replacing the 2′ and 3′ adenosine ribose hydroxyl group with substituents extending in the direction of these changed amino acids. Preliminary results indicated that a derivative of adenosine thus designed, 2′-deoxy-2′(3-methoxybenzamido) adenosine, inhibits the human enzyme only marginally but enhances inhibition of the parasite enzyme 45-fold

from that of adenosine to an estimated IC_{50} value of 2.2 mM (110). Although potency of the inhibitor still requires considerable improvement and in vivo testing against trypanosomes with this compound needs to be done, this finding is encouraging for future rational drug designs.

Trypanosomes do not have lactate dehydrogenase. The glycosomal NAD^+ consumed during glycolysis must be regenerated from NADH via a dihydroxyacetone phosphate (DHAP) α-glycerophosphate (α-GP) shuttle consisting of a glycosomal NAD^+-dependent α-GP dehydrogenase and a mitochondrial α-GP oxidase (111). During anaerobiosis, the oxidase cannot function. α-GP, NADH, and ADP are accumulated to high concentrations in glycosome, which can revert the glycerol kinase (GK)–catalyzed reaction to convert the excessive α-GP and ADP to glycerol and ATP (111). Thus, the parasites can still survive under anaerobic conditions with 50% of the normal growing rate by converting a glucose molecule to pyruvate and glycerol in order to generate one ATP molecule (95). α-GP oxidase can be inhibited by salicylhydroxamic acid (SHAM) to bring *T. brucei brucei* under an anaerobic-equivalent condition. Glycolysis is then stopped by inhibiting the GK-catalyzed reverse reaction with glycerol to bring the parasite to cell lysis within minutes (27). The combined SHAM-glycerol chemotherapy suppressed parasitemia in animal models (112), but the curative dose of SHAM in the combination caused 44% animal mortality owing to acute toxicity (113). A hydrophobic derivative of SHAM, *p*-n-tetradecyloxy-benzhydroxamic acid, was later synthesized and found to be 70 times more potent as an inhibitor of α-GP oxidase and 10- to 20-fold more effective as an antitrypanosomal agent in vitro than SHAM (114). But the eventual success in this elegant rational approach to antitrypanosomal chemotherapy may have to rely on better understanding of the properties of the unique *T. brucei* enzyme α-GP oxidase in the future.

The value of glycosome as a potential target for antitrypanosomal chemotherapy can also be attributed to the unique mechanisms underlying the import of proteins into the organelle (98). Recent successful development of efficient expression vectors for transfection of *T. brucei brucei* procyclic form (115) has made it possible to express reporter proteins in the organism and monitor their import into the glycosome. Sommer et al (116) used the technique to express firefly luciferase in the stable transfectants of *T. brucei brucei* procyclic cells and demonstrated, by using biochemical means and electron microscopic immunogold stainings, that this foreign protein was imported almost exclusively into the glycosomes. Previously, firefly luciferase expressed in mammalian cells had been found imported into the peroxisome (117), a morphologically closely related single membrane–bound microbody organelle consisting of primarily catalase, fatty acid–metabolizing enzymes, etc (118). The peroxisomal import of luciferase depends on the very last three amino acids at the C terminus of the protein, serine-lysine-leucine (SKL), and an added



C-terminal SKL sequence is sufficient to target a cytosolic protein to peroxisomes (119, 120). Sommer et al (116) expressed the luciferase without its C-terminal SKL in *T. brucei brucei* and found the truncated protein only in the cytosol. Apparently, the glycosomal protein import machinery can use the same targeting signal for peroxisomal protein import. This conclusion agrees with a similar suggestion by Fung & Clayton (121), who expressed chloramphenicol acyltransferase (CAT) in *T. brucei brucei* with SKL tagged to its C terminus and found the fusion protein associated with a pelletable fraction containing the glycosomes.

The requirements for a C-terminal tripeptide to qualify as targeting signal for mammalian peroxisomal protein import are rather stringent (120). In the SKL sequence, S can be replaced only by cysteine or alanine, and K, by arginine or histidine, whereas L cannot be substituted at all in order to retain the signaling capability. Using a series of site-directed mutagenesis and in vivo glycosomal protein import studies, Sommer et al (116) demonstrated a much more degenerate C-terminal target signal for the glycosomes (Figure 2). With the SKL sequence as a starting point, S can be substituted by any other polar amino acid; small size is preferred. K can be replaced by those amino acids capable of forming H bonds, and L is replaceable by other hydrophobic amino acids. Further investigations indicated that a C-terminal SSL, which is not a targeting signal for peroxisomal import (122), is sufficient to direct the import of a cytosolic protein, β-glucuronidase (GUS), into glycosomes (123).

Among the seven *T. brucei brucei* glycosomal proteins whose primary structures have been determined, four have the C-terminal tripeptides apparently capable of targeting them for glycosomal import: GAPDH (AKL; 102), PGI (SHL; 103), PGK (SSL; 123), and PEPCK (SRL; 124). The other three proteins—TIM (ATQ; 104), ALDO (NTY; 100), and 56-kDa PGK (EKE; 99)—possess C-terminal tripeptides incapable of acting as targeting signal for glycosomal protein import (JM Sommer & CC Wang, unpublished data). These proteins may thus rely on N-terminal or internal peptide(s) as targeting signals. The N-terminal 10–amino acid peptide in mammalian 3-ketoacyl-CoA thiolase that serves as a targeting signal for peroxisomal import (125) does not function in glycosomal protein import (124). An internal 19–amino acid peptide in PEPCK #478–496 was recently identified as another targeting signal indepen-

Figure 2 Amino acid replacement in the C-terminal SKL tripeptide of firefly luciferase and import of the protein into the glycosomes of *T. brucei*. The serine, lysine, and leucine residues at the C terminus of luciferase were individually replaced by all 19 other possible amino acids using site-directed mutagenesis of the gene. (*A*) The amino acids replacing the serine residue in SKL are ordered as a function of increasing amino acid volume. (*B*) Amino acids replacing lysine residue in SKL are grouped according to the hydrogen-bonding capability of their amino acid side chains. (*C*) The amino acids replacing the C-terminal leucine are ordered according to their relative hydrophobicity (TOTFT scale). (From Reference 98.)

dent of the C-terminal SRL and capable of directing 50% of PEPCK to the glycosome (124). Apparently, much remains to be learned on the subject of glycosomal protein import.

The distinction in C-terminal targeting signals between mammalian peroxisomal and *T. brucei brucei* glycosomal protein import should provide an opportunity for selective inhibition of glycosomal import, which will prove lethal to the parasite. An analogue of the degenerated C-terminal tripeptide glycosomal targeting signal could be designed and synthesized in the form of peptoids (126) to avoid proteolytic digestion and tested for antitrypanosomal activity. That some of the glycosomal proteins do not use C-terminal tripeptides for targeting may actually work to the advantage of this strategy, because an antagonist of the C-terminal signal may block the import of only some of the glycosomal proteins while allowing the others to be imported normally, thus breaking down the process of glycolysis completely.

On a related subject, bloodstream *T. brucei brucei* imports glucose by facilitated diffusion (127), and the uptake of glucose apparently represents the rate-limiting step in glycolysis (128). Genes encoding the glucose transporters are tandemly arranged in a multigene family consisting of two homologous groups, THT1 and THT2 (129). THT1-encoded glucose transporters are preferentially expressed in the bloodstream form. They demonstrated a moderate sensitivity to cytochalasin B and recognized D-fructose as substrate, thereby distinguishing themselves from the human erythrocyte glucose transporter. They can be classified as potential targets for antitrypanosomal chemotherapy.

RNA Processing

There is a general lack of specific promoters capable of controlling gene transcriptions during the development of *T. brucei brucei*. The promoters for the VSG genes and the gene encoding procyclin, a major surface antigen of the procyclic forms of *T. brucei brucei,* are largely constitutive (130). The rates of transcription initiation of these genes are similar in both bloodstream and procyclic forms (131). Control of gene expression in *T. brucei* is thus most likely accomplished at the posttranscriptional level. Many household genes in trypanosome are arranged in random repeats and expressed in the form of polycistronic transcripts (132). Thus, *trans*-splicing of the large precursor mRNAs to form mature mRNAs provides the first important step in regulating gene expression in trypanosomes (47). The uniqueness and importance of *trans*-splicing should make it an ideal target for antitrypanosomal chemotherapy. Antitrypanosomal diamidines and phenanthridiniums ought to be tested on *trans*-splicing (see the section on Pentamidine). The method of using antisense oligonucleotides to the splice leader RNA to disrupt the *trans*-splicing in *T. brucei brucei* was tried previously without much success (133). It could perhaps be tested again with more careful planning of the use of various

chemical derivatives of the antisense oligonucleotides (134). Additional activities involved in transcript processing are polyadenylation and mRNA degradation. They have not yet been investigated in depth enough among the trypanosomes to be qualified as potential targets for chemotherapy. RNA editing in kinetoplastidae is both unique and interesting (43), but it appears to be confined to the mitochondrial gene transcripts only and thus cannot be viewed as a target for treating trypanosomiasis in mammals.

Protein Degradation

Posttranslational control, involving programmed protein degradation, may provide another means of regulating gene expression in trypanosomes. We have witnessed a peculiar lack of degradation of ODC in *T. brucei brucei,* which leads to a selective action on trypanosome by a general suicide inhibitor of ODC, DFMO (13). This fortuitous discovery of a new drug could be turned into a general strategy for developing new antitrypanosomal agents (135). Many mammalian proteins are known to have relatively short half-lives. Among their counterparts in trypansomes, some of them may have very long half-lives and perform crucial functions for the survival of the parasite. These proteins could be viewed as potential targets for suicide inhibitor design with the purpose in antitrypanosomal chemotherapy. One advantage to this approach is that once the difference in protein stability has been identified, one may proceed to the general design of a suicide inhibitor without being concerned with the need for a specific inhibitor acting selectively on the parasite enzyme.

The critical role of the C-terminal 36-amino acid PEST peptide in triggering rapid in vivo degradation of mouse ODC was verified by further investigations (136). A mutant mouse ODC with 37 amino acids deleted from its C terminus was expressed in ODC-deficient Chinese hamster ovary (CHO) cells and found to have a prolonged half-life. When *T. brucei brucei* ODC was expressed in the CHO cells, it also demonstrated a long half-life (137), but when the 36–amino acid PEST peptide was linked to the C terminus of *T. brucei brucei* ODC and expressed in the CHO cells, the fusion protein became rapidly degraded (137). However, when mouse ODC was overexpressed in *T. brucei brucei* procyclic form, it demonstrated an extraordinarily long half-life, though the same expressed protein showed unchanged rapid in vitro degradation in rabbit reticulocyte lysate (138). Thus, it appears that the machineries regulating protein degradation in *T. brucei brucei* are somewhat different from those in the mammalian cells.

Protein degradations have been studied in *T. brucei brucei* in a couple of instances. The glycolytic enzymes in glycosome are turned over slowly with an average half-life of ~48 h (29). Cytochrome c, synthesized at similar rates in both bloodstream and procyclic trypanosomes, has a half-life of ~1 h in the

bloodstream form but barely turns over at all in the procyclic form of *T. brucei brucei* (139). Two polymorphic polyubiquitin genes were identified in *T. brucei brucei* (140, 141), and ubiquitin ligase activity was detected in the cytoplasms of bloodstream *T. brucei brucei* (142). Thus, a ubiquitin-mediated protein degradation system is probably present in trypanosome.

Mammalian ODC is degraded by the 26S proteasome without ubiquitination (143). The degradation is accelerated by ODC antizyme, an inhibitory protein induced by polyamines (144). It forms a high-affinity, enzymatically inactive and reversible complex with ODC (145). Mouse ODC is very stable in trypanosomes (138), and the stability is unaffected by exogenous polyamines (SB Hua, XQ Li, P Coffino & CC Wang, unpublished data). Trypanosomes do not have an antizyme-like protein. Thus, by expressing both mouse ODC and rat antizyme in the same trypanosome cell, Hua et al (SB Hua, XQ Li, P Coffino & CC Wang, unpublished data) intended to see if a 26S proteasome-equivalent is present in the organism to degrade the mouse ODC. The mouse ODC–rat antizyme complex was found in trypanosome, resulting in partial inhibition of the enzyme activity but without degradation of mouse ODC. Gel electrophoresis with fluorogenic peptide staining indicated the presence of 20S proteasome in trypanosome but not the 26S proteasome. The mammalian 20S proteasome cannot perform controlled proteolysis and cannot digest ubiquitin conjugates (146) or ODC (143). It will be interesting to find out how trypanosomes regulate their protein degradations with the apparent presence of only 20S proteasome. This aspect of trypanosomal metabolism may provide opportunities for selective inhibition.

Polyamine Metabolism

The ability to synthesize polyamines is apparently of vital importance for the proliferation of bloodstream African trypanosomes in an environment deficient in polyamines (72). Thus, in addition to ODC, S-adenosylmethionine decarboxylase (SAMDC) and spermidine synthase in trypanosome must also serve crucial functions (17) and can be qualified as potential targets for antitrypanosomal chemotherapy, provided that they also have unique features for selective inhibition. Not much is known about trypanosomal SAMDC except that it was unable to cross-react with a human SAMDC antiserum (148). However, a synthetic chemical MDL 73811, 5′-[(Z-4-amino-2-butenyl)methylamino]-5′-deoxyadenosine, was recently found to be irreversibly inhibitory to the SAMDC activity in *T. brucei brucei*, with a K_i of 1.5 μM (149). It elevated the intracellular concentrations of putrescine and SAM and decreased the level of spermidine in the treated trypanosomes (150). Most impressively, it is also active against trypanosome infections in animal models (151). Since detailed comparisons between mammalian and trypanosomal SAMDCs have not yet been done, the mechnism behind the therapeutic effect of MDL 73811 is

unknown. A recent study, however, suggested that the drug is efficiently imported into trypanosome to a high intracellular concentration of 1.9 mM by a parasite-specific adenosine transporter not present in mammalian cells (57, 152). This observation suggests that the primary mechanism of MDL 73811 action may reside in the blockage of adenosine import in trypanosomes.

Trypanothione Metabolism

Trypanothione, a conjugate between glutathione and spermidine, trypanothione reductase, and the synthetic enzymes of trypanothione found in trypanosomatids exemplify the truly unique features of these organisms (54). Like many other unusual biological phenomena observed in them, the presence of trypanothione metabolism in trypanosomes defies obvious rationalization. It is not that the trypanosomes cannot synthesize enough glutathione for their need; the synthesis of trypanothione has to start from glutathione. Trypanothione and glutathione have such similar redox potentials that thiol-disulfide exchange reactions between the two can occur nonenzymatically (153). The only major difference between the two compounds is that the reduced trypanothione remains in one molecule, whereas reduced glutathione is separated into two. Due to the long-distance between the two cysteine residues, the reduced trypanothione is not an ordinary dithiol. Its association with trivalent arsenicals is much weaker than that of 2,3-dimercaptopropanol or lipoic acid (53). Thus, in short, no obvious task can be performed by trypanothione and not by glutathione. The presence of trypanothione remains a mystery.

The existence of trypanothione reductase instead of glutathione reductase in trypanosome makes trypanothione an essential cofactor mediating the redox balance in the organism and trypanothione reductase a potential target for antitrypanosomal chemotherapy. Considerable in-depth studies on the structure of this enzyme have been carried out (54). The enzyme was partially purified from *T. brucei brucei* (154), and the gene encoding this enzyme in *T. brucei brucei* was identified and characterized (155). Detailed X-ray characterizations of the three-dimensional structure were completed on the recombinant trypanothione reductase from *Crithidia fasciculata,* a related kinetoplastida parasitizing insects (156). The crystal structure, refined to 2.6 Å resolution (157), revealed considerable resemblance to that of glutathione reductase, except that the binding site for trypanothione is more open due to the rotations of two helical domains forming part of the active site. Also, the highly positively charged and hydrophilic region of glutathione reductase, where the glycine carboxylate of glutathione interacts with the enzyme, is replaced by a hydrophobic and negatively charged pocket in trypanothione reductase (156). Presumably, the glutamic acid–17 in the latter could either form a hydrogen bond with one of the amide linkages between the spermidine and glycine carboxylate or interact with the positively charged secondary amine of spermidine. Such

well-defined distinctions between glutathione and trypanothione reductases provide good opportunities for designing specific trypanothione reductase inhibitors via computer graphic modelings.

Overexpressions of trypanothione reductase were recently accomplished in two other related kinetoplastidae, *Trypanosoma cruzi* and *Leishmania donovani,* by DNA transfections (158). The transfectants did not demonstrate any enhanced resistance toward the drugs thought to act on trypanosomes by inducing oxidative stress or any increased ability to metabolize hydrogen peroxide (158). This finding may suggest that by inhibiting the activity of trypanothione reductase in trypanosomes, the latter may not become supersensitive to oxidative stress either, thus questioning the validity of maintaining trypanothione reductase as an antitrypanosomal chemotherapeutic target.

The trypanothione biosynthetic enzyme glutathionylspermidine synthetase has been generally accepted as another target for antitrypanosomal chemotherapy (54). However, the previous finding already showed that significant reduction in the level of trypanothione in trypanosome by DFMO did not make the organisms any more susceptible to oxidative stress. They only became dormant after polyamine depletion, as expected (see discussions above on DFMO). It is difficult to imagine how a more specific inhibitor of trypanothione synthesis would act any differently from DFMO, except that polyamines would no longer be depleted. One possible explanation for all the puzzling observations may be that trypanosomes only require very low levels of trypanothione and trypanothione reductase to catalyze the intracellular redox balances with glutathione as the de facto substrate. Inhibition of synthesis or reduction of trypanothione must then reach near completion to exert discernable antitrypanosomal effect, which is usually difficult to achieve in chemotherapeutic practices. A separate approach in this situation would be the design of subversive substrates for these enzymes (159). This is a standard deviation one takes whenever a unique target in an infectious organism turns out either nonessential for the organism or not readily inhibitable. One then tries to design a unique false substrate for this target enzyme with the expectation that the product may turn out to be a poison. The prospect of this alternate approach will depend heavily on the effectiveness of the poison thus generated.

Purine Salvage

All the parasitic protozoa studied to date have been found to lack the capability of de novo synthesis of purine nucleotides (159). African trypanosomes are no exception (58). Interestingly, Carter & Fairlamb (57) found that P1 and P2 adenosine transporters in *T. brucei brucei* have unusual substrate specificities. Melarsen oxide and MDL 73811 are apparently false substrates of these trans-

porters, providing good clues for designing new inhibitors, even in the absence of structural information on the two transporters.

The purine salvage enzymes in trypanosomes are also obvious targets for antitrypanosomal chemotherapy. However, a general phenomenon associated with purine salvage is the existence of multiple pathways for the same purpose (160). Thus, in the absence of a thorough metabolic analysis, it will be risky to focus on one salvage enzyme and assume that it must be a target for antitrypanosomal chemotherapy because it was proven to be so in another parasitic protozoan. A detailed analysis of purine salvage in *T. brucei brucei* has not yet been performed. However, purine salvage enzymes have been observed in a related parasitic protozoon, *L. donovani*. It was reported to possess hypoxanthine-guanine phosphoribosyltransferase (HGPRTase), adenine phosphoribosyltransferase (APRTase), xanthine phosphoribosyltransferase (XPRTase) (161), and purine nucleoside phosphotransferase (162). Should *T. brucei brucei* have a similar spectrum of purine salvage enzymes, it will take more than one specific enzyme inhibitor to block off all the incoming precusors of purine nucleotides. One may then resort to the tactic of using subversive substrates with the expectation that they will only be recognized by the parasite enzyme and that the nucleotide products thus generated will turn out poisonous to the parasite. The most successful example of this approach was the identification of antileishmanial activity in allopurinol (163), a hypoxanthine analogue and a relatively nontoxic antigout drug for human use (164). Apparently, allopurinol cannot serve as a substrate for human HGPRTase, but it can be recognized by the HGPRTase from *L. donovani* as well as the rest of the parasite enzymes involved in converting IMP to ATP and incorporating ATP into RNA (165). Allopurinol ribonucleoside monophosphate is an inhibitor of GMP reductase and IMP dehydrogenase, whereas the aminopurinol ribonucleotides can increase the catabolism of RNA in *L. donovani* (165). All of these toxic effects could have contributed to the antileishmanial activity of allopurinol, but the clinical efficacy of this drug is, unfortunately, quite weak (166).

The gene encoding HGPRTase from *T. brucei brucei* was recently cloned, sequenced, and overexpressed in *E. coli* (167). The recombinant enzyme has a subunit molecular weight of 26,000 and is probably present in the form of a dimer in its native state. Its primary structure shares only 21% sequence identity with that of human HGPRTase. A logical next step would be a close comparison of the three-dimensional structures between the host and parasite enzymes for computer-aided subversive substrate design. Fortunately, the crystal structure of human HGPRTase with bound GMP has been determined recently and refined to 2.5 Å resolution by Eads et al (168). This achievement should facilitate the effort to resolve the three-dimensional structure of HGPRTase from *T. brucei brucei*. However, since the parasite and the host

enzymes share only 21% sequence identity, elucidation of the three-dimensional structure of *T. brucei brucei* HGPRTase by molecular replacement will be too risky. An X-ray crystallographic analysis of the parasite enzyme is still necessary.

The Glycolipid Anchor for Variant Surface Glycoprotein

The VSG of bloodstream African trypanosomes is anchored to the cell surface by a glycosyl phosphatidylinositol (GPI) that contains myristate as its only fatty acid component (169). The pathway of GPI biosynthesis in trypanosomes has been elucidated in a cell-free system that forms the glycan portion of the GPI from endogenous phosphatidylinositol (PI) plus uridine-5′-diphosphate-N-acetylglucosamine (UDP-GlcNAc) and guanosine-5′-diphosphate-mannose (GDP-Man) (170, 171). This GPI precursor contains two fatty acids more hydrophobic than myristate, which are sequentially replaced by two myristate molecules via a process termed fatty acid remodeling (172). Even in the absence of de novo GPI biosynthesis, myristate is still incorporated into pre-existing glycolipid precursor by a presumed acyl exchange process (172). Why trypanosomal VSG GPI-anchor requires strictly myristate as its fatty acid component is still unclear. Assuming that such a structural specificity is essential for the antigenic variations enabling the trypanosomes to evade the host immune response, it should then qualify as an attractive target for anti-trypanosomal therapy. A blockade of the process of fatty acid remodeling or acyl exchange or introduction of a subversive substrate to replace the myristate from GPI may well result in suppressing development of trypanosomes in mammalian blood. Doering et al (173) studied incorporations of heteroatom-containing analogues of myristate into the GPI both in a cell-free system and in intact trypanosomes. Results indicated that the specificity of fatty acid incorporation into the GPI depends on chain length rather than hydrophobicity. One of the analogues that was found efficiently incorporated into the GPI in the cell-free system, 10-(propoxy) decanoic acid, was tested on the bloodstream *T. brucei brucei* in the in vitro culture. At a concentration of 10 μM, this analogue caused gross morphological alterations of the trypanosomes and killed most of them within a few hours. With an estimated LD_{50} value of less than 1 μM on bloodstream trypanosomes, this compound, nevertheless, has little effect on the procyclic forms of trypanosomes and no effect on the growth of human T lymphocytes at 100 μM (174). However, it is unclear whether the specific cytotoxic effect of this myristate analogue on trypanosomes is attributed to its incorporation into the GPI-anchor, because it is incorporated only up to 10% of that of myristate in the intact parasites, presumably due to poor import. No difference in coat morphology was observed between cells treated with myristate and the analogue. Thus, one can not rule out the possibility at the moment that the myristate analogue may act on the trypanosome by a

mechanism other than being a subversive substrate in the GPI synthesis. Furthermore, in vivo testings of this compound will be necessary for evaluating the potential in this new approach to antitrypansomal chemotherapy.

CONCLUSION AND OUTLOOK

I have thus far reviewed many intriguing biological features in African trypanosomes, many of which may turn out to be attractive targets for antitrypanosomal chemotherapy. In view of the seriousness of the diseases caused by the parasites and the pitiful state of the currently existing antitrypanosomal drugs, it is indeed an exciting and significant challenge to all the researchers in this area today to derive new means of controlling this disease using knowledge of these organisms. Much better communication must be established between the molecular biologists and the drug testers so that opportunities such as testing the antitrypanosmal diamidines on mRNA *trans*-splicing will not be missed. The validation of a target for chemotherapy must be thorough in order to avoid wasting precious time and efforts on hopeless endeavors. The two requirements for a target to be both distinctively unique and essential for the survival of parasites must be strictly observed. However, in the enthusiastic pursuit of new antitrypanosomal agents, one often sees that these two rules are not always adhered to. Thus, some enzymes known to be crucial for the trypanosome to survive have been extensively investigated without questioning how distinctive they are from the mammalian enzymes. Conversely, certain very unique enzymes in trypanosomes are being studied intensively without being scrutinized for any indication of their indispensibility for the well-being of trypanosomes. Possible polymorphisms in the genes that encode the therapeutic targets should be another important concern in view of the prevalence of multicopy genes in trypanosome.

Finally, in the current rushes to design inhibitor with computer graphic modelings (175), one should be constantly reminded that even if the computer program has been perfectly crafted, it can only function as well as the information fed into it. The crystal structure of a protein molecule does not contain all the necessary information for designing specific inhibitors. More thorough studies on the structure and function of a protein are necessary to improve the chance of success. Otherwise, computer modeling will be just another form of random screening, except that it uses computers instead of randomly synthesized chemicals or massive collections of fermented soil samples. Predicting which form of screening is more effective in finding a new drug would then be difficult. Screening is the result of a lack of sound knowledge of the organism one intends to control. Hopefully the accumulation of enormous amounts of interesting biological information on trypanosomes in the past will

enable us to use the parasite as a model for truly rational drug design in the future.

ACKNOWLEDGMENTS

The author wishes to thank Drs. Jürg Sommer and Alice Lee Wang for their kind assistance and critical reading of the manuscript. Research conducted in the author's laboratory is supported by NIH grant AI-21786.

Literature Cited

1. Hoare CA. 1972. *The Trypanosomes of Mammals. A Zoological Monograph.* Oxford: Blackwell
2. Jordan AM. 1986. *Trypanosomiasis Control and African Rural Development,* pp. 44–45. New York: Longman
3. Kuzoe FAS. 1993. Current situation of African trypanosomiasis. *Acta Trop.* 54: 153–62

3a. Lumsden WHR, Evans DA, eds. 1976. *Biology of the Kinetoplastida.* London: Academic

4. Vickerman K. 1976. The diversity of kinetoplastid flagellates. See Ref. 3a, pp. 1–34
5. Bowman IBR, Flynn IW. 1976. Oxidative metabolism of trypanosomes. See Ref. 3a, pp. 435–76
6. Hill GC. 1976. Electron transport systems in kinetoplastida. *Biochim. Biophys. Acta* 465:149–93
7. Vickerman K, Tetley L, Hendry KAK, Turner CMR. 1988. Biology of African trypanosomes in the tsetse fly. *Biol. Cell* 64:109–19
8. Donelson JE, Rice-Ficht AC. 1985. Molecular biology of trypanosome antigenic variation. *Microbiol. Rev.* 49: 107–25
9. Miller EN, Turner MJ. 1981. Analysis of antigenic types appearing in first relapse populations of clones in *Trypanosoma brucei. Parasitology* 82:63–80
10. Kinabo LDB. 1993. Pharmacology of existing drugs for animal trypanosomiasis. *Acta Trop.* 54:169–83
11. Suryanarayana C, Gupta SL, Singh RP. 1985. Therapeutic and prophylactic effect of Tribexin prosalt and Gilpol against experimental *Trypanosoma evansi* infection in donkeys. *Indian J. Vet. Med.* 5:20–22
12. Zhang ZQ, Giroud C, Baltz T. 1991. *In vivo* and *in vitro* sensitivity of *Trypanosoma evansi* and *T. equiperdum* to diminazene, suramin, Mel Cy, quinapyramine and isometamidium. *Acta Trop.* 50:101–10
13. Phillips MA, Coffino P, Wang CC. 1987. Cloning and sequencing of the ornithine decarboxylase gene from *Trypanosoma brucei:* Implications for enzyme turnover and selective difluoromethylornithine inhibition. *J. Biol. Chem.* 262:8721–27

13a. McCann PP, Sjoerdsma A, eds. 1987. *Inhibition of Polyamine Metabolism, Biological Significance and Basis for New Therapies.* New York: Academic

14. Bey P, Danzin C, Jung M. 1987. Inhibition of basic amino acid decarboxylases involved in polyamine biosynthesis. See Ref. 13a, pp. 1–31
15. Hawking F. 1978. Suramin with special reference to onchocerchiasis. *Adv. Pharmacol. Chemother.* 15:289–322
16. Vansterkenburg ELM, Coppens I, Wilting J, Bos OJM, Fischer MJE, et al. 1993. The uptake of the trypanocidal drug suramin in combination with low-density lipoproteins by Trypanosoma brucei and its possible mode of action. *Acta Trop.* 54:237–50
17. Fairlamb AH, Bowman IBR. 1980. *Trypanosoma brucei* maintenance of concentrated suspensions of bloodstream trypomastigotes *in vitro* using continuous dialysis for measurement of endocytosis. *Exp. Parasitol.* 49:366–80
18. Fairlamb AH, Bowman IBR. 1980. Uptake of the trypanocidal drug suramin by bloodstream forms of *Trypanosoma brucei* and its effect on respiration and

growth rate *in vivo. Mol. Biochem. Parasitol.* 1:315–33
19. Misset O, Bos OJ, Opperdoes FR. 1986. Glycolytic enzymes of *Trypanosoma brucei:* Simultaneous purification, intraglycosomal concentrations and physical properties. *Eur. J. Biochem.* 157: 441–53
20. Hanggi D, Carr P. 1985. Analytical evaluation of the purity of commercial preparations of Cibacron Blue F3GA and related dyes. *Anal. Biochem.* 149:91–104
21. Jaffe JJ, McCormack JJ, Meymariam E. 1972. Comparative properties of schistosomal and filarial dihydrofolate reductases. *Biochem. Pharmacol.* 21:719–31
22. Chello PL, Jaffe JJ. 1972. Comparative properties of trypanosomal and mammalian thymidine kinases. *Comp. Biochem. Physiol. B* 43:543–62
23. Wilson M, Callens M, Kuntz DA, Perié J, Opperdoes FR. 1993. Synthesis and activity of inhibitors highly specific for the glycolytic enzymes from *Trypanosoma brucei. Mol. Biochem. Parasitol.* 59:201–10
24. Wierenga RK, Swinkels B, Michels PAM, Osinga K, Misset O, et al. 1987. Common elements on the surface of glycolytic enzymes from *Trypanosoma brucei* may serve as topogenic signals for import into glycosomes. *EMBO J.* 6:215–21
25. Opperdoes FR, Borst P. 1977. Localization of nine glycolytic enzymes in a microbody-like organelle in *Trypanosoma brucei:* The glycosome. *FEBS Lett.* 80:360–64
26. Opperdoes FR, Baudhuin P, Coppens I, De Roe C, Edwards SW. 1984. Purification, morphomatric analysis and characterization of the glycosomes (microbodies) of the protozoan haemoflagellate. *J. Cell Biol.* 98:1178–84
27. Clarkson AB, Brohn FH. 1976. Trypanosomiasis: an approach to chemotherapy by the inhibition of carbohydrate catabolism. *Science* 194:204–6
28. Hart DT, Baudhuin P, Opperdoes FR, De Roe C. 1987. Biogenesis of the glycosome in *Trypanosoma brucei:* The synthesis, translocation and turnover of glycosomal polypeptides. *EMBO J.* 6: 1403–11
29. Clayton CE. 1987. Import of fructose biphosphate aldolase into the glycosomes of *Trypanosoma brucei. J. Cell Biol.* 105:2649–54
30. Neal RA. 1987. Experimental chemotherapy. In *The Leishmaniases in Biology and Medicine. Clinical Aspects and Control,* ed. W Peters, R Killick-Kendrick, 2:793–845. London: Academic
31. Sands M, Kron MA, Brown RB. 1985. Pentamidine: a review. *Rev. Infect. Dis.* 7:625–34
32. Kapusnik JE, Mills J. 1987. Pentamidine. In *Antimicrobial Agents Annual,* ed. PK Peterson, J Verhoef, 2:271–81. Amsterdam: Elsevier Sci.
33. Edwards KJ, Jenkins TC, Neidle S. 1992. Crystal structure of a pentamidine-oligonucleotide complex: implications for DNA binding properties. *Biochemistry* 31:7104–9
34. Hajduk SL. 1978. Influence of DNA complexing compounds on the kinetoplast of trypanosomatids. *Prog. Mol. Subcell. Biol.* 6:158–200
35. Newton BA. 1974. The chemotherapy of trypanosomiasis and leishmaniasis: Towards a more rational approach. In *Trypanosomiasis and Leishmaniasis,* pp. 285–307. Amsterdam: Elsevier/Excerpta Medica/North Holland
36. Delain E, Brack C, Riou G, Festy B. 1971. Ultrastructural alterations of *Trypanosoma cruzi* kinetoplast induced by the interaction of a trypanocidal drug (hydroxystilbamidine) with the kinetoplast DNA. *J. Ultrastruct. Res.* 37:200–18
37. Shapiro TA, Englund PT. 1990. Selective cleavage of kinetoplast DNA minicircles promoted by antitrypanosomal drugs. *Proc. Natl. Acad. Sci. USA* 87: 950–54
38. Berger BJ, Carter NS, Fairlamb AH. 1993. Polyamine and pentamidine metabolism in African trypanosomes. *Acta Trop.* 54:215–24
39. Liu Y, Tidwell RR, Leibowitz MJ. 1994. Inhibition of *in vitro* splicing of a group I intron of *Pneumocystis carinii. J. Eukaryot. Microbiol.* 41:31–38
40. Cech TR. 1990. Self-splicing of group I introns. *Annu. Rev. Biochem.* 59:543–68
41. Dujon B. 1989. Group I introns as mobile genetic elements: facts and mechanistic speculations—a review. *Gene* 82: 91–114
42. Benne R. 1989. RNA editing in trypanosome mitochondria. *Biochim. Biophys. Acta* 1007:131–39
43. Blum B, Bakalara N, Simpson L. 1990. A model for RNA editing in kinetoplastid mitochondria: "Guide" RNA molecules transcribed from maxicircle DNA provide the edited information. *Cell* 60:189–98
44. Van der Spek H, Arts G-J, Zwaal RR, Van den Burg J, Sloof P, Benne R. 1991. Conserved genes encode guide RNAs

in mitochondria of *Crithidia fasciculata*. *EMBO J.* 10:1217–24
45. Cech TR. 1991. RNA editing: world's smallest introns? *Cell* 64:667–69
46. Woodson SA, Cech TR. 1989. Reverse self-splicing of the Tetrahymena group I intron: implication for the directionality of splicing and for intron transposition. *Cell* 57:335–45
47. Agabian N. 1990. *Trans* splicing of nuclear pre-mRNAs. *Cell* 61:1157–60
48. Bruzik JP, Van Doren K, Hirsh D, Steitz JA. 1988. *Trans* splicing involves a novel form of small nuclear ribonucleoprotein particles. *Nature* 335:559–62
49. Damper D, Patton CL. 1976. Pentamidine transport and sensitivity in *brucei*-group trypanosomes. *J. Protozool.* 23: 349–56
50. Flynn IW, Bowman IBR. 1974. The action of trypanocidal arsenical drugs on *Trypanosoma brucei* and *Trypanosoma rhodesiense*. *Comp. Biochem. Physiol. B* 48:261–73
51. Van Schaftingen E, Opperdoes FR, Hers H-G. 1987. Effects of various metabolic conditions and of the trivalent arsenical melarsen oxide on the intracellular levels of fructose-2,6-bisphosphate and of glycolytic intermediates in *Trypanosoma brucei*. *Eur. J. Biochem.* 166:653–61
52. Misset O, Van Beeumen J, Lambeir AA, van der Meer R, Opperdoes FR. 1987. Glyceraldehyde-phosphate dehydrogenase from *Trypanosoma brucei:* Comparison of the glycosomal and cytosolic isoenzymes. *Eur. J. Biochem.* 162:501–7
53. Fairlamb AH, Henderson GB, Bacchi CJ, Cerami A. 1987. In vivo effects of difluoromethylornithine on trypanothione and polyamine levels in bloodstream forms of *Trypanosma brucei*. *Mol. Biochem. Parasitol.* 24:185–91
54. Fairlamb AH, Carter NS, Cunningham M, Smith K. 1992. Characterization of melarsen-resistant *Trypanosoma brucei brucei* with respect to cross-resistance to other drugs and trypanothione metabolism. *Mol. Biochem. Parasitol* 53:213–22
55. Cunningham ML, Zvelebil MJ, Fairlamb AH. 1994. Mechanism of inhibition of trypanothione reductase by trivalent organic arsenicals. *Eur. J. Biochem.* 221: 285–95
56. Johnston RM. 1963. The arsenicals. In *Metabolic Inhibitors*, ed. RM Hochster, JH Quastel, 2:99–118. New York/London: Academic
57. Carter NS, Fairlamb AH. 1993. Arsenical-resistant trypanosomes lack an unusual adenosine transporter. *Nature* 361: 173–76
58. Hammond DJ, Gutteridge WE. 1984. Purine and pyrimidine metabolism in the *Trypanosomatidae*. *Mol. Biochem. Parasitol.* 13:243–61
59. Plagemann PGW, Wohlhueter RM, Woffendin C. 1988. Nucleoside and nucleobase transport in animal cells. *Biochim. Biophys. Acta* 947:405–43
60. Tabor CW, Tabor H. 1984. Polyamines. *Annu. Rev. Biochem.* 53:749–90
61. Schechter PJ, Sjoerdsma A. 1986. Difluoromethylornithine in the treatment of African trypanosomiasis. *Parasitol. Today* 23:223–24
62. Milord F, Pepin J, Loko L, Ethier L, Mpia B. 1992. Efficacy and toxicity of eflornithine for the treatment of *Trypanosoma brucei gambiense* sleeping sickness. *Lancet* 340:652–55
63. Poulin R, Lu L, Ackermann B Bey P, Pegg AE. 1992. Mechanism of the irreversible inactivation of mouse ornithine decarboxylase by α-difluoromethylornithine: characterization of sequences at the inhibitor and coenzyme binding sites. *J. Biol. Chem.* 267:150–58
64. Hickok NJ, Seppnen PJ, Gunsalus G, Jänne OA. 1987. Complete amino acid sequence of human ornithine decarboxylase deduced from complementary DNA. *DNA* 6:179–87
65. Giffin BF, McCann PP, Bitonti AJ, Bacchi CJ. 1986. Polyamine depletion following exposure to DL-α-difluoromethylornithine both *in vivo* and *in vitro* initiates morphological alterations and mitochondrial activation in a monomorphic strain of *Trypanosoma brucei brucei*. *J. Protozool.* 33:238–43
66. Bitonti AJ, McCann PP, Sjoerdsma A. 1986. Necessity of antibody response in the treatment of African trypanosomiasis with α-difluromethylornithine. *Biochem. Pharmacol.* 35:331–34
67. Bacchi CJ, McCann PP. 1987. Parasitic protozoa and polyamines. See Ref. 13a, pp. 317–44
68. Phillips MA, Coffino P, Wang CC. 1988. *Trypanosoma brucei* ornithine decarboxylase: enzyme purification, characterization and expression in *Escherichia coli*. *J. Biol. Chem.* 263: 17933–41
69. Gupta M and Coffino P. 1985. Mouse ornithine decarboxylase. *J. Biol. Chem.* 260:2941–44
70. Rogers S, Wells R, Rechsteiner M. 1986. Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. *Science* 234:364–68
71. Bacchi CJ, Yarlett N, Goldberg B,

Bitonti AJ, McCann PP. 1991. Polyamine metabolism in African trypanosomes. In *Biochemical Protozoology*, ed. G Coombs, M North, pp. 469–81. London: Taylor & Francis

72. Gahl WA, Pitot HC. 1979. Putrescine-oxidase activity in adult bovine serum and fetal bovine serum. *In Vitro* 15:252–57
73. Fairlamb AH, Henderson GB, Cerami A. 1989. Trypanothione is the primary target for arsenical drugs against African trypanosomes. *Proc. Natl. Acad. Sci. USA* 86:2607–11
74. Bass KE, Sommer JM, Cheng Q-L, Wang CC. 1992. Mouse ornithine decarboxylase is stable in *Trypanosoma brucei*. *J. Biol. Chem.* 267:11034–37
75. Bayles JD, Harrison SM, Mbwabi DL, Schechter PJ. 1989. Treatment of arsenical refractory Rhodesian sleeping sickness in Kenya. *Ann. Trop. Med. Parasitol.* 83(Suppl. 1):111–14
76. Phillips MA, Wang CC. 1987. A *Trypanosoma brucei* mutant resistant to α-difluoromethylornithine. *Mol. Biochem. Parasitol.* 22:9–17
77. Bellofatto V, Fairlamb AH, Henderson GB, Cross GA. 1987. Biochemical changes associated with α-difluoromethylornithine uptake and resistance in *Trypanosoma brucei*. *Mol. Biochem. Parasitol.* 25:227–38
78. Browning CH, Morgan GT, Robb JUM, Walls LP. 1938. The trypanocidal action of certain phenanthridinium compounds. *J. Pathol. Bacteriol.* 46:203–4
79. Jensch H. 1955. 4,4′-Diaminodinodiazoaminobenzol, ein neues Mittel gegen Trypanosomen- und Babesien-Infectionen. *Arzneim. Forsch.* 5:634–35
80. Wragg WR, Washbown K, Brown KN, Hill J. 1958. Metamidium: a new trypanocidal drug. *Nature* 182:1005–6
81. MacGregor JT, Johnson IJ. 1977. *In vitro* metabolic activation of ethidium bromide and other phenanthridinium compounds: mutagenic activity in *Salmonella typhimurium*. *Mutat. Res.* 48: 103–7
82. Opperdoes FR. 1985. Biochemical peculiarities of trypanosomes, African and South American. *Br. Med. Bull.* 41:130–36
83. Fairlamb AH. 1990. Interaction of trypanocidal drugs with the metabolism and function of trypanothione. In *Chemotherapy for Trypanosomiasis. Proc. Workshop, Nairobi, Kenya, 1989*, ed. AS Peregrine, pp. 25–31. Nairobi: Int. Lab. Res. Anim. Dis.
84. Scott JM, Pegram RG. 1974. A high incidence of *Trypanosoma congolense* strains resistant to homidium bromide in Ethiopia. *Trop. Anim. Health Prod.* 6:215–21
85. Hu S, Weisz K, James TL, Shafer RH. 1992. ^{1}H-NMR studies on d(GCTT-AAGC)2 and its complex with berenil. *Eur. J. Biochem.* 204:31–38
86. Lane AN, Jenkins TC, Brown T, Neidle S. 1991. Interaction of berenil with the *Eco*R1 dodecamer d(CGCGAATTCG-CG)$_2$ in solution studied by NMR. *Biochemistry* 30:1372–85
87. Rushigajiki PKB, Mayende JSP, Guloba A, Wilson AJ. 1986. Maintenance of a herd of breeding cattle in an area of trypanosome challenge. *Bull. Anim. Health Prod. Afr.* 34:149–55
88. Osman AS, Jennings FW, Holmes PH. 1992. The rapid development of drug resistance by *Trypanosoma evansi* in immunosuppressed mice. *Acta Trop.* 50: 249–57
89. Ndoutamia G, Moloo SK, Murphy NB, Peregrine AS. 1993. Derivation and characterization of a quinapyramine-resistant clone of *Trypanosoma congolense*. *Antimicrob. Agents Chemother.* 37:1163–66
90. Peregrine AS, Mamman M. 1993. Pharmacology of diminazene: a review. *Acta Trop.* 54:185–204
91. Schönefeld A, Röttcher D, Moloo SK. 1987. The sensitivity to trypanocidal drugs of *Trypanosoma vivax* isolated in Kenya and Somalia. *Trop. Med. Parasitol.* 38:177–80
92. Kinabo LDB, Bogan JA. 1988. The pharmacology of isometamidium. *J. Vet. Pharmacol. Ther.* 11:233–45
93. Gimbi AA, Kinabo LDB. 1992. Influence of atropine on the acute toxicity of isometamidium. *Vet. Hum. Toxicol.* 34:398–400
94. Sutherland IA, Holmes PH. 1993. Alterations in drug transport in resistant *Trypanosoma congolense*. *Acta Trop.* 54:271–78
95. Brohn FH, Clarkson AB Jr. 1980. *Trypanosoma brucei brucei:* Patterns of glycolysis at 37°C *in vitro*. *Mol. Biochem. Parasitol.* 1:291–305
96. Visser N, Opperdoes FR, Borst P. 1981. Subcellular compartmentation of glycolytic intermediates in *Trypanosoma brucei*. *Eur. J. Biochem.* 118:521–26
97. Grant PT, Sargent JR. 1960. Properties of L-glycerol-3-phosphate oxidase and its role in respiration of *Trypanosoma rhodesiense*. *Biochem. J.* 76:229–37
98. Sommer JM, Wang CC. 1994. Targeting proteins to the glycosomes of African trypanosomes. *Annu. Rev. Microbiol.* 48:105–38

99. Osinga KA, Swinkels BW, Gibson WC, Borst P, Veeneman GH, et al. 1985. Topogenesis of microbody enzymes: a sequence comparison of the genes for the glycosome (microbody) and cytosolic phosphoglycerate kinases of *Trypanosoma brucei. EMBO J.* 4:3811–17
100. Clayton CE. 1985. Structure and regulated expression of genes encoding fructose biphosphate aldolase in *Trypanosoma brucei. EMBO J.* 4:2997–3003
101. Alexander K, Parsons M. 1991. A phosphoglycerate kinase-like molecule localized to glycosomal microbodies: evidence that the topogenic signal is not at the C-terminus. *Mol. Biochem. Parasitol.* 46:1–10
102. Michels PAM, Poliszczak A, Osinga KA, Misset O, Van Beaumen J, et al. 1986. Two tandemly linked identical genes code for the glycosomal glyceraldehyde-phosphate dehydrogenase in *Trypanosoma brucei. EMBO J.* 5:1049–56
103. Marchand M, Kooystra U, Wierenga RK, Lambeir AM, Van Beeumen J, et al. 1989. Glucosephosphate isomerase from *Trypanosoma brucei:* Cloning and characterization of the gene and analysis of the enzyme. *Eur. J. Biochem.* 184: 455–64
104. Swinkels BW, Gibson WG, Osinga KA, Kramer R, Veeneman GH, et al. 1986. Characterization of the gene for the microbody (glycosomal) triosephosphate isomerase of *Trypanosoma brucei. EMBO J.* 5:1291–98
105. Kueng V, Schlaeppi K, Schneider A, Seebeck T. 1989. A glycosomal protein (p60) which is predominantly expressed in procyclic *Trypanosoma brucei:* Characterization and DNA sequence. *J. Biol. Chem.* 264:5203–9
106. Parsons M, Smith JM. 1989. *Trypanosome* glycosomal protein p60 is homologous to phosphoenolpyruvate carboxykinase (ATP). *Nucleic Acids Res.* 17: 6411
107. Broman K, Knupfer AL, Ropars M, Deshusses J. 1983. Occurrence and role of phosphoenolpyruvate carboxykinase in procyclic *Trypanosoma brucei brucei* glycosomes. *Mol. Biochem. Parasitol.* 8:79–87
108. Wierenga RK, Kalk KH, Hol WGJ. 1987. Structure determination of the glycosomal triosephosphate isomerase from *Trypanosoma brucei* at 2.4 Å resolution. *J. Mol. Biol.* 198:109–21
109. Vellieux FMD, Hajdu J, Verlinde CLMJ, Groendijk H, Read RJ, et al. 1993. Structure of glycosomal glyceraldehyde-3-phosphate dehydrogenase from *Trypanosoma brucei* determined from Laue data. *Proc. Natl. Acad. Sci. USA* 90:2355–59
110. Verlinde LMJ, Callens M, Van Calenbergh S, Van Aerschot A, Herdewijn P, et al. 1994. Selective inhibition of trypanosomal glyceraldehyde-3-phosphate dehydrogenase by protein structure-based design: towards new drugs for the treatment of sleeping sickness. *J. Med. Chem.* 37:3605–13
111. Visser N, Opperdoes FR. 1980. Glycolysis in *Trypanosoma brucei. Eur. J. Biochem.* 103:623–32
112. Evans DA, Brightman CJ, Holland MF. 1977. Salicylhydroxamic acid/glycerol in experimental trypanosomiasis. *Lancet* 2:769
113. van der Meer C, Versluijs-Broers JAM, Opperdoes FR. 1979. *Trypanosoma brucei:* Trypanocidal effect of salicylhydroxamic acid plus glycerol in infected rats. *Exp. Parasitol.* 48:126–34
114. Grady RW, Bienen EJ, Clarkson AB Jr. 1986. p-Alkyloxybenzhydroxamic acids, effective inhibitors of the trypanosome glycerol-3-phosphate oxidase. *Mol. Biochem. Parasitol.* 19:231–40
115. Lee MGS, Van der Ploeg LHT. 1990. Homologous recombination and stable transfection in the parasitic protozoan *Trypanosoma brucei. Science* 250: 1583–87
116. Sommer JM, Cheng QL, Keller G-A, Wang CC. 1992. *In vivo* import of firefly luciferase into the glycosomes of *Trypanosoma brucei* and mutational analysis of the C-terminal targeting signal. *Mol. Biol. Cell* 3:749–59
117. Keller G-A, Gould S, Deluca M, Subramani S. 1987. Firefly luciferase is targeted to peroxisomes in mammalian cells. *Proc. Natl. Acad. Sci. USA* 84: 3264–68
119. Gould SJ, Keller G-A, Hosken N, Wilkinson J, Subramani S. 1989. A conserved tripeptide sorts proteins to peroxisomes. *J. Cell Biol.* 108:1657–64
120. Gould SJ, Keller G-A, Subramani S. 1988. Identification of peroxisomal targeting signals located at the carboxy terminus of four peroxisomal proteins. *J. Cell. Biol.* 107:897–905
121. Fung K, Clayton CE. 1991. Recognition of a peroxisomal tripeptide entry signal by the glycosomes of *Trypanosoma brucei. Mol. Biochem. Parasitol.* 45: 261–64
122. Blattner J, Swinkels B, Dorsam H, Prospero T, Subramani S, Clayton CE. 1992. Glycosome assembly in trypanosomes: variations in the acceptable degeneracy of a COOH-terminal micro-

body targeting signal. *J. Cell Biol.* 119: 1129–36
123. Sommer JM, Peterson G, Keller G-A, Parsons M, Wang CC. 1993. The C-terminal tripeptide of glycosomal phosphoglycerate kinase is both necessary and sufficient for import into the glycosomes of *Trypanosoma brucei*. *FEBS Lett.* 316:153–58
124. Sommer JM, Nguyen TT, Wang CC. 1994. Phosphoenolpyruvate carboxykinase of *Trypanosoma brucei* is targeted to the glycosomes by a C-terminal sequence. *FEBS Lett.* 350:125–29
125. Swinkels BW, Gould SJ, Bodnar AG, Rachubinski RA, Subramani S. 1991. A novel, cleavable peroxisomal targeting signal at the amino-terminus of the rat 3-ketoacyl-CoA thiolase. *EMBO J.* 10: 3255–66
126. Simon RJ, Kania RS, Zuckermann RN, Huebner VD, Jewell DA, et al. 1992. Peptoids: a modular approach to drug discovery. *Proc. Natl. Acad. Sci. USA* 89:9367–71
127. Eisenthal R, Game S, Holman GD. 1989. Specificity and kinetics of hexose transport in *Trypanosoma brucei*. *Biochim. Biophys. Acta* 985:81–89
128. Ter Kuile BH, Opperdoes FR. 1991. Glucose uptake by *Trypanosoma brucei:* Rate limiting steps in glycolysis and regulation of the glycolytic flux. *J. Biol. Chem.* 266:857–62
129. Bringaud F, Baltz T. 1993. Differential regulation of two distinct families of glucose transporter genes in *Trypanosoma brucei*. *Mol. Cell. Biol.* 13:1146–54
130. Pays E, Coquelet H, Tebabi P, Pays A, Jefferies D, et al. 1990. *Trypanosoma brucei:* Constitutive activity of the VSG and procyclin gene promoters. *EMBO J.* 9:3145–51
131. Jefferies D, Tebabi P, Pays E. 1991. Transient activity assays of the *Trypanosoma brucei* variant surface glycoprotein gene promoter: control of gene expression at the post-transcriptional level. *Mol. Cell. Biol.* 11:338–43
132. Borst P. 1986. Discontinuous transcription and antigenic variation in trypanosomes. *Annu. Rev. Biochem.* 55:701–32
133. Pascolo E, Blonski C, Shire D, Toulmé JJ. 1993. Antisense effect of oligodeoxynucleotides complementary to the mini-exon sequence of the protozoan parasite *Leishmania amazonensis*. *Biochimie* 75:43–47
134. Matsukura M, Zon G, Shinozuka K, Robert-Guroff M, Shimada T, et al. 1989. Regulation of viral expression of human immunodeficiency virus *in vitro* by an antisense phosphorothioate oligodeoxynucleotide against *rev (art/trs)* in chronically infected cells. *Proc. Natl. Acad. Sci. USA* 86:4244–48
135. Wang CC. 1991. A novel suicide inhibitor strategy for antiparasitic drug development. *J. Cell. Biochem.* 45:49–53
136. Ghoda L, van Daalen Weters T, Macrea M, Ascherman D, Coffino P. 1989. Prevention of rapid intracelluar degradation of ODC by a carboxy-terminal truncation. *Science* 243:1493–95
137. Ghoda L, Phillips MA, Bass KE, Wang CC, Coffino P. 1990. Trypanosome ornithine decarboxylase is stable because it lacks sequence found in the carboxyl-terminus of the mouse enzyme that is targeted for intracelllular degradation. *J. Biol. Chem.* 265:11823–26
138. Bass KE, Wang CC. 1991. The *in vitro* differentiation of pleomorphic *Trypanosoma brucei* from bloodstream into procyclic form requires neither intermediary nor short-stumpy stage. *Mol. Biochem. Parasitol.* 44:261–70
139. Torri AF, Bertrand KI, Hajduk SL. 1993. Protein stability regulates the expression of cytochrome c during the developmental cycle of *Trypanosoma brucei*. *Mol. Biochem. Parasitol.* 57:305–16
140. Wong S, Campbell DA. 1989. A polyubiquitin gene from *Trypanosoma brucei*. *Mol. Biochem. Parasitol.* 37: 147–50
141. Wong S, Elgort MG, Gottesdiener K, Campbell DA. 1992. Allelic polymorphism of *Trypanosoma brucei* polyubiquitin gene. *Mol. Biochem. Parasitol.* 55:187–96
142. Lowrie DJ Jr, Giffin BF, Ventullo RM. 1993. The ubiquitin-ligase system in *Trypanosoma brucei brucei*. *Am. J. Trop. Med. Hyg.* 49:545–51
143. Murakami Y, Matsufuji S, Kameji T, Hayashi S, Igarashi K, et al. 1992. Ornithine decarboxylase is degraded by the 26S proteasome without ubiquitination. *Nature* 360:597–99
144. Fong WF, Heller JS, Canellakis ES. 1976. The appearance of an ornithine decarboxylase inhibitory protein upon the addition of putrescine to cell culture. *Biochim. Biophys. Acta* 428:456–65
145. Murakami Y, Fujita K, Kameji T, Hayashi S. 1985. Accumulation of ornithine decarboxylase-antizyme complex in HMO_A cells. *Biochem. J.* 225: 689–97
146. Rechsteiner M, Hoffman L, Dubiel W. 1993. The multicatalytic and 26S proteases. *J. Biol. Chem.* 268:6065–68
147. Bitonti AJ, Kelley SE, McCann PP. 1984. Characterization of spermidine

synthase from *Trypanosoma brucei brucei. Mol. Biochem. Parasitol.* 13:21–28

148. Tekwani BL, Bacchi CJ, Pegg AE. 1992. Putrescine activated S-adenosylmethionine decarboxylase from *Trypanosoma brucei brucei. Mol. Cell. Biochem.* 117:53–61
149. Bitonti AJ, Byers TL, Bush TL, Casara PJ, Bacchi CJ, et al. 1990. Cure of *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense* infections in mice with an irreversible inhibitor of S-adenosylmethionine decarboxylase. *Antimicrob. Agents Chemother.* 34: 1485–90
150. Byers TL, Bush TL, McCann PP, Bitonti AJ. 1991. Antitrypanosomal effects of polyamine biosynthesis inhibitors correlate with increases in *Trypanosoma brucei brucei* S-adenosyl-L-methionine. *Biochem. J.* 274:527–33
151. Bacchi CJ, Nathan HC, Yarlett N, Goldberg B, McCann PP, et al. 1992. Cure of murine *Trypanosoma brucei rhodesiense* infections with an S-adenosylmethionine decarboxylase inhibitor. *Antimicrob. Agents Chemother.* 36: 2736–40
152. Byers TL, Casara P, Bitonti AJ. 1992. Uptake of the antitrypansomal drug 5′-(Z-4-amino-2-butenyl) methylamino-5′-deoxyadenosine (MDL. 73811) by the purine transport system of *Trypanosoma brucei brucei. Biochem. J.* 283:755–58
153. Fairlamb AH, Cerami A. 1992. Metabolism and functions of trypanothione in the kinetoplastida. *Annu. Rev. Microbiol.* 46:695–729
154. Fairlamb AH, Henderson GB. 1987. Metabolism of trypanothione and glutathionylspermidine in trypanosomatids. In *Host-Parasite Cellular and Molecular Interactions in Protozoal Infections,* ed. K-P Chang, D Snary, pp. 29–40. Berlin: Springer-Verlag/NATO ASI Ser. Vol. H11
155. Aboagye-Kwarteng T, Smith K, Fairlamb AH. 1992. Molecular characterization of the trypanothione reductase gene from *Crithidia fasciculata* and *Trypanosoma brucei*: comparison with other flavoprotein disulfide oxidoreductases with respect to substrate specificity and catalytic mechanism. *Mol. Microbiol.* 6: 3089–99
156. Kuriyan J, Kong X-P, Krishna TSR, Sweet RM, Murgolo NJ, et al. 1991. X-ray structure of trypanothione reductase from *Crithidia fasciculata* at 2.4 Å resolution. *Proc. Natl. Acad. Sci. USA* 88:8764–68
157. Bailey S, Smith K, Fairlamb AH, Hunter WN. 1993. Substsrate interactions between trypanothione reductase and N^1-glutathionylspermidine disulfide at 0.28 nm resolution. *Eur. J. Biochem.* 213:67–75
158. Kelly JM, Taylor MC, Smith K, Hunter KJ, Fairlamb AH. 1993. Phenotype of recombinant *Leishmania donovani* and *Trypanosoma cruzi* which overexpress trypanothione reductase: sensitivity towards agents that are thought to induce oxidative stress. *Eur. J. Biochem.* 218: 29–37
159. Henderson GB, Ulrich P, Fairlamb AH, Rosenberg I, Pereira M, et al. 1988. "Subversive" substrates for the enzyme trypanothione disulfide reductase: alternative approach to chemotherapy of Chagas' disease. *Proc. Natl. Acad. Sci. USA* 85:5374–78
160. Wang CC. 1984. Parasitic enzymes as potential targets for antiparasitic chemotherapy. *J. Med. Chem.* 27:1–9
161. Tuttle JV, Krenistsky TA. 1979. Purine phosphoribosyltransferases from *Leishmania donovani. J. Biol. Chem.* 255: 909–16
162. Nelson DJ, LaFon SW, Tuttle JV, Miller WH, Miller RL, et al. 1979. Allopurinol ribonucleoside as an antileishmanial agent. *J. Biol. Chem.* 254:11544–49
163. Marr JJ, Berens RL. 1983. Mini Review: pyrazolopyrimidine, metabolism in the pathogenic trypanosomatidae. *Mol. Biochem. Parasitol.* 7:339–56
164. Hitchings GH. 1975. Pharmacology of allopurinol. *Arthritis Rheum.* 18:863–70
165. Looker DL, Marr JJ, Berens RL. 1986. Mechanisms of action of pyrazolopyrimidines in *Leishmania donovani. J. Biol. Chem.* 261:9412–15
166. Kager PA, Rees PH, Wellde PT, Hockmeyer WT, Lyerly WH. 1981. Allopurinol in the treatment of visceral leishmaniasis. *Trans. R. Soc. Trop. Med. Hyg.* 75:556–59
167. Allen T, Ullman B. 1993. Cloning and expression of the hypoxanthine-guanine phosphoribosyltransferase gene from *Trypanosoma brucei. Nucleic Acids Res.* 21:5431–38
168. Eads JC, Scapin G, Xu Y, Grubmeyer C, Sacchettini JC. 1994. The crystal structure of human hypoxanthine-guanine phosphoribosyltransferase with bound GMP. *Cell.* 78:325–34
169. Ferguson MAJ, Cross GAM. 1984. Myristylation of the membrane form of a *Trypanosoma brucei* variant surface glycoprotein. *J. Biol. Chem.* 259:3011–15
170. Thomas JR, Dwek RA, Rademacher TW. 1990. Structure, biosynthesis, and

function of glycosylphosphatidylinositols. *Biochemistry* 29:5413–22
171. Masterson WJ, Doering TL, Hart GW, Englund PT. 1989. A novel pathway for glycan assembly: biosynthesis of the glycosyl-phosphatidylinositol anchor of the trypanosome variant surface glycoprotein. *Cell* 56:793–800
172. Masterson WJ, Raper J, Doering TL, Hart GW, Englund PT. 1990. Fatty acid remodeling: a novel reaction sequence in the biosynthesis of trypanosome glycosyl phosphatidylinositol membrane anchors. *Cell* 62:73–80
173. Doering TL, Raper J, Buxbaum LU, Adams SP, Gordon JI, et al. 1991. An analog of myristic acid with selective toxicity for African trypanosomes. *Science* 252:1851–54
174. Bryant ML, Heuckeroth RO, Kimata JT, Ratner L, Gordon JI. 1989. Replication of human immodeficiency virus 1 and Moloney murine leukemia virus is inhibited by different heteroatom-containing analogs of myristic acid. *Proc. Natl. Acad. Sci. USA* 86:8655–59
175. Ring CS, Sun E, McKerrow JH, Lee GK, Rosenthal PJ, et al. 1993. Structure-based inhibitor design by using protein models for the development of antiparasitic agents. *Proc. Natl. Acad. Sci. USA* 90:3583–87