ANTIPARASITE CHEMOTHERAPY

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The attention of medical science in our era of sophisticated technology is largely directed toward the "physiologic" and neoplastic diseases. However, in appraising the health of the entire community of man one finds that animal parasites, the protozoa and helminths, remain, as they have for countless millennia, the major etiologic agents of infection. Parasitoses are rarely fatal although malaria may still be a direct major cause of mortality in some endemic areas. Furthermore, parasitic infections, particularly intestinal helminthiases, are often so prevalent in a tropical population as to be accepted as a natural phenomenon. This complaisance is hardly warranted as these infections undoubtedly constitute a drain on human energy resources, particularly when aggravated by malnutrition and concurrent diseases common in the tropical world. It is questionable whether a nation whose people are so burdened can readily advance from a subsistence agricultural existence to a manufacturing, technical economy.

The chemotherapeutic armamentarium against parasitic infections is, in general, adequate although there are notable deficiencies such as the lack of an effective drug against *Trypanosoma cruzi*. The physician can usually treat the individual patient satisfactorily although the therapeutic regimen may be extended and in some instances necessitate hospitalization. In addition to treatment of the individual patient, chemotherapy and chemoprophylaxis continue to play an important role in the control of some parasitic infections. In many instances control by chemotherapeutic agents is the only practical approach. Vector control may be too costly or too arduous, or both; health education, while effective, is a slow process and requires changes in cultural practices by a usually conservative tribal society. For many viral and bacterial diseases artificial immunization has been the means by which populations have been protected without necessitating changes in the socio-environmental order. However a practical method of immunization against any of the parasitic diseases infecting man has never been realized. Until methods can be devised to confer an artificial protective immunity, drugs will play a highly important role in the control and treatment of these infections.

It is beyond the scope of this article to provide an exhaustive review of antiparasite chemotherapy. Current therapeutic treatment for the various

parasitoses can be found in the recent editions of textbooks on medical parasitology such as that by Brown (1). Experimental aspects of parasite chemotherapy is the subject of a book edited by Schnitzer & Hawking (2). Pharmacologists can refer to Cutting's Handbook of Pharmacology (3) for a list of current antiparasite compounds. The busy medical practitioner can find an excellent synoptic guide to treatment in the Medical Letter on Drugs and Therapeutics issued in 1966 (4). The chemotherapy of parasites of domestic animals has been reviewed recently by Douglas & Baker (5). It is the purpose of this review to consider special problems associated with the antiparasite chemotherapy and discuss the efficacy of new drugs that have been developed during the past ten years.

Wuchereria Bancrofti and Brugia Malayi Filariasis:

Prior to the discovery by Hewitt and his colleagues in 1947 (6) that one of the piperazine series, diethylcarbamazine (DEC), was highly active against *Litosomoides carinii,* a filarial worm of the cotton rat, no practical therapeutic agent was available for the treatment of filariasis. Shortly after this observation DEC was applied and found to be successful in the treatment of Bancroftian filariasis (7, 8). Wilson (9) subsequently confirmed that the drug was equally effective against *Brugia malayi.* Since these first clinical trials DEC has been the sheet anchor for the treatment of filariasis caused by *W. bancrofti* and *B. malayi.* Moreover, it is the only available agent that can be safely used against these parasites.

The action of DEC is mainly directed against the microfilaria. A single intravenous injection into a cotton rat causes 80% of *L. carinii* microfilariae to disappear from the peripheral circulation within 2 minutes (10). A similar clearing action occurs in *W. bancrofti* and *B. malayi* infections following oral dosing. Experimental studies on *L. carinii* indicate that DEC is not directly microfilaricidal. After treatment, the microfilariae concentrate in the small vessels of the liver (10). Macrophages are seen to congregate about the entrapped microfilariae and destruction, presumably by phagocytosis, is completed within about 18 hours. Hawking (11, 12) suggests that DEC may act in a manner similar to that of an opsonin, sensitizing the parasites to the action of the phagocytic cells of the reticuloendothelial system.

DEC has a less marked effect on stages of the filarial worm other than the microfilaria. It will prolong the development of the larval forms in the insect vector host but most of the drug-exposed larvae will still proceed to maturity (13). Adult worms are not very susceptible to DEC, but may be killed over a relatively long period of time during which repeated courses of the drug are administered.

Although DEC displays a remarkable in vivo effect on microfilariae, there is no observable in vitro activity. The supposition that this phenomenon is due to the microfilariacidal action of a metabolite rather than DEC itself is not supported by experimental evidence. Neither serum nor urine from a DEC-treated animal have any in vitro effect on microfilariae (10).

Following intraperitoneal inoculation of tritium labelled DEC into mice and cotton rats, maximum radioactivity is present about 20 minutes later in the liver, kidney, adrenal, muscle, gastrointestinal tract, brain, and lymph glands. After 20 minutes the concentration of drug in these tissues rapidly decreases. However, in the lung and stomach wall the drug is concentrated and retained (14). DEC is rapidly absorbed from the gut when given by mouth, a peak blood level appearing after 3 to 4 hours. The blood level then decreases until at 48 hours it is no longer detectable (15). Drug excretion is almost entirely via the urine. About 90% of the excreted drug is in the form of four different metabolites. The piperazine ring of the metabolites remains intact but the side chains exhibit degradative changes (16, 17).

At therapeutic doses, DEC is not seriously toxic. Side reactions to the drug depend upon whether the individual receiving the drug is infected or not, and the species of the offending filarial worm. In uninfected persons receiving 10–20 mg/kg, DEC may produce mild gastro-intestinal disturbances. Filariasis patients, being treated at similar dosages, may experience edema, papular rash, headache, hyperpyrexia, intense itching, lymph node tenderness, and tachycardia. The reactions are generally more severe in patients with high microfilaremia than in those with low microfilarial densities. These untoward effects are more liable to occur in patients, particularly older individuals, with *B. malayi* than in cases of *W. bancrofti*. The cause of the reactions is not known with any certainty (18–20) but it appears to be an allergic reaction in sensitized patients. Possibly the rapid destruction of the microfilariae leads to the release of soluble antigens which then combine with antibodies at cell fixed sites. This in turn would lead to the release of pharmacologically active substances such as histamine and kinins. Antihistamines have a moderating effect but do not completely abolish the side reactions. Experimental inquiry as to the actual mechanism for these drug-induced effects and their logical pharmacological management has not been adequately made despite the obvious importance for doing so. In a number of instances the failure to control filariasis by mass DEC administration to the population can be directly attributed to the drug's side effects and consequent rejection by the population.

With varying degrees of success, mass DEC administration has been used in a number of filariasis control programs (21–27). Such administration causes a rapid decrease in the microfilaria rates, providing all individuals receive the full course totalling 72 mg/kg. It is suggested that individual doses should not exceed 8 mg/kg taken at daily, weekly, or monthly intervals. The individual dose of 6 to 8 mg/kg tends to decrease the adverse reactions. Total eradication of the infection has not been accomplished by any of the filariasis programs and mass drug administration must be continued for a number of years if the gains of the control measures are to be maintained. The cost and labor necessary to distribute DEC to an entire population over a period of many years is obviously high and it is often difficult for governments of tropical nations, with their limited technical and

financial resources, to provide the necessary recurrent surveillance and drug administration. A promising solution to this problem is to achieve control by continuous DEC administration via medicated salt and other foods such as commercially prepared soups and orangeade. Pilot control programs employing medicated food have proved that the method is of great potential promise (28–31).

In addition to DEC several other compounds have been reported to possess filaricidal activity. Prior to the introduction of DEC, antimonials, arsenicals and cyanine compounds had been used to treat filarial infections (18, 32). The antimonial compounds, tartar emetic, neostibosan (a pentavalent antimonial), and lithium antimonythiomalate were all effective but general usage was precluded by the severe toxic reactions they were liable to produce. Unlike DEC these metallic compounds have a direct effect on the adult worm. Of the arsenicals, a water soluble melarsen derivative, Mel W (pentylthiarsaphenylmelamine) seems to be most effective. However, this compound is also toxic, albeit less so than other arsenicals. Treatment with Mel W may also produce a delayed hypersensitivity characterized by a fever, rash, and lymphangitis, two weeks or more after treatment. The delayed hypersensitivity is probably due to the antigens released by the dying adult worms. Mel W may also act as a complete antigen and per se initiate a hypersensitive state (33).

Chari & Hiremath (34) reported that a combination of sulphaproxyline and sulphamerazine improved the symptoms (edema and lymphagitis) of patients suffering from acute filariasis. Recently Hawking & Worms (35) reported a curative effect of the antimitotic agents methotrexate, 6-mercaptopurine, and cyclophosphamide in experimental *L. carinii* in the cotton rat.

A nontoxic chemotherapeutic agent active against microfilariae and adult filariids is urgently required. Until such a compound is discovered DEC will remain, despite its shortcomings, as the drug of choice for the treatment of filariasis.

Intestinal Nematodes

Although intestinal nematode infections are usually thought of as being peculiar to the tropical world, these parasites have, in fact, a cosmopolitan distribution. Pinworm (*Enterobius vermicularis*) for example, is common throughout the temperate areas of the world. Soil transmitted helminths (*Ascaris*, hookworm, *Trichuris, Strongyloides, Trichostrongylus*) are particularly prevalent where indiscriminate defecation is practised, where human feces is used as fertilizer, and in over-crowded towns of nonindustrial centers. It has been estimated (36) that 25% of the world's population is infected with *Ascaris lumbricoides*. Infections with intestinal parasites are rarely fatal but the morbidity may be of considerable public health significance, particularly in young children. Attention is now being directed to the control of soil transmitted helminths by means of environmental measures or mass chemotherapy (36). The relative ease of mass drug administration

as compared to implementation of sanitary changes make it an attractive method of control, particularly when the technical and economic public health resources of a country are limited. Infection with more than one species of parasite occurs regularly in most tropical populations. Therefore, chemotherapeutic control necessitates the use of broad spectrum antihelminthics of low toxicity and acceptable palatability. They must also be of sufficiently low cost to make widespread distribution possible. Although the antihelminthic armamentarium has been greatly strengthened and expanded during the past 20 years there is still no single drug that fulfills all the criteria.

This review is primarily concerned with advances in the chemotherapy of intestinal helminthiasies made during the last 15 years. Information on the subject prior to 1957 has been reviewed by Bueding & Swartzwelder (37) and Bueding & Most (38). During the 1950's, drugs of choice were piperazine salts (diethylenediamine) for ascariasis, and enterobiasis and tetrachloroethylene for ankylostomiasis. Within the last 10 years a number of new antihelminthic agents of diverse chemical classes have been developed which have largely replaced these older drugs. The antihelminthic activity of these newer compounds is summarized in Table 1.

The cyanine compounds are derived from dyes which contain the amidium ion system. The nitrogen ions in this system are incorporated into two

TABLE 1. THE ACTIVITY OF RECENTLY INTRODUCED ANTIHELMINTHICS ON INTESTINAL NEMATODES

	Enterobius vermicularis	*Ascaris lumbricoides*	*Trichuris trichiura*	*Hookworm*	*Strongyloides stercoralis*
Pyrvinium pamoate	++++	−	+	+	+ ++
Stilbazium iodide	++++	++	++	+−++	+−++
Iodo-thymol	?	++	?	++	?
Phenylene di-iso-thiocyanate	?	++	?	++	?
1 bromo-2-naphthal	?	++−+++	++	++−+++	?
Mantomide	?	?	?	+++	?
Dymanthine hydrochloride	?	?	?	++−+++	++−+++
Pyrantel pamoate	++++	++++	−	+++	−
Dichlorvos	?	++++	++++	++++	?
Tetramisole	++++	++++	−	++?	?
Bephenium hydroxynaphthoate	?	+++	+−++	+++ (*A. duodenale*) ++ (*N. americanus*)	−
Thiabendazole	++++	+++	+	++−+++	++++

++++ = 90% − 100% cure
+++ = 89% − 60% cure
++ = 59% − 30% cure
+ = 29% − 10% cure
− = <10% cure

heterocyclic rings in which a quaternary N is separated from a trivalent N by a resonating chain of alternating double and single bonds. The first cyanine antihelminthic to be put to clinical use was dithiazanine iodide (3, 3-diethylthiadicarbocyanine iodide). While this compound proved to be an effective broad-spectrum antihelminthic, particularly for such drug-recalcitrant nematodes as *S. stercoralis* and *T. trichiura,* its toxicity was such that it was withdrawn from the market. Several deaths associated with abnormal drug absorption have been caused by dithiazanine. Pyrvinium pamoate [*bis*-6-dimethylamino-2 (2-[2, 5-dimethyl-1-phenyl-3-pyrolyl] vinyl)-1-methyl-quinolium salt of pamoic acid] is not appreciably absorbed from the gut and therefore is of low toxicity. It is highly effective for the treatment of enterobiasis; 95% to 100% cure can be expected with a single 2 mg–5 mg/kg dose (39–41). Strongyloidiasis can be successfully and safely treated with pyrvinium pamoate providing a more prolonged treatment, 5 mg/kg daily for 5 days, is given (42–45). It has a low order of antihelminthic activity against *A. lumbricoides, T. trichiura,* and hookworm. The cyanine, stilbazium iodide, is a quaternary derivative of pyridine. A single dose of 3 mg/kg–10 mg/kg produces a high cure rate for the treatment of enterobiasis (41, 46–49). In multiple doses 40% to 80% cure rates for ascariasis and trichuriasis have been reported (50–52). There is also moderate activity against hookworms particularly in dealing with light infections (50). It has not had adequate clinical trial for strongyloidiasis, but the report of Campos et al (53) would indicate it not to be as effective as pyrvinium pamoate. In common with many other cyanine antihelminthics, stilbazium tends to induce mild to moderate toxic reactions, e.g. nausea, vomiting, and dizziness. For multiple dosage an enteric-coated formulation is recommended to reduce the adverse side effects.

A relatively new antihelminthic, tetramisole [2, 3, 5, 6, tetrahydro-6-phenyl-imidazo (2, 1-b) thiazole] is of interest because of its low toxicity and high activity against *A. lumbricoides* (54–58). A single dose of 2.5 mg/kg can be expected to achieve a cure rate of over 80%. Recent studies (59, 60) indicate that the (laevo (*l*) isomer) form of tetramisole is superior to the normal form as an antihelminthic. It is active against *E. vermicularis* and shows some activity against hookworm, but for the latter, extensive clinical trials are yet to be carried out. *T. trichiura* infections do not respond to tetramisole treatment (59).

An iodothymol compound (4-iodo-3-methyl-1-hydroxy-6-isopropyl benzine) has been used, mainly by Japanese workers, to treat *A. lumbricoides* and hookworm infections. Approximately 60%–80% cure rates can be obtained for light hookworm infections but the rate drops to 30% when dealing with heavy infections (61–63). Iodothymol gives approximately a 50% cure rate for ascariasis. In view of this relatively modest activity compared to other antihelminthics it probably will not find a permanent place in the chemotherapeutic armamentarium.

Another antihelminthic of apparently limited efficacity is phenylene-di-

iso-thiocyanate. It is a poorly absorbed compound that is mainly excreted via the feces. While it reduces the egg load in most cases of both ascariasis and *Necator americanus* hookworm infections, total cure is obtained in only about 30% of the patients treated (64, 65). It possibly has greater activity against *Ancylostoma duodenale,* Bhandari & Shrimali (66) having recently reported 26 of 30 cases to be totally cured with this compound.

The activity of 1-bromo-2-naphthal against intestinal round worms is not well documented. It is apparently a broad spectrum antihelminthic that produced approximately 60% cure rates for ascariasis, trichuriasis, and ancylostomiasis (67–71). This compound would seem to deserve further clinical trial.

Mantomide [N-(2–4-dichlorobenzyl)-N-(2-hydroxyethyl) dichloracetamide] was first used to treat amebiasis. Two clinical trials (71, 72) have shown it to be of considerable potential value against heavy hookworm burdens when given in multiple doses. It is reported to be well tolerated and to produce little or no toxicity at adult dosages of 750 mg t.i.d. for 3 days.

One of the most interesting compounds to appear on the local drug scene is the organophosphate, dichlorvos (2, 2-dichlorovinyl phosphate) which began its career as an insecticide. The mechanism of activity against insects is by inhibition of cholinesterase. When given to humans, dichlorvos depresses erythrocyte cholinisterase for 24 to 72 hours. However at therapeutic dosages there are no symptoms of organophosphate toxicity to obviate its usage. Dichlorvos is a broad spectrum antihelminthic that is highly effective even against the most drug-recalcitrant nematode, *T. trichiura.* A single dose of 12 mg-15 mgkg produces a 75% to 95% complete cure rate of ascariasis, trichuriasis, and ancylostomiasis (*N. americanus* and *A. duodenale*) (73–75). If further trial confirms its lack of toxicity, it obviously is a drug of great potential value.

Two clinical trials (76, 77) have been carried out on the antihelminthic activity of dymanthinc hydrochloridc (dimethylocta-decyclamine hydrochloride). The rate of cure of hookworm and strongyloidiasis patients was about 60%. The drug also is ovicidal and the helminth eggs from patients not totally cleared failed to hatch. Dymanthine is believed to exert its vermicidal effect by absorption through the cuticle of the worm as well as by ingestion into its digestive tract.

Pyrantel pamoate (*trans*-1, 4, 5, 6-tetrahydro-1-methyl-2[2-(2thienyl) vinyl] pyrimidine pamoate) is a unique new antihelminthic. It is water insoluble and not absorbed in the digestive tract. Bumbalo et al (78) reported a 100% cure rate of children infected with *E. vermicularis.* In a recent clinical trial Desowitz et al (79) demonstrated pyrantel's high order of activity against *A. lumbricoides* and *N. americanus.* A single 5 mg/lb dose effected a 90% cure rate in patients with ascariasis. The cure rate following a 2-day course at this dosage was 97%. A 3-day course of 5 mg/lb daily was necessary to treat hookworm infection. Approximately 85% of hookworm patients, all with light to moderate burdens, were cured by this regimen. There

was no antihelminthic activity against *S. stercoralis* or *T. trichiura*. Pyrantel is nontoxic and highly palatable. It is predicted that it will become one of the drugs of choice for the treatment of enterobiasis, ascariasis and, possibly, hookworm infection.

Bephenium hydroxynaphthoate (benzyldimethyl-2-phenoxethyl-ammonium hydroxynaphthoate) is a crystalline quaternary ammonium antihelminthic. Since the first clinical trial by Goodwin et al (80) documentation regarding this drug is now so extensive that a complete review is beyond the scope of this contribution. The main application of bephenium is for the treatment of hookworm. There is however a disparity in its effect upon *A. duodenale* as compared to that on *N. americanus*. A single 5 gm dose in adults can be expected to give a cure rate of 80% to 90% for the former infection but only about a 45% rate against the latter (80–86). Bephenium is also active against *A. lumbricoides*, giving a cure rate of approximately 65%. It has little or no activity against *S. stercoralis* (81, 86). A prolonged treatment of 4 days produces some effect against trichuriasis. Bephenium is relatively nontoxic but it does cause a relatively high incidence of vomiting following its use.

Thiabendazole (2-[4-thiazolyl]-benzimidazole) is a new broad-spectrum antihelminthic that has had extensive clinical trial. Over 100 papers have been written regarding its use for the treatment of a variety of helminthiases (reviewed by Campbell & Cuckler, 87). Side effects such as dizziness, nausea, and vomiting are common after taking thiazendazole and for this reason it probably will not become the drug of choice for many intestinal helminthiases when less toxic antihelminthics are available. Enterobiasis responds very well to treatment with thiabendazole. Between 80% and 100% cure rate can be expected after 50 mg/kg dose (88–90). Cure rates for ascariasis ranging between 41% to 90% have been reported. Multiple doses are required to achieve cure (84, 91, 92). The results of thiabendazole treatment of hookworm infections have not been consistent. Radical cure, in various trials, has been achieved in anywhere from 30% to 100% of the treated cases (87, 93). The drug is most effective when given at a schedule of 25 mg/kg b.i.d. for 2 or 3 days. It also seems to be less effective against heavy burdens than for light infections (94). Thiabendazole is probably now the drug of choice for the treatment of strongyloidiasis. Clinical trials (87, 95) have shown that presumptive radical cure can be obtained in 90% or more of the patients treated for two days with 25 mg/kg daily. The response of *T. trichiura* to thiabendazole has been erratic and it is not considered an effective drug for this infection (87, 92).

The Relationship of Antihelminthic Activity to the Chemical Physiology of the Parasite

The ultimate aim of pharmacological research is the logical design of chemotherapeutic agents that selectively act on the chemical metabolism of the offending parasite. In the case of antihelminthics there is not sufficient

knowledge either of helminth chemical physiology or the actual mechanism(s) of drug action to make this possible. The problem is further complicated for animal parasites in that their progressive metamorphic development is usually accompanied by profound physiological alterations.

Thus the larva of a nematode is a different metabolic animal than the adult which it will eventually become. A number of vermicides are stage specific, e.g. DEC, but for the most part it is not known whether this is due to a selective activity or merely that the developmental stages are unaffected because of their location in the tissues inaccessible to the drug. Antihelmintics such as pyrvinium pamoate that are not appreciably absorbed from the intestine are generally active only against the adult forms living in the lumen, while systemic drugs such as thiabendazole and tetramisole are ovicidal and larvacidal as well as being effective against the adult stages. Ovicidal drugs are of potential value in mass treatment compaigns as they tend to reduce the transmission of soil-transmitted nematode parasites.

The relationship between antihelminthic effects and chemical physiological mechanisms has been the subject of a comprehensive review by Saz & Bueding (96). The biochemistry of *A. lumbricoides* has been reviewed by Fairbairn (97). The reader should consult these reviews for a full account of the subject. The following is a synoptic account of the present state of knowledge.

The physiological effects of only two drugs, piperazine and the cyanines, have been studied sufficiently to allow speculation as to their mode of antihelminthic action. Piperazine does not seem to be directly vermicidal but rather causes a flaccid paralysis of *A. lumbricoides* which is then expelled by the normal peristaltic movement of the intestine. Several in vitro studies (98–100) have indicated that piperazine blocks neuromuscular functions by interfering with acetylcholine formation at the myoneural junction. In a series of elegant experiments Del Castillo et al (100, 101) showed that when microelectrodes were placed in *A. lumbricoides* resting muscle cells, four electrical discharges per second were recorded. When a piperazine concentration of 1/10,000 was added to the fluid medium bathing the worm these cyclical electrical variations disappeared and the resting electrical potential increased. It was posited by these investigators that piperazine is similar to a substance produced by inhibitory nerve fibers present in *A. lumbricoides*. Succinate formation is inhibited in piperazine-treated ascarids. However this does not appear to be caused by any direct action of the drug on muscle metabolism but rather reflects the decreased energy requirement of the paralyzed muscle (102).

The confusion that presently exists in attempting to relate a specific drug-induced biochemical lesion to its antihelminthic activity is exemplified by the present state of knowledge of cyanine activity. Cyanine dyes have a marked lethal effect on *L. carinii*, in vivo and in vitro. Oxygen consumption is greatly decreased in the presence of low concentrations of these compounds but glycolysis is enhanced (103). In keeping with what is known of

the biochemical effect of cyanide compounds on other enzyme systems it could be assumed that oxidative metabolism of *L. carinii* is disturbed by interference of cytochrome oxidase. However it has not been possible, as yet, to demonstrate the presence of a cytochrome system in this filariid. Morcover, cyanine compounds have no therapeutic effect on *W. bancrofti.* This is yet another example of the many differences in the response to chemotherapeutic agents between "model" parasites in experimental animals and the parasites of man.

In view of the fact that cyanine compounds such as dithiazanine and pyrvinium have a marked effect on intestinal helminths, organisms which are essentially anaerobes, it is probable that these compounds act on a metabolic pathway other than the cytochrome c system. Bueding et al (104) has demonstrated that dithiazanine irreversibly inhibits glycolysis in *Trichuris vulpis,* the dog whipworm. The specific enzymes concerned with anaerobic glycolysis of these worms, which are attacked by the cyanines, have not as yet been studied.

It is obvious that there is a vast gap in our knowledge of the biochemical mechanisms of antihelminthics. Prerequisite to further advances in this field is the acquisition of more information on the chemical physiology of the parasites.

Malaria

During the early 1950s the World Health Organization boldly conceived a plan for the global eradication of man's ancient enemy, malaria. As originally formulated, transmission of the disease was to be interrupted by reducing the anopheline population. The repeated insecticide spraying, mainly with DDT, was to be maintained for a sufficient length of time until all infections had naturally become "burnt-out" cases. Antimalarial drug administration was to play only a minor role, dealing with localized problem areas. However the rapid emergence of resistance to the chlorinated hydrocarbon insecticides in many parts of the world resulted in a greater dependence on mass administration of antimalarials in order to achieve eradication of the disease. The chemoprophylactic and therapeutic mainstay since World War II has been chloroquine, a 4-amino-quinoline compound. Despite widespread use, there were no reports of chloroquine resistance until 1961 when Moore & Lanier (105) reported the first two cases from Colombia. Since that time numerous instances of chloroquine resistance have been documented as occurring in South America and South East Asia. The problem is aggravated by the fact that *P. falciparum* may also become resistant to other chemoprophylactics, e.g. proguanil and pyrimethamine. Malarial drug resistance has been the subject of several extensive reviews (106–109) and the interested reader should consult these publications for a fuller account of the problem.

The ambitious hope that by the 1960s malaria would be relegated to the

status of a medical curiosity has not been realized. There recently have been disturbing resurgent epidemics in Ceylon and India. The prospect for malaria eradication in Africa is at present rather bleak. The exigencies of war in South East Asia and the widespread presence of chloroquine resistant strains of *P. falciparum* in that area have caused a problem that is very much an American concern. This current crisis in malaria is being met by renewed, burgeoning research activity, particularly in the search for new classes of antimalarials effective against chloroquine resistant strains. It is of great importance that this reawakened interest in malaria be sustained; the stop and start research that is reflective of crisis or complacency cannot provide the answers by which malaria will be controlled and finally eradicated.

Mechanisms of chloroquine action and resistance.—Chloroquine is a blood schizontocide, i.e. it has little or no effect on exoerythrocytic liver stages or on the sexual stages. Relatively little is known of the manner in which chloroquine exerts its chemotherapeutic effect. It is believed that chloroquine interferes with parasite replication rather than being directly lethal. The drug has a strong affinity for nucleoproteins and combines with DNA (110). In the presence of chloroquine, P^{32} labeled phosphate incorporation into plasmodial DNA and RNA is inhibited (111, 112) giving further evidence of a deranged enzymatic synthesis of these substances. Hahn (113) postulates the possibility that the heterocyclic rings of chloroquine and the acridines become intercalated between bases of DNA, which results in a multiple misreading of the genetic code followed by abortive protein synthesis.

There is evidence that chloroquine's antimalarial effect is not exclusively the result of a reproduction-inhibiting effect but that it may also be broadly detrimental to the parasite's metabolism. Experiments with *P. fallax,* a malarial parasite of turkeys, demonstrated that chloroquine interferes with fatty acid incorporation into phospholipids (114). Malarial parasites synthesize significant amounts of phospholipids. Since these substances are concerned with reactivation of energy enzymes, a chloroquine-induced derangement of phospholipid metabolism would have a deleterious effect on parasite growth. Chloroquine also has an effect on glycolysis; both hexokinase and glutamic dehydrogenase are inhibited (115, 116). Similar to many other plant and animal cells, plasmodia fix CO_2 to contribute to the free amino acid pool (117). Siu (118) has demonstrated that chloroquine inhibits carboxykinase and carboxylase of *P. berghei,* two enzymes participating in CO_2 fixation and eventual conversion of phosphoenolpyruvate to amino acids.

Recent work (119, 120) however indicates that chloroquine does in fact have an injurious effect on the parasite. Light
veal that chloroquine causes a progressive aggregation of malarial pigment within the parasite. These aggregations are included in vesicles (lyso-

somes) that have been described as occurring in other injured cells. There is a progressive degeneration of the chloroquine treated parasites until at 48 hours electron micrographs reveal Palade granules separated from the membranous component of the endoplasmic reticulum, abnormal myelin figures, and a decreased electron density of the cytoplasm. During this latter phase the pigment-containing vesicles gradually disappear. Little is known about the relationship of chloroquine to malarial pigment. The problem is complicated by uncertainty as to the exact nature of hemozoin. Malarial pigment (hemozoin) is a metabolite produced by the parasite's digestion of host erythrocyte hemoglobin. Hemozoin is a heme-containing protein which is distinctly different from hemoglobin and hematin (121, 122). It is a complex mixture, as revealed by the presence of at least three antigenic components (122). Chloroquine binds strongly to ferrihemic acid (123, 124) and it has been argued that enhanced conjugation would reduce the drug's antimalarial activity. Sherman et al (121, 122) have rejected this hypothesis on the grounds that they failed to detect ferrihemic acid as a component of hemozoin. The observation of Peters et al (125) that chloroquine resistant strains of *P. berghei* contain very little pigment as compared to normal strains is further indication of altered hemoglobin breakdown as a mechanism (or consequence) of chloroquine resistance. This was construed as evidence of a failure of chloroquine-resistant parasites to conjugate ferrihemic acid to a protein moiety. However, this hypothesis for drug resistance cannot be applied to all species of plasmodia, since it has been shown that chloroquine-resistant *P. falciparum* produces a normal amount of pigment (126).

A peculiar characteristic of chloroquine is its ability to concentrate selectively within parasitized erythrocytes. Using a *P. berghei*-white mouse system, Macomber et al (127) found that 4 hours after a subcutaneous chloroquine administration the level of chloroquine in infected erythrocytes was 100 times greater than that of the plasma. Of considerable interest is the observation that red cells infected with chloroquine-resistant *P. berghei* accumulate only one half to one seventh the amount of drug that erythrocytes infected with sensitive parasites do (127–129). Fitch (129) believes that the high-affinity binding site is within the parasite and that chloroquine resistance is due to a decrease in the number, affinity, or accessibility of a constituent of the malarial parasite. The differential accumulation of chloroquine between sensitive and resistant strains has recently been confirmed as occurring in *P. falciparum* (130).

Recent developments in malaria chemotherapy.—Despite the intensive search, particularly that supported by the Medical Research and Development Command of the United States Army, for new classes of antimalarial compounds, there have been disappointingly few drugs that have shown sufficient promise to warrant clinical trial. In the absence of new classes of

antimalarials effective against chloroquine-resistant *P. falciparum,* attention has turned again to the sulfonamides. There was considerable interest in the therapeutic activity of these agents during the 1940s. However since the sulfonamides were less active than chloroquine, quinine, or pyrimethamine they were not employed in clinical practice. The observations made in the 1950s (131, 132) that pyrimethamine potentiates the antimalarial activity of sulphonamides and sulphones has only recently been put to practical exploitation. The value of sulphonamide and sulphone-pyrimethamine combinations in effecting radical cure of chloroquine-resistant plasmodia was first demonstrated for *P. berghei* (133) and shortly thereafter confirmed for *P. falciparum* (134–136). Sulfadiazine, sulfamethoxypyridazine, sulphorthomidine, sulfisoxazole, sulphormethoxine, and diaminodiphenylsulfone (DDS) successfully, either alone or in combination with pyrimethamine, have been used to treat normal and multi-resistant strains of malaria. As would be expected, the combination of sulfonamide and pyrimethamine is of greater therapeutic value than either drug alone. The most effective sulfonamide appears to be sulphorthomidine (Fanzil, Fanasil), particularly when combined with pyrimethamine (134, 136, 137). Berman (138) recommends the "blunderbuss" regimen of chloroquine-pyrimethamine-sulfisoxazole for the treatment of *P. falciparum* malaria in nonimmune patients. This combination produced a rapid and radical cure of all 76 patients as treated.

The status of the sulfones as antimalarials is still somewhat uncertain. Early trials by Basu et al (139) in India indicated that a single 240 mg dose of DDS cleared all *P. falciparum* infections but a similar regimen was ineffective in 6 out of 17 *P. vivax* cases. Chin et al (140) also noted the failure of DDS to act as a suppressive against the Chesson strain of *P. vivax*. Trials in American troops in South Vietnam indicate that sulfones are of some promise as a prophylactic against *P. falciparum* although they can also be used as a therapeutic supplement to quinine-chloroquine-pyrimethamine regimens.

The initial fear of sulfonamide-sulfone toxicity has not as yet been realized and so far therapeutic regimens of these drugs have been well tolerated. Daily doses of over 200 mg of DDS can cause hemolysis in individuals with glucose-6-phosphate-dehydrogenase deficiency (141), however there have been no reports from the field as yet of its causing hemolysis at prophylactic regimens. The ease with which sulfonamides induce drug resistance in microbes and experimental malarias leads to the apprehension that their widespread use would rapidly cause the development of resistant strains of human malaria. Sulfonamide-sulfone resistant malarias have not as yet been reported from the field. Perhaps the combination with pyrimethamine makes the parasites less liable to become drug-fast than either drug used alone. Nevertheless until more is known regarding drug resistance to these compounds their use should be reserved for carefully controlled therapeusis.

The only new chemical class of antimalarial to reach clinical trial is the pyrimidine derivative, trimethoprim [2, 4-diamino-5 (3′, 4,5′-trimethoxybenzyl]. This drug acts primarily as a dihydrofolate inhibitor. Martin & Arnold (142) reported that trimethoprim combined with the long acting sulfalene radically cured 8 of 8 normal *P. falciparum* infections and 10 of 11 infections of chloroquine-pyrimethamine-quine-resistant *P. falciparum*. The therapeutic dose was well-tolerated and caused rapid clearance of parasites with concomitant alleviation of clinical manifestations. Obviously this combination of antimalarials is of great potential value providing further clinical trials confirm its efficacity.

Many new types of potential antimalarials are now being screened through the experimental system. It is to be expected that some of these will prove effective at clinical trial in man and add to the reserve of antimalarials to be used against drug resistant strains. Meanwhile, however, it is a peculiar commentary on the science of pharmacology that the antimalarial of last resort is still quinine, a drug that has already celebrated its 300th birthday.

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