Preface

We who are engrossed in the study of antimalarial chemotherapy are fond of repeating certain maxims. Malaria is one of the most important disease problems in the world. The control of malaria is increasingly limited by resistance to available drugs. New strategies for treating malaria are urgently needed. We should strive to identify new targets for antimalarial agents. Each of these maxims has reached the status of a cliché, but is nonetheless compelling. The complex biology of malaria parasites and extreme poverty in most malarious regions have locked us into an unrelenting continuation of endemic malaria in most of the tropical world. Meanwhile, drug resistance worsens, and it appears that the speed of efforts to develop new treatment strategies may not keep pace with the resourceful parasites. This rather bleak scenario presents us with major challenges. For the short term, as drug resistance worsens and standard therapies fail, how will we utilize existing agents to prevent worsening of worldwide malaria? Which strategies are likely to provide effective new antimalarial drugs? And for the future, how can we develop strategies incorporating old and new therapies and other control modalities to begin to lessen the worldwide burden of malaria?

Although challenges for the effective treatment and control of malaria are great, so are current opportunities. Our understanding of the biology of malaria parasites is growing rapidly. The entire genome of *Plasmodium falciparum* will soon be sequenced. New molecular technologies are allowing us to definitively assess the biological roles of key parasite molecules. There is good reason to believe that these advances will speed up the pace of antimalarial drug discovery and development. As is discussed throughout this book, recent progress has been impressive. New insights into the appropriate use of existing drugs and optimal means of attacking known targets are being gained, and new potential drug targets are being identified.

At this point, it seems appropriate to collect our current understanding of antimalarial chemotherapy in a single volume. Much has changed since earlier classic references on this subject. We have moved to a more rational approach to antimalarial chemotherapy, where we are attempting to logically use existing agents and to develop new drugs designed to target specific parasite pathways. For this approach, a much better understanding of parasite biology is needed.

Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery offers detailed discussions from experts in many areas. As background, chapters on the biology of parasites highlight two key areas, the plasmodial food vacuole and plasmodial transport mechanisms. The public

health consequences of current problems in antimalarial chemotherapy are also reviewed. Established antimalarial drugs and new agents under development are then discussed in detail. Our emphasis is not on summarizing established drug usages, but rather to present current understanding of the mechanisms of action and resistance of existing agents in order to help us design new strategies to use these or related compounds. The last section of the book presents information on new compounds. These include agents that are related to existing effective antimalarials and some new targets. The chosen targets represent a small sample of potential new avenues for chemotherapy. It is hoped that the discussions of parasite biology and chemotherapy provided in this book will help to stimulate additional ventures in this direction. As is often mentioned (in yet another cliché), additional funding for research on malaria will be essential for the breadth of study required to develop multiple new drugs.

My editing of *Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery* has been rather time-consuming, but very rewarding in allowing me the opportunity to work with world leaders in all areas of malaria chemotherapy, and in providing me with a privileged look at the status of cutting edge research in this field. I wish to thank all of the authors for their hard work in preparing excellent discussions on their respective topics. Thanks are also in order to those who have helped me to choose specific topics and authors and offered advice through the course of the book preparation process. I'm afraid that I will certainly neglect some contributors, but special thanks go to Steve Meshnick, Irwin Sherman, Hagai Ginsburg, Ioav Cabantchick, Terrie Taylor, Peter Bloland, Chris Plowe, David Fidock, Tom Wellems, Mike Gottlieb, Lou Miller, Steve Ward, Leann Tilley, and Piero Olliaro. I thank members of my laboratory at UCSF and my collaborators in Kampala, Uganda, for their inspiration and useful ideas. I remain indebted to the late Jim Leech, who was the perfect mentor to start me on a path of antimalarial drug discovery. Lastly, I thank my wife, Kandice Strako, for her indulgence and support during the hectic and seemingly never-ending editing process. My hope is that this book will offer a useful review for those who study malaria, and, more importantly, an entry point into antimalarial chemotherapy for those new to this field. If this is the case, and we can help to expand efforts toward antimalarial drug discovery and development, our labors will certainly have been worthwhile.

Philip J. Rosenthal, *MD*

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The History of Antimalarial Drugs

Steven R. Meshnick and Mary J. Dobson

INTRODUCTION

Physicians have diagnosed and treated fevers for thousands of years. Until Robert Koch, Louis Pasteur, and their contemporaries uncovered the "germs" that cause most febrile illnesses, fevers were considered diseases, not results of diseases. Fevers were treated with a variety of remedies, such as bloodletting or herbs, most of which were ineffective. Malaria-like febrile illnesses (with names like "the ague" or "paludism") have been described since Hippocrates as fevers that were periodic and associated with marshes and swamps. The word "malaria" comes from the Italian "mal'aria" for "bad airs." It was not until the 1880s and 1890s that Alphonse Laveran, Ronald Ross, Battista Grassi, and others were able to identify the malaria parasite and link the transmission of malaria to mosquitoes. Although the understanding of the mosquito cycle led to a number of new approaches in vector control in the early 20th century, malaria prophylaxis and therapy continued to draw on earlier remedies. Indeed, what is remarkable about malarial fevers is that two herbal treatments, cinchona bark and qinghao, were used to treat malaria effectively for hundreds of years prior to the understanding of the mosquito cycle. Today both quinine (derived from the cinchona bark) and artemisinin (from qinghao) remain of prime importance in the control of malaria.

The practice of Western medicine changed dramatically during the 19th and 20th centuries, as herbal remedies were gradually replaced by pure chemical compounds and, later, synthetic drugs. So, too, did the treatment of malaria undergo important scientific developments. Malaria was among the first diseases to be treated by a pure chemical compound—quinine—isolated from the cinchona bark in 1820. It was, subsequently, the first disease to be treated by a synthetic compound—methylene blue. In addition, malaria parasites were among the first pathogenic microbes to out-smart medical intervention and become drug resistant.

Malaria was one of the best-studied diseases in Western medicine until the middle of the 20th century. Until that time, malaria was still endemic in North America and Europe. It also had great importance because it represented an obstacle to the expansion of European nations into the tropical world. It also played an important role in the major wars of both the 19th and 20th centuries. The situation has changed, and, until recently, interest in malaria in Western nations has waned even though the disease at a global scale has not.

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QUININE

The compound quinine occurs naturally in the bark of Cinchona trees, originally found in high altitudes of South America (Fig. 1). Cinchona bark was introduced into Europe as a treatment for the ague in the early 17th century by Jesuit priests returning from Peru. Peruvian Indians chewed on cinchona bark—but, as far as we know, not to treat malaria. Indeed, malaria may not have existed in the New World prior to Columbus *(1,2)*. There have been many speculations about the South American Indians' knowledge and use of the cinchona bark. According to one account, Indians used it while working in cold streams in Spanish-owned mines in order to stop shivering. This effect was probably the result of quinine's direct effects on skeletal muscle and neuromuscular junctions *(3)*. Some physicians and Jesuit priests in Peru reasoned that the bark might be able to stop the shivering associated with attacks of the ague. They tried the bark of the "fever tree" on malarial patients and found that the feverish symptoms of ague sufferers were relieved.

The Countess of Chinchon and her husband are credited with bringing the bark back to Spain (Fig. 2) although like several of the myths and mysteries associated with the early history of cinchona, this story is probably fallacious. Linnaeus in 1742 named the tree, cinchona, after her, although the bark was more commonly known as Jesuits' powder or Peruvian bark *(4)*.

A number of European physicians and quack doctors had remarkable successes with the bark *(5)*, but its use initially met with a great deal of skepticism. First, many people were skeptical of anything associated with the Jesuits. In fact, Oliver Cromwell suffered severely from malaria, apparently because he refused to ingest "the powder of the devil" *(6)*. Second, because merchants were frequently unable to distinguish cinchona from other trees, many types of bark were used as long as they were bitter. Finally, different cinchona species vary greatly in quinine content *(7)* and there was considerable confusion concerning the "best" bark to administer. These last two factors made therapeutic results inconsistent.

Richard Morton, who published his *Pyretologia* in 1692, became a firm advocate of Peruvian bark. He claimed it was a "Herculean antidote" to the poison of intermittent fevers and, when given in proper dosage, usually returned the patient to health immediately *(8)*. He also used the therapeutic results of the bark as a guide to diagnosis. His ideas were further developed by Francesco Torti who, in his classic work of 1712, *Therapeutice Specialis*, designed a "tree of fevers." Different fevers, shown as branches of the tree, were divided into categories: On the left were those that responded to cinchona (shown as branches covered with bark) and on the right were those that did not (depicted as denuded leafless branches) *(8)*. Torti's classification and differentiation of fevers and his recognition that only certain fevers could be treated by cinchona was of major importance. By the late 18th century, formulations became more standardized and cinchona was more widely accepted as a treatment for specific intermittent fevers *(3,9)*.

Cinchona had become so popular by the eighteenth century that several species of cinchona trees were becoming extinct *(6,10)*. In 1820, two young French chemists, Pierre Pelletier and Joseph Caventou, isolated the alkaloids quinine and cinchonine from cinchona bark. Within a year, several French physicians were successfully using pure quinine to treat patients with intermittent fever *(11)*. Explorers and scientists then

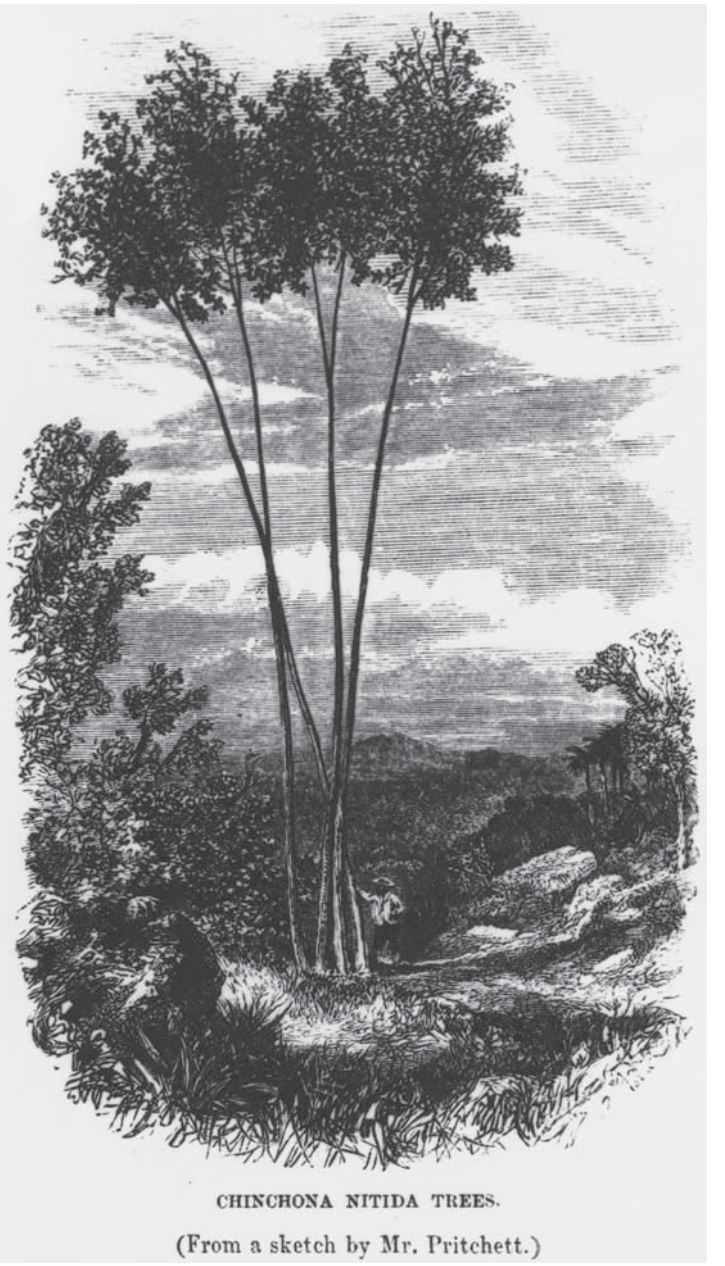

Fig. 1. Cinchona tree. (From Markham, CR. Peruvian Bark: The Introduction of Chinchona Cultivation into British India. London: John Murray, 1860. Reproduced with the permission of the Wellcome Institute Library, London, UK.)

began to search for the cinchona species with the highest quinine content. Charles Ledger and his faithful Bolivian servant, Manuel Incra Mamani, found a variety of cinchona with a high quinine content (*Cinchona ledgeriana*); after the British rejected Ledger's offer, he sold some seeds to the Dutch government for a few guilders in 1865

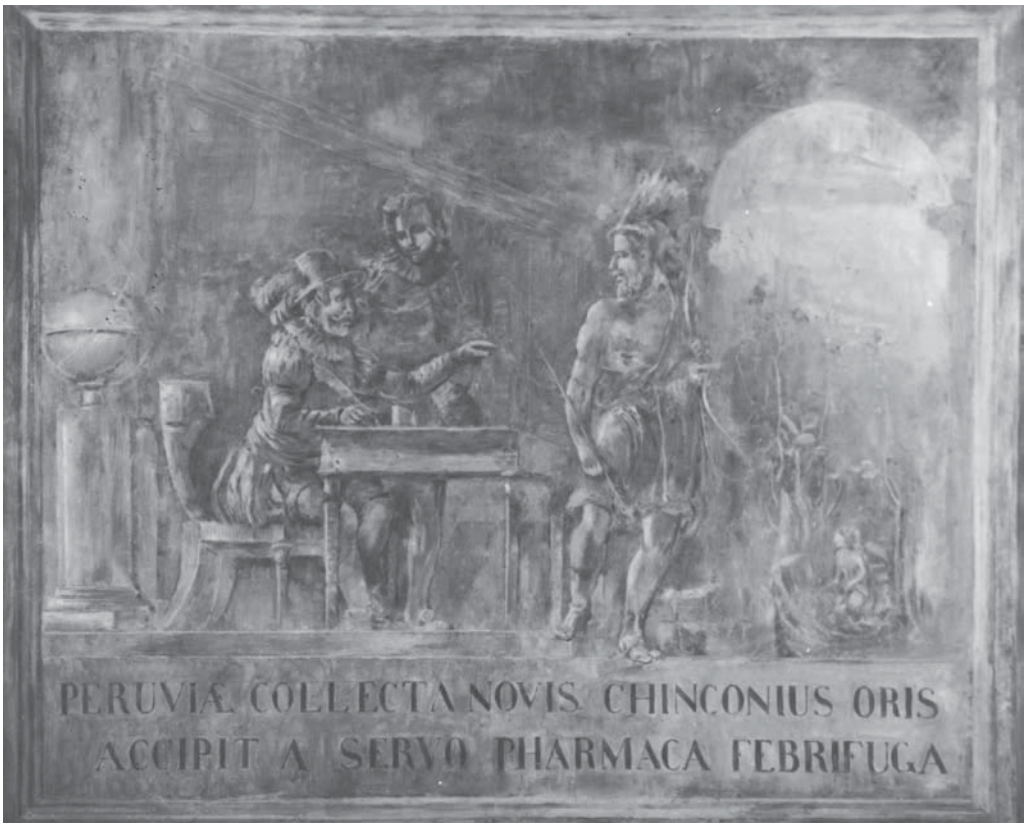

Fig. 2. The Count of Chinchon receives cinchona. (From a fresco in the Ospedale di Santo Spirito in Rome. Reproduced with the permission of the Wellcome Institute Library, London, UK.)

(9,12). These seeds were one of the best investments in history. Within a short time, the Dutch plantations of Java were producing 97% of the world's supply of quinine and had a virtual monopoly, producing in the 1930s about 10 million kilograms of bark a year (Fig. 3). From the mid-19th century to the 1940s, quinine became the standard therapy for intermittent fever throughout the world.

Prior to the isolation of quinine, the bark was usually administered as a suspension in wine or spirits to counteract its bitterness. This recipe may have evolved into the gin and tonic, a daily staple of British colonialists throughout the world *(13)* (Fig. 4). Tonic water today only contains 15 mg of quinine per liter *(14)*, so the drink has little antimalarial benefit.

SYNTHETIC ANTIMALARIALS

The science of synthetic organic chemistry underwent a revolution in the late 19th century, partly in response to the need for new antimalarials. In 1856, William Henry Perkins, an 18-yr-old English chemist, set out to synthesize quinine, but failed. (Indeed, the synthesis of quinine was not accomplished until 1944 and, even to this day, has not been achieved on a commercially economic scale.) However, Perkins succeeded in

Fig. 3. Photograph of a warehouse in Amsterdam, cases filled with cinchona bark. (Reproduced with the permission of the Wellcome Institute Library, London, UK.)

synthesizing "mauve," the first synthetic textile dye that did not wash off in water. This advance sparked the development of a huge German synthetic dye industry *(6)*.

The new dye industry helped promote the advancement of medicine. When microbial pathogens were first identified, they were difficult to see under the microscope. Newly synthesized dyes were then used by microbiologists as stains to enhance visualization and classification. Paul Ehrlich, a German scientist, noticed that methylene blue was particularly effective in staining malaria parasites. He reasoned that because the parasite avidly took up the dye, it might be poisoned by it in vivo. In 1891, Ehrlich cured two patients of malaria using methylene blue, the first time a synthetic drug was ever used in humans *(15)*.

Bayer, one of the leading German dye companies, soon became a leading pharmaceutical company. A team of chemists and biologists was assembled by Bayer to develop new synthetic antimalarials using methylene blue as a prototype. In 1925, they developed plasmoquine (also called pamaquine). Plasmoquine, the first 8-aminoquinoline, proved to be the first compound capable of preventing relapses in vivax malaria. In 1932, they developed mepacrine (atebrine) which was effective against falciparum malaria.

In 1934, H. Andersag, working at the Elberfield labs of Bayer IG Farbenindustrie AG developed a compound known as resochin *(16,17)*. Although the compound looked promising, it was felt to be too toxic. In 1936, Andersag synthesized a derivative of resochin known as sontochin, which seemed to be less toxic.

TAKE an ounce of the beſt jeſuits bark, Virginian ſnake-root, and orange-peel, of each half an ounce; bruiſe them all together, and infuſe for five or ſix days in a bottle of brandy, Holland gin, or any good ſpirit; afterwards pour off the clear liquor, and take a wine-glaſs of it twice or thrice a-day. This indeed is recommending a dram; but the bitter ingredients in a great meaſure take off the ill effects of the ſpirit.

M 4 Thoſe

Fig. 4. An early recipe for the gin and tonic. (From Buchan W. Domestic Medicine: or, a Treatise on the Prevention and Cure of Diseases by Regimen and Simple Medicines. London: W. Strachan & T. Cadell, 1781. Reproduced with the permission of the Wellcome Institute Library, London, UK.)

During World War II, the world supply of quinine was cut off as the Japanese took over Java. Plasmoquine and mepacrine (atebrine) were manufactured and widely used by both sides. As part of the war effort, American, British, and Australian scientists cooperated in a large-scale attempt to develop new synthetic antimalarials. Sixteen thousand compounds were synthesized and tested. Surprisingly, Allied scientists had been informed about resochin and it was one of the first tested compounds. It had the acquisition number SN-183. For the second time, it was considered too toxic and dropped. Meanwhile, French Vichy physicians were carrying out clinical trials on sontochin in Tunis. After the allies captured North Africa, they obtained samples of sontochin and data from the study. Interest was rekindled in resochin, which was renamed chloroquine (and renumbered SN-7618). By 1946, US clinical trials showed that this compound was far superior to atebrine *(16,17)*. The eventual recognition of chloroquine as a powerful antimalarial is one of the most fascinating stories in the history and development of synthetic drugs. As Coatney has commented, "the main story of chloroquine, 1934 to 1946, involves investigators of six countries on five continents and embraces its initial discovery, rejection, re-discovery, evaluation and acceptance" *(17)*.

Chloroquine proved to be the most effective and important antimalarial ever and was used widely throughout the world. In the 1950s, Mario Pinotti in Brazil introduced the strategy of putting chloroquine into common cooking salt as a way of distributing the drug as a prophylactic on a wide scale. This medicated salt program (using either chloroquine or pyrimethamine) became known as "Pinotti's method" and was employed in South America as well as parts of Africa and Asia. Chloroquine was the main drug of choice in the WHO Global Eradication Programme of the 1950s and 1960s, and although somewhat overshadowed by the widespread use of the residual insecticide DDT, chemoprophylaxis with chloroquine tablets or chloroquine-medicated salt was an important supplementary component of eradication and control programs

in many areas of the world. Its use was only curtailed beginning in the 1960s with the advent of chloroquine resistance in *Plasmodium falciparum* (which may have been caused, in part, by the medicated salt program). Chloroquine resistance has now spread to many of the areas of the world where the infection is endemic *(18)*.

Chloroquine was one of many antimalarials resulting from scientific advances made during World War II. The American effort also included attempts to make more effective versions of the 8-aminoquinoline plasmaquine. Soon after the war, primaquine was introduced, and proved to be the standard drug for the prevention of relapses in vivax malaria *(16)*. Interestingly, 50 yr later, American military scientists have found yet another promising 8-aminoquinoline, WR 238605 or tafenoquine *(19)*.

The British war effort led to the development of proguanil (Paludrine). After the war, proguanil served as a prototype for the development of pyrimethamine (Daraprim) in 1950 by Burroughs–Wellcome *(16)*. Pyrimethamine, in combination with sulfadoxine, was introduced in the 1970s and named Fansidar *(20)*. Fansidar is still in wide use, particularly in Africa.

Several compounds discovered during the American war effort later served as prototypes for the development of other antimalarials. One such compound, SN 10275, was a prototype for mefloquine, which was introduced in the mid-1970s *(21)*. Mefloquine (Lariam) is widely used throughout the world. Another class of compound developed during World War II were the 2-hydroxynaphthoquinones. These served as prototypes for a drug that was introduced only recently—atovaquone. Atovaquone is now being manufactured by Glaxo-Wellcome Pharmaceuticals in combination with proguanil and sold as Malarone *(22)*.

ARTEMISININ

Artemisia annua—sweet wormwood or qinghao (pronounced "ching-how")—was used by Chinese herbal medicine practitioners for at least 2000 yr, initially to treat hemorrhoids. In 1596, Li Shizhen, a famous herbalist, recommended this herb for fever, and specified that the extract be prepared in cold water *(23)*.

In 1967, the government of the People's Republic of China established a program to screen traditional remedies for drug activities *(24)* in an effort to professionalize traditional medicine. Qinghao was tested in this program and found to have potent antimalarial activity. In 1972, the active ingredient was purified and named qinghaosu (essence of qinghao). Qinghaosu and derivatives were then tested on thousands of patients. Summaries of these studies were published in the late 1970s and early 1980s *(25,26)*. Artemisinin derivatives are now widely used in Southeast Asia and are starting to be used elsewhere (reviewed in ref. *27*).

ANTIMALARIALS AND THEIR IMPACT ON HISTORY

Quinine had a profound influence on modern colonial history and a number of historians have highlighted the importance of this single drug as one of the "tools of empire" *(28)*. Falciparum malaria was a major problem for missionaries, explorers, colonists, and the military in many parts of the tropical and subtropical world. As Europeans began to settle the coasts, penetrate the interiors, and colonize the lands of Africa and Asia, they were frequently struck down with tropical diseases, including malaria.

Malaria in West Africa, a region often typified as "White Man's Grave," was especially severe. For example, almost half of the British soldiers stationed in Sierra Leone between 1817 and 1836 died of infectious disease, mostly malaria *(28)*. The introduction of quinine, however, contributed to a marked reduction of colonial military mortality in certain areas from the mid-19th century *(29)*. Its use was encouraged by some, although not all, doctors as imperative for survival in the tropics. In Alexander Bryson's text of 1847, *Report on the Climate and Principal Diseases of the African Station*, he recommended and noted the importance of quinine as a prophylaxis amongst Europeans:

> So general has the use of quinine now become, that there is hardly any part of Western Africa, where there are resident Europeans, in whose houses it is not to be found; it is in fact considered to be one of the necessaries of life, where life is of all things the most uncertain.

Later, when malaria and other tropical diseases were shown to be the result of infectious agents rather than an inherently bad climate, the use of quinine as a prophylactic and effective treatment for malaria was advocated in the scientific literature, advice manuals, and travel guides for Europeans venturing into the tropics. Although death rates in the early 20th century remained high among Europeans in malarial areas, the use of quinine, as well as mosquito control, bed-nets, screening, and other forms of prevention and protection, helped alleviate the misery caused by malaria. Indigenous populations were often viewed as "reservoirs" of infection and it was suggested by a number of leading malariologists in the early twentieth century that the "immune" adult did not suffer from the debilitating effects of malaria. However, as Europeans increasingly relied on local and imported labor forces to work on European plantations, estates, and mines, it was soon recognized that malaria was a problem for indigenous as well as colonial populations. Gradually, the use of quinine was recommended more widely for humanitarian as well as economic reasons and demands for the drug increased in the first half of the twentieth century.

Concerns about the correct dosage, the cost, and the side effects of long-term prophylaxis with quinine, and especially its possible connection with blackwater fever, however, gave rise to scientific debates concerning its use. Moreover, for many of the poorest rural populations of the world, the drug was not readily available. In 1925, the Malaria Commission of the Health Committee of the League of Nations estimated that no less than 26,441,000 kg of quinine would be required annually in order to provide a therapeutic dose of 2.6 g to every malaria case in the world. The actual consumption remained considerably less, reaching a figure of only 610,000 kg in the 1930s *(30)*.

It has been particularly during the military campaigns and the major wars of the past 150 yr that quinine and, later, synthetic antimalarials have been employed most rigorously and on a wide scale (Fig. 5). Quinine played an important role in American military history. The American Civil War might have ended in its first year if malaria had not ravaged General McClellan's troops invading Virginia *(31)*. Although Union troops vastly outnumbered Confederate troops, "Chickahominy fever," a combination of malaria and typhoid, made Union troops unable to fight. During the war, the Union army used over 25,000 kg of quinine or other cinchona products *(9)*.

Antimalarials also played a crucial role in World War II, especially in the Southwest Pacific. In many cases, malaria posed a far greater health risk than battlefield injuries

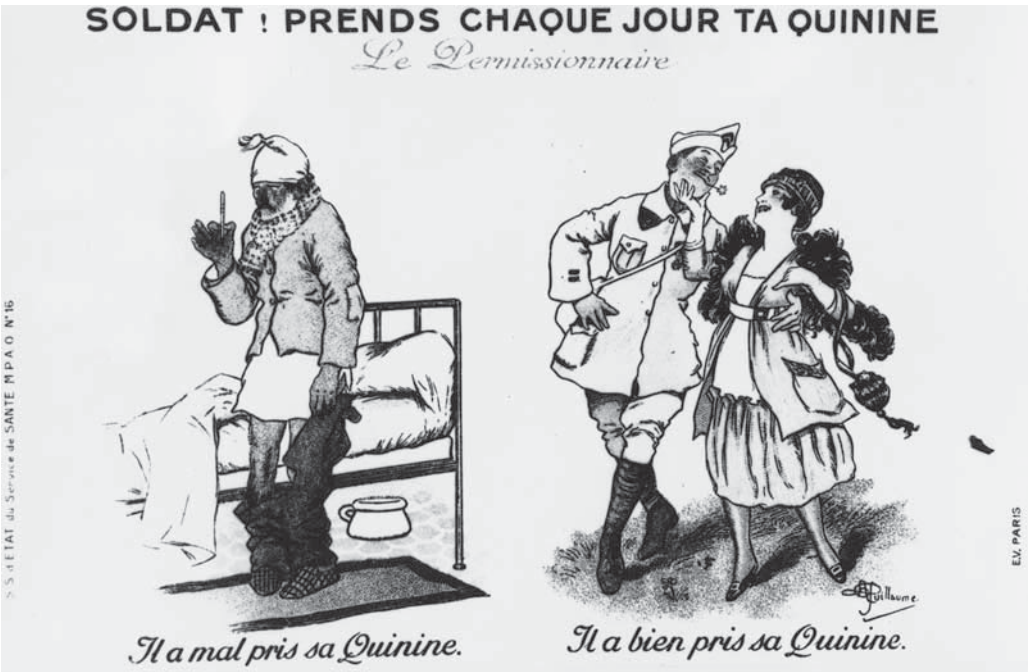

Fig. 5. The results of taking quinine contrasted with the results of not taking it. (Postcard, Paris circa 1914. Reproduced with the permission of the Wellcome Institute Library, London, UK.)

(32). Daily prophylaxis with atebrine was required for all Allied troops, even though it turned the skin yellow and was reputed to cause impotence. This drug helped protect the health of the Allied troops fighting in some of the most malarious areas of the world and, as Bruce-Chwatt has said, "there is no exaggeration in saying that this probably changed the course of modern history" *(33)*. Interestingly, Japanese troops fighting in this area also used atebrine, but at an inadequate dose. This may have contributed to the development of atebrine resistance in New Guinea *(32)*.

During the Vietnam War, malaria was the single greatest cause of casualties even though all troops received prophylaxis with chloroquine and primaquine *(21)*. An estimated 390,000 sick days were lost to malaria among the American forces and the emergence of strains of falciparum that were resistant to available antimalarial drugs caused considerable concern and a renewed interest in the search for new antimalarial agents at the Walter Reed Army Institute of Research in Washington *(16)*.

The American military maintained a strong program in antimalarial drug development through the early 1990s. They synthesized and tested over 250,000 compounds *(21)*. Several drug companies such as Wellcome and Roche also maintain active programs. However, the economics of drug development have changed dramatically in recent years as have methods of warfare. As a result, the American military's antimalarial development program has been cut, and many drug companies have stopped attempting to develop new antimalarials. As fears of drug resistance are becoming more pervasive, it is essential that new drugs or combinations of older drugs be developed for the future.

CONCLUSION

As the development costs of pharmaceuticals have escalated, the Western pharmaceutical industry has lost interest in antimalarial development. Once resistance to artemisinin and Malarone develop, there may be no new antimalarials ready to take their places.

In the last few years there has been a renewed concern for malaria as a global problem with programs such as WHO's Roll Back Malaria and the Multilateral Initiative on Malaria. History tells us that many of our past breakthroughs in malaria control were driven by the needs of the military and of the colonial powers. Can malaria control in the tropical and subtropical parts of the world advance in the absence of war or colonialism?

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