Malaria and Victory in Vietnam

The first battle against drug-resistant malignant malaria is described.

Walter Modell

Battles with malaria are not new or unusual for the Armed Forces of the United States. Tropical medicine is a military medical specialty. The military has a long history of major contributions to the understanding and treatment of tropical diseases (two Nobel laureates for their work in malaria). It is a paradox that the diseases to which medical military establishments have made their greatest contributions are not the common diseases of their homelands but exotic diseases, and that the important nations of modern times, which are not in the tropics, should have made their contribution to the malarias, yellow fever, scrub typhus, leishmaniasis, shigellosis, filariasis, schistosomiasis, hookworm, ascariasis, amebiasis, Chagas' disease, and others caused by smaller and larger parasites.

The military establishments active in research in tropical medicine in modern times were those defending wealthy and powerful nations; in the main, Germany, France, Great Britain, and the United States—none of which has much endemic tropical disease. Although these countries maintain first-rate hospitals in their homelands, the research in endemic diseases by their military establishments is not of the same high caliber, nor is it pursued with the same intensity.

This is not perversity on the part of the military, but the logical consequence of the nature of military action transporting forces into foreign environments, often tropical or subtropical, and exposing them to infectious organisms with which they have not had any previous contact and to which they are therefore without any natural or acquired resistance which develops or is inherent in the indigenous populations. Thus fresh troops invading new lands are often more quickly and effectively laid low by local diseases than by military resistance from the natives.

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Research in Tropical Medicine

Tropical medicine has attracted the military of the United States since the Spanish-American War, with steadily increasing interest, beginning with the building of the Panama Canal and continuing through World War I, World War II, the Korean War, and now the conflict in Vietnam. Adequate funds are readily available when military danger is imminent or suspected. With such support, the military can call on civilian scientists to assist in problems in tropical medicine. Military establishments have thus supported research which has found answers to difficult problems that might have remained unresolved for many years. For example, research done during World War II led to the resolution of the problems of malaria (recounted below), and of infected war wounds (by the practical extraction of penicillin). The penicillin produced by Fleming's mold in St. Mary's Hospital in London and elsewhere was unavailable for more than 10 years after its discovery because of the lack of financial support for designing a method for extracting it. When it became a military necessity, virtually unlimited funds from Great Britain and the United States were promptly placed at the disposal of cooperating groups of scientists. In a few years, Fleming's prediction of a generation before was a practical reality. Thus, the military need forcing medical scientists to produce penicillin resulted in a great gift to mankind. Beyond that, the success of the penicillin program led to further searches for practical antibiotics, although before the war such searches had not been stimulated by Fleming's prediction that there was an effective antibacterial agent in his culture of *Penicillium*, or by Waksman's reports of antibiosis in the soil, or even by Dubos' actual isolation of the antibiotic, tyrothricin.

Some of the outstanding practical and theoretical achievements in science have resulted from military needs which stimulated research in areas otherwise beyond reach and impractical. The military has funded projects handsomely, and the pace of accomplishment seems miraculous. The unleashing of atomic energy, the explorations of the surface and the interior of the earth, of the bottom of the sea, and of the outer reaches of space, of planes and rockets, of ships and submarines, of solid-state physics, of sound waves, light waves, and waves beyond our sensorium, and of weather, are all spin-offs of the process by which the military has made science fiction come true. So too has the military brought about a miracle in malaria.

Effective treatment of rampant malaria among our troops is a necessity in Vietnam, and is similar to the problem which 20 years ago was believed to have been solved. Our problem, which threatens to envelop the world, involves malignant or falciparum malaria; formerly such patients responded well and promptly to chloroquine, a drug developed during World War II. However, a newly emerging but rapidly spreading malignant form of malaria is chloroquine-resistant. Until recently this resistant form of malaria caused much more disability, hospitalization, and demoralization among American soldiers in Vietnam than enemy fire did. Now, we have apparently achieved a means of curing the infection rapidly and effectively.

Malaria is a disease which travels widely and rapidly, and, therefore, any new form of malaria can quickly become serious (*1*). Although in nature malaria is far more common in birds (several hundred species), amphibia, and reptiles than in mammals (*2–4*), man is the only mammal in which it is an important cause of morbidity. The plasmodia causing malaria in man are not found in other animals, and only very recently has successful transmission to lower animals been possible. This fact has hampered research because test models are needed in searches for drugs and no small, easily housed and husbanded mammal that can be infected by the same species of plas-

modium that causes malaria in man is available.

The first specific remedy for malaria used in Europe came from the Western Hemisphere. Since there was no malaria in the New World, it was not until cinchona bark was brought to Europe, where it was used as a febrifuge, that its antimalarial properties were discovered. It is less well known and not generally cited that the Chinese used the first antimalarial drug, Ch'ang shan, an herb which contains among other alkaloids the active antimalarial febrifugine. Malaria is an ancient disease of the Old World and was brought to the New World by the slaves of the Dutch, Portuguese, French, and English traffickers (*4*). Malaria spread rapidly in the New World mostly because there was a large indigenous population of *Anopheles quadrimaculatus*, a mosquito vector. By 1526, malaria was so well established in the New World that, together with hunger and other allies, it defeated an attempt to colonize North Carolina. In the early 17th century malaria was a clinical problem as far north as Virginia. It first appeared in New England around 1650. The invasion of the two American continents by the malarial parasite continued rapidly. Serious epidemics of malaria occurred even in the northern parts of the eastern American colonies (*1, 3*). Malaria can spread rapidly and far, and modern methods of transportation and the increased amount of world travel adds markedly to this potential danger.

Effective measures for control of malaria depended on an understanding of the mode of its transmission and the biology of the malaria parasite and the medical details essential to the effective use of quinine. As such understanding developed, many important local successes were accomplished through measures of control of *Anopheles* as well as effective use of quinine. Associated with the contribution of Walter Reed to the understanding of yellow fever was the control, by William Gorgas (*5*), of mosquito-borne disease rampant during the building of the Panama Canal.

Methods for containing malaria did not change much between 1900 and 1940. During this time, malaria was kept within bounds in the more temperate parts of the United States and was uncommon in civilians except those who traveled abroad. Malaria was endemic in the southern and semitropical parts of the United States where control of *Anopheles* was less complete.

At the outset of World War II, quinine and quinacrine (Atabrine) were used to treat malaria, and pyrethrum was used to control *Anopheles*. Used well, however, they could be very effective, as the following will show. In Brazil, in March 1930, R. Shannon found a specimen of the notorious African *Anopheles gambiae* that had somehow crossed the Atlantic (*3*). By 1938, *A. gambiae* had caused the greatest epidemic of malaria of the Americas. In 6 months it had caused over 100,000 cases with 14,000 deaths, and this African invader threatened other American countries. By a concerted effort to combat *A. gambiae* rather than malaria, the entire infected area was cleared so that in November 1940 there was no evidence of it in South America. No further difficulty with malaria transmitted by this species has occurred since (*3*).

Malaria Control during World War II

During World War II, with the movement of our troops into North Africa, Sicily, the Far East, and Polynesia, better measures for control and treatment than those accepted in peacetime were needed. Our troops were in special danger because the only sources of quinine (which was not then and has never become a commercial synthetic product) were the Dutch plantations in the Southwest Pacific that had been taken over by the Japanese. Although the civilian supply of quinine was severely restricted in the United States, our stockpile was inadequate for our expanding military needs [chlorophenothane (DDT) had not yet been introduced as an insecticide, so that control of *Anopheles* still depended on 50-year-old methods]. Only Germany had anticipated the need of a quinine substitute as well as for better antimalarial therapy before World War II (*6*): pamaquin was developed in 1926 and quinacrine in 1930; quinacrine proved to be an effective antimalarial drug. Since the turn of the century (except for a modest effort in 1939 by Lyndon Small) the United States did nothing much about malaria until World War II (*6*).

Quinacrine has many clinical advantages over quinine, but even in prophylactic doses, it stains the skin yellow and sometimes causes gastrointestinal distress. Although J. A. Shannon developed an effective prophylactic program with quinacrine, something better was needed for use in the field (*7*). In the meantime, the War Production Board stimulated the production of quinacrine in this country so that during World War II there always was an adequate supply of quinacrine. In several tropical South American countries cinchona plantations were revitalized. A fairly crude extract of the bark, totaquina, a mixture of all the natural cinchona alkaloids, was used to treat malaria. This was preferred by some because it did not have the unpleasant side effects of quinacrine.

There was also a prompt response of the scientific medical community to the call for better drugs for malaria. During World War II the Office of Scientific Research and Development organized an antimalarial program involving scientists from universities, industry, the Army, the Navy, and the Public Health Service, and with an effective liaison with similar operative groups in Great Britain. In 1943, all these were unified under the Board for the Coordination of Malarial Studies, a task force geared to find effective antimalarial drugs. Wiselogle gives a full account of the effort (*8*).

The cooperative program provided (i) information on the general biology of the malarial parasite; (ii) effective and reliable methodology for the appraisal of antimalarial drugs; and (iii) therapy and synthetic drugs (*9*). During its 2 years of operation the program was responsible for the screening of about 16,000 chemical compounds for clinical antimalarial activity. Among these were the 4-aminoquinolines, the 8-aminoquinolines, and the sulfonamides, and entirely new chemical entities, as well as variations on older structural themes. Out of this effort there came (some postwar) new antimalarial drugs such as chloroquine, amodiaquine, primaquine, pyrimethamine (Daraprim), and chloroguanide (Paludrine, proquanil). Americans associated with the cooperative effort include Alving, Berliner, Coatney, Coggeshall, Dieuaide, Dochez, Earle, Elderfeld, Loeb, Marshall, Most, Schmidt, Shannon, Zubrod, and others (*6, 8, 10*).

The screening program set new standards for the search for new drugs that, by present standards, are classic examples of basic pharmacology as well as of clinical pharmacologic investigations of drugs.

In drug-screening programs in which lower animals are used, a drug useful for man may fail to be revealed or a drug

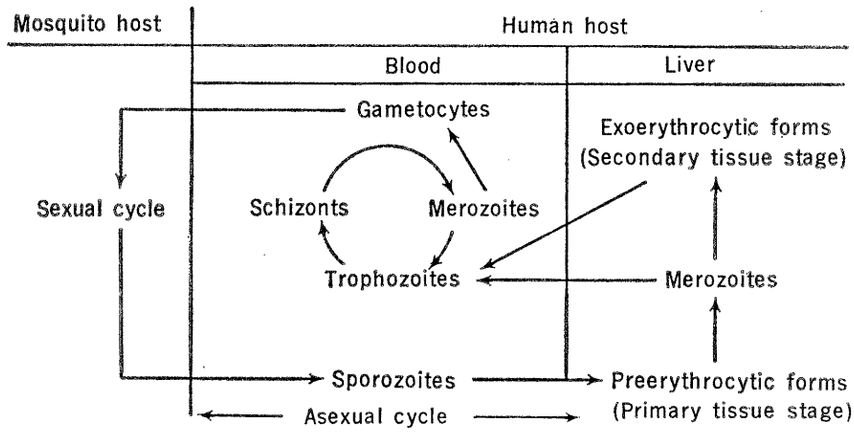

Fig. 1. Cycles of the development of the human malarial parasite in man and in the *Anopheles* mosquito.

which is not effective in man may be recommended. The effectiveness of the screening process used in the United States program is dramatized by the fact that chloroquine (the outstanding antimalarial agent found) was the same chemical the Germans had rejected 10 years previously (called Resochin by them). The Germans had decided, on the basis of a test in one species of avian malaria and a few tests in paretic patients with malaria, that it was less effective and more toxic than quinacrine and hence not a useful drug (*6*). There was further brief exploration by the Germans of related 4-aminoquinolines, after which the program was abandoned, and all confidence was placed in the well-established quinacrine. We chose to use chloroquine after we tested it. Of the 8-aminoquinolines screened, only primaquine was added; the others were too toxic (pamaquine had long been established) (*6*).

From the end of World War II until the recent outbreak of chloroquine-resistant falciparum malaria, chloroquine was recognized as the most effective antimalarial agent available. Amodiaquine, a closely related 4-aminoquinoline, was discovered in a screening process in 1951 and was shown to be as effective as chloroquine (*4*). Both are still the best drugs for all other forms of malaria, and no important distinction between them can be made; chloroquine is simply the prototype.

The screening program which established the effectiveness of chloroquine and other drugs in malaria deserves special attention, not only because it was a well-coordinated drug search, but also because it is the basis of the current search for a drug for chloroquine-resistant falciparum malaria. The coordinated screening program in World War II was conducted in six species of avian malaria in three species of birds and in the single natural species of monkey malaria (*Plasmodium knowlesi*) then available for laboratory trials. These strains of plasmodia are not the natural causes of malaria in man and at the time no nonhuman plasmodium had been transmitted to man. Because there are well-known examples of drugs more effective against human malaria than against some species of avian malaria, and because drugs very effective in birds may be ineffective in man, some investigators (notably Marshall) tended to denigrate the usefulness of the avian screen and to suggest that only tests in man would provide useful leads (*9*). The simian screen was of limited use because the rhesus monkey is so large and difficult to handle and to husband that the numbers demanded by testing 16,000 chemicals made this species feasible for only the special cases. Also only one species of simian plasmodium *P. knowlesi* (identified in 1933) was in use, although *Plasmodium inui* and *Plasmodium cynomolgi* have been known since 1907.

Human and Nonhuman Malaria

Differences between nonhuman and human malaria and malarial parasites were first recognized in 1928 and were appreciated by all workers in the coordinated program. Sharp differences between strains of the same species of parasite were also recognized. The avian screen was continued on the general hypothesis that although there were many differences there were also many similarities, and if a sufficient number of different species of the plasmodium were used, data which applied to most avian parasites were also likely to apply to the human parasite. Thus, with full appreciation of differences generated by species and strain, the major portion of the screening program was based on avian malaria.

In drugs which passed the avian and monkey malaria screen, toxicity tests were carried out in dogs, rabbits, monkeys, mice, and rats. On the balancing of toxicity against the effects in avian and simian plasmodia, decisions as to whether to test the drug in man were made. In only about 80 of the 16,000 drugs screened was therapeutic and prophylactic effectiveness tested in human volunteers with vivax, malariae, ovale, and falciparum malaria, induced and contracted naturally. The coordinated program provided information relative to the toxicity and usefulness of a new drug that surpassed anything previously used in the search for new drugs, and established that a drug discarded by the Germans was the outstanding antimalarial agent of all time—victory for the scientific method.

The investigations also provided information on the safest and most effective way of using drugs prophylactically as well as therapeutically (*6–8, 11, 12*). It established guidelines concerning the prevention of the spread of malaria by human nonsymptomatic carriers. The tests also made it clear that despite chloroquine for the treatment of the acute disease there were many aspects of malaria for which other drugs were still necessary. Though many new and effective drugs had been introduced, it was appreciated that each of them, chloroquine included, had specific limitations, and that there was no single drug which solved the whole problem; the metamorphic forms of the parasite were so different that only by using highly selective drugs for each form could the infestations be completely controlled.

In view of the demonstrated effectiveness of the drugs available for therapy and of the newly introduced DDT and other chemicals for controlling the mosquito vectors of the disease, after World War II there was a tendency to consider the antimalarial problem as permanently resolved, in that all the essential elements were known and the necessary drugs were available. Through the effective treatment, prevention, and pesticides it seemed reasonable to assume that malaria would soon be a disease of man's past (*13–15*). However, the increasing resistance of the

Anopheles mosquito to insecticides as well as of the plasmodium to drugs was not being taken into account (*16*).

Dubos has noted, and it applies well to the complex cycle of the malarial parasite and the drugs for its destruction within the human host, that, "Selectivity is never absolute. Even in the case of enzymes and antibodies there is always some overlapping of enzymatic and immunological activity. As it is most unlikely that drugs can be made more specific than enzymes or antibodies, thus selectivity will at best remain relative" (*17*). Hence even a highly selective drug is likely to react with more than one structure, and with some with which reaction is undesirable.

Chloroquine is still the best antimalarial drug available. But like many other antimalarials it cannot destroy tissue schizonts; therefore after chloroquine is administered relapses in vivax, malariae, and ovale malaria occur. Chloroquine destroys the gametocytes, but it fails to do so in falciparum malaria; hence its use alone produces carriers of malignant malaria. The same limitations apply to amodiaquine (1951). Other new drugs also had their limitations.

A brief status report on the other antimalarial drugs available in 1952 follows. At first chloroguanide (1945) was apparently truly curative for vivax and a relatively nontoxic suppressive treatment for all malarias, but its use was rapidly followed by the development of resistance. Pamaquine (1926), also called plasmochin, was useful only for prevention of relapses of vivax and other relapsing forms as a secondary tissue schizontocide. Primaquine (1952) was useful only to suppress gametocytes, hence to prevent the development of carriers. Pyrimethamine (Daraprim, 1951) was sometimes curative in falciparum, occasionally in vivax, but was too toxic and rapidly led to development of resistance. Combinations of these drugs seemed to provide for all the needs for the treatment of malaria. Between one drug for primary schizonts, another for erythrocytic forms, another for secondary schizonts, and primaquine for gametocytes, all necessary therapeutic forces seemed to be available (*18*).

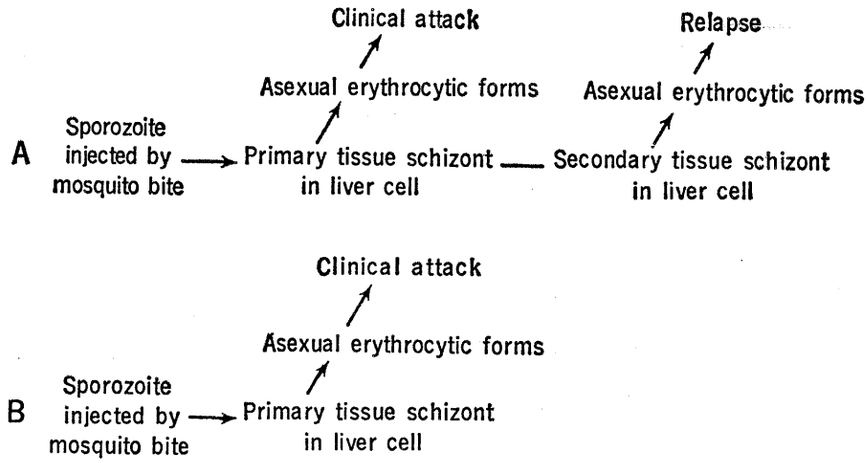

Fig. 2. Differences in the asexual cycles of the human malarial parasite showing basis for clinical relapse. (A) *Plasmodium vivax, P. ovale,* and *P. malariae*; (B) *Plasmodium falciparum.*

Development of Resistance to Antimalarial Drugs

Resistance to effective drugs was accepted with equanimity at first, perhaps because there was no resistance to chloroquine, and the loss seemed tolerable in that other drugs and effective insecticides were still available. From 1948 to 1950 the World Health Organization reports that resistance to chloroguanide was detected in the malaria parasite in Malaysia. From 1950 to 1959 resistance to other antimalarial drugs, including quinine, was reported in many parts of the world in which malaria was indigenous. In 1960, the first instance of resistance to chloroquine by the *Plasmodium falciparum* was recognized (in South America) (*3*). In 1961, troops of the United States and the Commonwealth and some American scientists contracted chloroquine-resistant falciparum malaria in Vietnam, Thailand, Cambodia, and Malaysia (*19*). In 1962, chloroquine resistance was identified in the falciparum parasite in Brazil. Very recently, chloroquine-resistant falciparum malaria has been identified about 40 kilometers from Singapore. Malaria travels far and rapidly, yet no organized campaign was started against chloroquine-resistant falciparum malaria (although it was first noted in 1960) until it became a serious threat to our military in Vietnam.

At first quinine could be used effectively, although not as effectively as chloroquine, in chloroquine-resistant *P. falciparum*. But whereas the first chloroquine-resistant strains seemed to respond reasonably well to quinine, in 1963 to 1964 carefully controlled studies indicated that strains of *P. falciparum* were appearing which were resistant to chloroquine and all synthetic antimalarial drugs, and to quinine as well—to all known antimalarials. Thus 20 years after some considered the problem solved the emergence of resistant strains of falciparum malaria changed the picture. Apart from its immediate military importance in the Vietnam conflict (*20*), its rapid spread makes it a threat to all parts of the globe in which the malarial parasite and its vectors are able to exist.

As its name implies, malignant malaria is a far more rapidly debilitating, more serious, and more frequently fatal disease than any of the other forms of human malaria. A few details of the life cycle of the malarial parasites in man and in the *Anopheles* mosquito must be given to correlate the details of the antimalarial program with the several aspects of the disease malaria (Figs. 1 and 2). Until now no distinction has been drawn in this account between falciparum malaria and the other human forms—ovale, vivax, and malariae—since it has not been essential to the discussion of the general problem which preceded the current crisis. But it does relate to the specific problem which arose in Vietnam when resistant falciparum malaria emerged, not only because other types of malaria continue to respond to chloroquine but also because falciparum malaria is clinically and biologically different from other forms. There are apparently many strains of *P. falciparum* with considerable variation in virulence. Falciparum malaria also differs from other forms in the nature of its response to drugs.

If the falciparum infection responds well clinically, most patients are cured and are not likely to suffer from relapse, whereas in all other forms of malaria even the best clinical response is likely to be followed, sooner or later,

by relapse. This is due to an important basic difference in the parasitic forms which develop in man. In the human phase of its cycle, *P. falciparum* does not form secondary tissue schizonts, whereas the other malaria plasmodia do. This is why, unlike other malarias, in the falciparum form there is not the same tendency for relapse if the acute stage is well treated. But we cannot explain why this form of malaria is so much more devastating than those caused by parasites which develop tissue schizonts.

The secondary tissue schizont causes relapses and is resistant to most drugs effective against the erythrocytic forms of the parasite that cause the acute clinical symptoms. In the malarias with secondary tissue schizonts, if suppressive therapy is discontinued, an erythrocytic phase of the cycle sooner or later develops and acute malarial relapses occur (Fig. 2). Drugs acting selectively on the metamorphic forms of the plasmodium are necessary to prevent relapses. This is not the case with malignant malaria, and, from the point of view of the pharmacologic attack, it is therefore a simpler disease to deal with than the less serious malarias. Thus a dose of chloroquine which ameliorates the acute attack of falciparum malaria cures the patient, whereas in vivax malaria such an effect, no matter how salubrious, is likely to be followed by a relapse.

Program for Treatment of Chloroquine-Resistant Falciparum Malaria

On recognition of the nature of the new problem with falciparum malaria in Vietnam, a program for the development of its effective treatment was instituted at the Walter Reed Army Institute of Research under the leadership of General William D. Tigertt, an outstanding malariologist. This program, unlike the World War II Coordinated Program on which it was based, dealt with a single type of malaria, malignant falciparum malaria resistant to chloroquine. In addition to work done in the laboratories of the Walter Reed Army Institute of Research, the program was carried out with the cooperation of many other laboratories. Two advisory committees of consultants, one of chemists and the other of pharmacologists, assisted the Walter Reed group in designing its plans.

While the basic approach of the World War II coordinated program was used, the new plan incorporated advances in malariology (*14*), greater knowledge of the metabolic processes of the parasite, newer approaches to toxicity of drugs in man, and the much larger number of strains of avian and mammalian plasmodia, and *P. falciparum* experimentally transmitted to the chimpanzee and the gibbon. Despite failure to find or transfer malaria to a Noah's Ark of animals—marmosets, capybaras, agouti, paca, nutria, chinchilla, guinea pigs, groundhogs, prairie dogs and opossum, many species of monkey, and other mammals—there was considerable expansion because of experimental transfer of plasmodia to new species. Transfer of *P. knowlesi* from Malayan to the Indian rhesus was useful because it is a devastating infection in the latter and benign in the former. Studies were also conducted on the infected *Anopheles* mosquito and on preparations in vitro of several varieties of plasmodia. The findings of malaria in hippopotamus, water buffalo, and a few other large mammals were not useful discoveries (*3*). Unfortunately, the susceptibility of the small monkey *Aotus* (South American night, or owl monkey) to the resistant falciparum plasmodium was discovered so recently that it has played no role in the major therapeutic accomplishment of this program until now.

Toxicologic studies were carried out on several mammals, including monkeys, with the drugs which had passed the antimalarial screens. These studies included not only the usual pathologic and physiologic observations, but careful examinations of effects on bone marrow and studies of drug metabolism and metabolic effects. Depression of folic acid by these drugs was determined by means of the effects of drugs on the growth of sensitive bacteria and their subsequent revival after the addition of folic acid.

In nearly 4 years, the program operating at the Walter Reed Army Institute of Research has resulted in the screening of more than 110,000 chemicals, and now about 1000 chemicals per month are being screened. Many of these are old drugs, some variations on old chemical themes, and some entirely new chemical structures. Results have been, as might be expected, mostly negative. But they found one treatment they were looking for. From the few promising new drugs a combination of drugs has been made that is a definitive answer to one of the problems that started the project—how to treat the acute phase of resistant falciparum malaria.

Although the mechanism of the antimalarial action of chloroquine still is unknown (DNA binding is suspected), and the mechanism by which the parasite develops resistance to it is unknown, there were leads to what was needed in a chloroquine substitute. A broad routine screening of a large variety of chemicals, including the 4-aminoquinolines, was in order. A good clue to a new treatment (recounted below) lay in the known mechanism of antimalarial action of chloroguanide and pyrimethamine—their depressant action on folic acid. Knowledge and understanding turned out to be profitable even though the need justified the great cost of the routine screening of more than 100,000 chemicals without any special promise and which turned out to have little or no use.

Folic Acid Cycle

The lead to the new approach to the therapy of resistant falciparum malaria came from the recognition of the sensitivity of protozoa to deficiency of folic acid (*21, 22*). The limitation of this therapeutic approach is that depression of folic acid is always a serious threat to formation of blood in man (*22, 23*). For this reason, unless one is dealing with as desperate a condition as cancer, depression of folic acid by a drug tends to discourage explorations of its clinical use. Many anticancer drugs are depressants of folic acid, useful because they ultimately interfere with synthesis of nucleic acid, sought because of this to prevent the mitotic activity of tumor cells, but where they are exceedingly dangerous because of this action, causing through it depression of formation of blood cells in bone marrow.

Depression of folic acid seemed defensible as a therapeutic approach in malaria only if some means of protecting the human host was developed. It was already established that drugs such as chloroguanide and pyrimethamine acted as antimalarials because they were depressants of folic acid. So too, the sulfonamides had an antimalarial effect due to an "antifol" action, and there was evidence in an observation reported in 1950 that the combination of a sulfonamide and pyrimethamine was therapeutically effective in malaria (*22*). The recognition that sites of this action in the two

drugs differed made their combined use a way to obtain a much higher degree of "antifol" action in the plasmodium than a simple summation of effects. That one of these effects did not ordinarily occur in man led to further exploration of the combination because it not only made the combination more effective against the parasite but it also made it safer for man.

The action of the sulfonamide (Fig. 3) is to prevent the synthesis of folic acid from para-aminobenzoic acid. This is the basis of the antibacterial and modest antimalarial effects of the sulfonamides. This action develops in the rat as the consequence of "antifol" action on beneficent intestinal bacteria and when it does it is the cause of the serious depression of the bone marrow. Although it has never been demonstrated to occur in man, in rare instances it may be a hazard of the therapeutic use of sulfonamide drugs.

Pyrimethamine (Fig. 3) prevents the conversion of folic acid to folinic acid (citrovorum factor) by depression of dihydrofolic acid reductase. This action develops in both the parasite and in man. Since the two sites of the two-step, or sequential, depression are present only in the parasite, it seemed reasonable that the combination was a far greater threat to the plasmodium than to man in whom only the second step occurred. This turned out to be the case. The combination is now also used in the therapy of coccidiosis and toxoplasmosis and has also been shown to be effective against some bacterial infections as well.

Better drugs of each type were sought for malaria. The common sulfonamides were highly protein-bound, hence feeble in action, and quickly eliminated, hence too brief in action. The eventual choice, sulfalene (2-sulfanilamido-3-methoxypyrazine) is a sulfonamide with an extremely long duration of action, a half-life of about 65 hours, and it is far less protein-bound (about 45 percent, whereas other long-acting sulfonamides are about 95 percent protein-bound). Thus sulfalene could be counted on for long-continued intense action in the parasite. Also to its credit is that it does not seem to cause the serious Stevens-Johnson syndrome in man which complicates the use of other long-acting sulfonamides.

A substitute for pyrimethamine was also needed because tolerance to it had developed in the field. It was found in trimethoprim [2,4-diamino-5-(3′,4′,5′-trimethoxybenzyl)-pyrimidine] which seems not to induce tolerance, which is less toxic than pyrimethamine, and is a very effective "antifol" in protozoa. The dihydrofolic acid reductase of protozoa is far more sensitive to trimethoprim than that of man. An excessive antifolic acid effect of trimethoprim in man could be reversed with folinic acid, whereas it did not reverse the process in the plasmodia (*24*). Thus trimethoprim appeared to provide the ideal second stage of the "antifol" action—a weak action in man and a potent one in protozoa.

A Treatment Is Found

The combination of sulfalene and trimethoprim was screened and tested in the laboratory, and, on the basis of these findings, was soon tried on human volunteers. The dramatic effects in the volunteers led to prompt clinical trials. Outstanding success in the treatment of resistant falciparum malaria contracted in Vietnam has been reported (*25*). One dose cures in almost all cases, and the one-dose combination of the two drugs contains much smaller amounts of either drug than has a clinical (or toxic) effect alone (*25*). This phase of the battle of resistant falciparum malaria seems to be a clear-cut victory.

Unfortunately the victory against the resistant falciparum is not unconditional. A prophylactic drug is still needed. Regular use of a folic acid depressant as a prophylactic would eventually depress blood formation in the human host. This has in fact already been demonstrated (*26, 27*). It is its one-shot (or two-shot) *coup de grâce* success that makes the combination safe as well as effective. So the search continues for a prophylactic drug. Also reserve drugs are sought, for although there are as yet no signs of development of tolerance to trimethoprim, the history of rapid development of resistance to chloroguanide and pyrimethamine suggest that it could happen and that we should be prepared for it.

Perhaps there will be an immunologic solution; resistance to malaria does develop in man and animals (*2, 28*) and is an important saving feature for the populations of endemic areas.

A new finding in this cooperative effort is strangely disturbing (*29*). Experiments have just been reported indicating that man can transmit the *P. falciparum* infection to the *Aotus* monkey by means of the natural *Anopheles* vector. There are also unreported experiments revealing that *Aotus* can transmit resistant falciparum malaria to man through the mosquito. We are now threatened, therefore, as troops (potential vectors) return from Vietnam, with a new parasite which travels rapidly, and we also have a new potential reservoir for infecting mosquitos in the South American night monkey *Aotus*. All our troops (unlike those of other

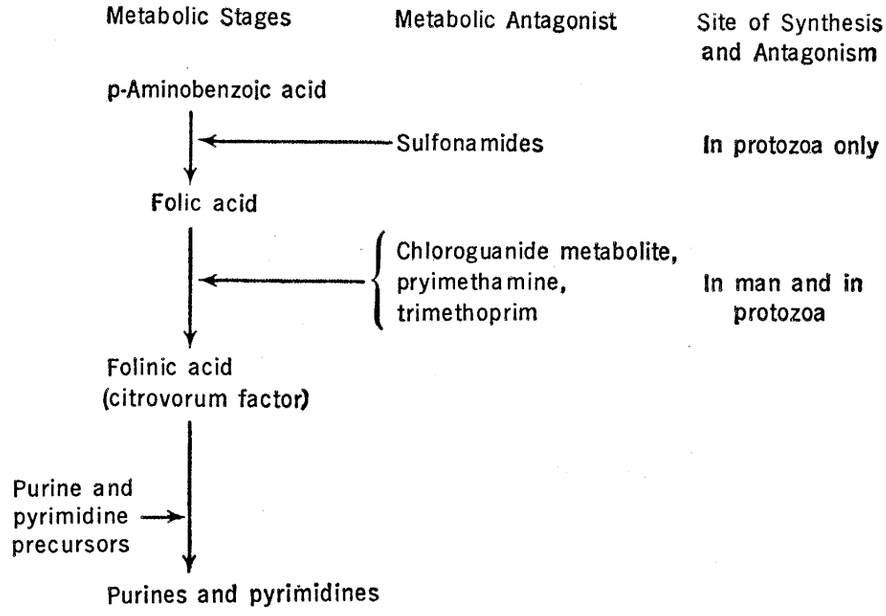

Fig. 3. Metabolic pathway of formation of purines and pyrimidines, essential to synthesis of nucleic acids; and sites of antagonism and drugs which will block at various stages in protozoa and in man.

countries) are given primaquin to destroy gametocytes which they may harbor, whether or not they had the clinical disease, to prevent them from returning to the homeland as carriers of chloroquine-resistant *P. falciparum*. It is to be hoped that this program will be carried out effectively. The treatment of acute chloroquine-resistant malignant malaria does not end at the battlefront. There will be other battles with *P. falciparum* as well as with other malarial parasites.

What has yet to be established in military medicine is where the battle lines of national defense should really be drawn, and whether the same full and prompt support of research in problems of national medical importance do not have a broader spectrum than is usually recognized and should not be applied to a more extensive horizon of natural enemies of mankind than a group of highly specific exotic tropical diseases. But in any event, the current military program of this country has solved a problem in malaria which will benefit all mankind: to date, our most important victory in Vietnam.

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Carbon Monoxide and Human Health

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Carbon monoxide (CO), a colorless, nonirritating gas, is generated by incomplete combustion. Its presence is a ubiquitous index of affluence, since it occurs in industry, in tobacco smoke, in household heating, and in motor vehicle exhaust. Because of the contribution of motor vehicle exhaust, carbon monoxide is one the most important of urban atmospheric pollutants.

To prevent adverse effects on human health as exposures to CO increase, adequate programs and policies must be adopted. Their formulation will require deliberate scientific judgment based on adequate information and the consideration of certain hypotheses, which are reviewed here.

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Sources of Exposure

Urgency is given the development of such policies by the large and rapidly growing number of motor vehicles, whose pollutants now have made the quantum jump from being a problem in the immediate vicinity of traffic to being a problem affecting the entire community. For example, in New York City *each day*, automobile traffic alone produces 8.3 million pounds (3.8 million kilograms) of CO; each car emits about 1/6 pound of CO per mile of travel at 25 miles (40 kilometers) per hour and about ⅓ pound per mile of travel at 10 miles per hour (*1*). An estimated 20 million pounds of CO per day were emitted by motor vehicles in Los Angeles during 1967.

In urban areas dependent on automobiles for commuting, a common pattern is observed. There is a relatively high peak of CO pollution in the morning and a flatter peak in the evening. A single day-long peak is observed in downtown New York City (*2*), reflecting saturation levels of traffic.

The first generation of exhaust control devices now required on new cars has reduced CO emissions, but the effectiveness of these systems is known to diminish as the vehicles are used. Since motor vehicle use is expected to increase by 70 percent by 1980, even 70-percent control—the goal of the existing program—would not produce an improvement over the present situation even if that goal were attained.

Carbon monoxide occurs in high concentration in cigarette smoke (> 2 percent), but an estimate of the average concentration in smoke inhaled into the lung is about 400 parts per million (0.04 percent).

The magnitude of exposure to CO from smoking has been estimated in a population of longshoremen (*3*) examined prior to the work shift and during a time when there was little community air pollution. The results therefore reflect primarily the effects of smoking. Exposure estimates were based on measurements of CO in expired air after the individual had held his breath for 20 seconds. Ringold *et al.* (*4*) have shown this to be a valid way to estimate the concentration of carboxyhemoglobin (COHb, the complex of carbon monoxide with hemoglobin in