

## CHAPTER 2

**Monkeys and Malaria**

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Malaria is one of the world's most devastating diseases. Its intermittent fevers have been recognized since the beginning of recorded history. Because the parasites which cause malaria are very small it was not possible to detect these pathogens until after the invention of the microscope. In fact the first description of the parasite was just over 90 years ago (Laveran, 1880). Four species of malaria in man are recognized now, the first three being described during the late 1800's and the fourth and last in 1922, viz., *Plasmodium vivax*, *P. falciparum*, *P. malariae*, and *P. ovale*.

About five years after the finding of the human malaria parasites, birds were found also to be infected with malaria. But it was not until 1898 that malaria was seen in monkeys (Koch, 1898; Kossel, 1899). It seems odd that bird malarias were found before the monkey malarias and that there was a lapse of 18 years from the discovery of the human parasite until they were found in these lower primates, animals which are recognized to have many similarities to man.

One of the earliest known attempts to transmit human malaria from man to nonhuman primates was by Koch (1900). He injected malarious blood apparently containing *P. vivax* and *P. falciparum* into orangutans and gibbons. The negative results led that famous scientist to conclude that manlike apes are not susceptible to human malaria, but that it should not be taken for granted that other animals farther from man could not harbor the human malaria parasites. Although we now know that he was partially wrong in his conclusion that human malaria will not grow

in the apes, he was correct in the prediction that they might grow in the lower primates farther removed from man. Chimpanzees and gibbons have been shown experimentally to be receptive to the human malarial, if splenectomized. At least six of the monkeys of the Western Hemisphere can be infected with one or more of the human malaria species and in some cases the infections can go back and forth between humans and non-human primates by inoculation with infected blood or with sporozoites from the mosquito (Young, 1970).

Benefits from the study of simian malarial can fall into two categories, i.e. (1) the immediately practical when used for the treatment of diseases or as a testing model for the development of new drugs; (2) the production of knowledge which relates to the disease condition in man and other primates.

The first and most dramatic practical benefit was the use of simian malaria for the therapy of central nervous system syphilis in man. Wagner von Juaregg (1922), a psychiatrist in Vienna, noted that, although neurosyphilitic patients usually died within a few years after the diagnosis was made, some of those with fevers lived considerably longer, and, in fact, some recovered. After experimenting with various pathogens causing fevers, he determined that infections with human malaria could be indeed a treatment for neurosyphilis, a late stage of syphilis in man which causes insanity. This was the first and, for many years, the only known specific treatment for a mental disease. After this became recognized, the use of the therapeutic malaria became widely established. *Plasmodium vivax* was the most widely used species, but as it would not infect negroes well, *P. malariae* was used for members of this race.

In 1932, Knowles and Das Gupta in India found that *P. knowlesi* from the macaque monkey could infect man. This monkey malaria then was used in several countries for the treatment of neurosyphilis. It appeared to have certain advantages over the human malarial as it produced a shorter and milder course of infection, and apparently there was less danger of its being transmitted by the local anopheline mosquitoes. However, some of these same characteristics were considered by some to be disadvantages. Often the course of the infection was not long enough to produce the 10 to 20 paroxysmal bouts considered optimum for the best results, the parasitemias often were low, and it showed some of the lower infectivity to Negroes that characterizes human *P. vivax* also.

Another practical application of the study of simian malarial is their use in nonhuman primate hosts for the testing of candidate compounds for antimalarial activity. This has been widely used during the past 25 years and has been useful in detecting compounds that are effective in humans against human malaria. For these studies much use has been made

of the macaque monkeys usually infected with *P. cynomolgi* or other malarias similar to *P. vivax* of man.

The other class of important benefits is the scientific knowledge resulting from the study of simian malarias in simians. The early facts concerning the biology of malaria tended to result from the study of the disease in man. After the discovery of the parasite in the red blood cells, it was determined that there were two stages of these pathogens, one causing the fever (the asexual forms), and the other forms (the sexual forms or gametocytes) harmless to man but producing infection in mosquitoes. It was then found that these mosquito forms produce a third type of parasite, the sporozoites, which, upon injection into man, would cause the infection. As there was a lag between the injection of the sporozoites into man and the appearance of parasites in the red blood cells, it became obvious that there had to be still other types of parasites living in other tissues.

In 1948, it was dramatically shown that this hidden cycle was in the parenchymal cells of the liver. Shortt and Garnham (1948) using *P. cynomolgi* in the rhesus monkey demonstrated such a developmental stage in the liver. *Plasmodium cynomolgi* closely resembles *P. vivax* of man. This led to experiments with the human *P. vivax* and it was found that this parasite grows in the liver cells of man just as does the *P. cynomolgi* in the monkeys. Subsequently, the liver stages for all of the four species of human malaria were discovered. These hidden cycles in the liver gave the first logical explanation for the missing stages between the bite of the infected mosquito and the appearance of the bloodstream parasites. It was shown further that these liver stages could persist for months in some cases, and years in others, after the first primary attack. An exacerbation of these forms released into the bloodstream would reinvade the red blood cells producing repeated separate attacks known as relapses.

Thus a great deal of knowledge was produced by the study of human malaria in humans, monkey malaria in humans, and monkey malarias in monkeys. As a result many chemical compounds were tested for anti-malarial activity, toxicity, pharmacologic activity, and other biologic parameters. Out of these investigations came better malarial drugs, some with remarkable advantages in the treatment of malaria. Coupled with this progress, was even more definitive progress in the control of malaria-bearing mosquitoes by insecticides in preventing the mosquito transmission of malaria. The demonstrated usefulness of the latter, together with the complementary benefits of drugs which reduced the supply of malaria parasites available to the mosquitoes led to the concept in 1955 that malaria eradication was a feasible concept and should be undertaken as an international, even global, goal. Much progress was made in eradicating

malaria in some areas and greatly reducing its prevalence in others. Millions of people were spared the malignant effects of malaria and there was a great improvement of the health and of the economy in many of the malarious areas of the world.

However, adverse conditions began to appear. Most serious was the appearance of resistance to the insecticides by the malaria vectors. This was then followed by the appearance of resistance of the malaria parasites to practically all of the recently developed drugs, which had had such good characteristics except for this weakness. However, some of the 4-aminoquinoline compounds were still excellent drugs for the treatment of clinical malaria and some of the 8-aminoquinolines would attack the liver stages of the parasite and thus reduce relapses. Confidence in these two types of compounds masked the difficulties of resistance appearing in the other drugs.

This confidence was greatly shaken when, in 1961 (Moore and Lanier, 1961; Young and Moore, 1961), it was shown that the worst of the malaria parasites, *P. falciparum*, could in some cases become resistant to the 4-aminoquinoline drugs which had for many years been the best agents against clinical malaria. Although the areas of resistance to these drugs were circumscribed, one being in upper South America and the other in Southeast Asia, nevertheless the presence of malaria-resistant parasites in the latter area was of major concern because of the war existing there and the large numbers of troops exposed to and contracting *P. falciparum* malaria, which often was resistant to many standard drugs.

Renewed investigations began to find better drugs for the prevention, treatment, and cure of the disease, hopefully for drugs which would not have the weakness of failing to cure all strains and types of malaria because of resistance. For the necessary testing of thousands of compounds to find better therapeutic agents, a large-scale program was put into effect using various models, including avian malaria in chicks, rat malaria in rodents, monkey malaria in nonhuman primates, and finally malaria in man. Although the lower test systems were useful, the final testing of the compounds had to be done in man, or hopefully, if possible, in nonhuman primates. Large monkeys were used extensively, especially the rhesus, but these were costly in acquisition and husbandry. The need for a better model was indicated. One greatly desired model was a small monkey in which human malaria could be grown.

Taliaferro and Taliaferro (1934) had tried to grow human malaria in Panama monkeys. Although the parasites of *P. falciparum* would persist for a few days, the shortness of the parasitemia and the failure to maintain the infections by serial transfers made this model impractical. Surprisingly few attempts had been made previous to this time or even after

this time to find a small monkey in which human malaria would grow. In 1965, workers at the Gorgas Memorial Laboratory in Panama renewed the attempts to grow human malaria in monkeys. Within one year it was shown that human *P. vivax* malaria could be grown in the small *Aotus* (night or owl) monkey, would infect mosquitoes, could be passed back to man by mosquito bites, and could be successfully maintained in these monkeys by serial passage by the injection of infected blood (Young *et al.*, 1966). This led to the use of this model by other investigators and as a result much valuable information is being produced in many diverse lines.

Further testing by the scientists at the Gorgas Memorial Laboratory showed that of the seven species of monkeys in Panama, six of them could be infected with one or more of the different species of human malaria.

Of immediate and urgent use of this human malaria-monkey host model is the possible employment for the final or almost final testing of chemical compounds for their use as antimalarial drugs. Hopefully it may be possible to substitute this system partially for the use of human volunteers. The use of human volunteers is restricted in scope, probably becoming more so, and is very expensive. Obviously their use does not have the experimental capabilities of a laboratory animal model.

However, before these monkey models can be used to their fullest capacity, a great deal of information must be produced. We need to know much more about the biology of the human malaria in the monkeys, especially on the points of important similarities and dissimilarities to the disease of human malaria in humans. Mosquito transmission of the disease resulting in the liver stages is a necessity to determine the effects of the drugs upon the persistent parasite stages in the liver which cause relapses. Therefore, the ability of various mosquitoes to become infected with and to transmit these human malaras between monkeys must be learned. This should lead to the development of techniques for the mass production of infected mosquitoes for this experimental work.

In addition to the obvious benefits of using this model for drug-testing, other information not readily available by the study of malaria induced in human volunteers can be produced. These would include the examination of internal tissue to follow the progress of the disease, experimental procedures which would attempt to change the response of the host to the disease, such as splenectomy, removal of the thymus, the use of immunosuppressant drugs, the effects of stress, and other procedures difficult or impossible to do in man. Such results could create knowledge in many lines such as immunology, pathogenesis, hematology, biology of the parasite, etc.

Other necessary research areas have to do with the potential insect vectors. There is only one known natural vector of monkey malaria at

present in the Western Hemisphere. To test mosquitoes adequately they must be colonized. This procedure itself is difficult and laborious. Only a few of the known mosquitoes of the world have been colonized. It is likely that vectors of monkey malaria in nature would be better vectors of human malaria in monkeys than are the presently known vectors of human malaria to humans. However, only one or two proven vectors of monkey malaria in nature are known, much less colonized. Once colonized, additional studies of human malaria in monkey hosts can be done.

The study of monkey malaria in monkeys has produced helpful information on the epidemiology of human malaria. Anopheline mosquitoes are the accepted vectors of mammalian malarias. In areas where human malaria is present it has been assumed that the naturally infected anopheline mosquitoes were the vectors. In areas where humans and monkeys live close together little is known about the vectors of the monkey malarias. Recently, workers in Malaysia (Warren and Wharton, 1963) have found that some of the mosquitoes thought to be vectors of human malarias were actually transmitters of monkey malarias and probably were not responsible for the human malarias as had been supposed previously. Such knowledge is helpful in developing control measures against human malaria.

Also important is the question of whether monkey malarias can be a zoonosis, i.e., will they infect man in nature? If so, what would be the impact on malaria eradication programs and would the monkeys serve as a reservoir of human malaria and a focus for reinfection after the human malaria was eradicated. It has been difficult to answer these questions because so few vectors of monkey malarias are known nor has the potential of the infection going from monkey to man been assessed adequately.

In view of our present knowledge, it seems improbable that the transmission of monkey malaria to man would occur very often, if at all, under ordinary conditions. But again our knowledge is so scant that more information needs to be obtained before the question can be answered satisfactorily. And in obtaining the answers to these questions, information would be obtained on the various mosquito vectors which would have immediate application to the epidemiology of human malaria.

Some scientists now believe that some of the malarias of man and monkey are the same species, viz., *P. malariae* of man is the same as *P. brasilianum* of the Western Hemisphere monkeys. Obviously this is an important point to elucidate.

There are many important problems which can, should, and probably will be investigated in this new monkey host-human parasite model. However, the major difficulties now have to do with the supply of the experimental animals, both quantitatively and qualitatively.

Because of the growing use of small monkeys for biomedical investigations, many thousands are taken from the jungles for export to research centers. Fears are being raised in some quarters that this will lead to a depletion and perhaps disappearance of certain species. However, the use of monkeys for biomedical research does not seem to be the most important factor, if indeed it is a factor, in the possible depletion of a species in the Western Hemisphere. It seems apparent that the numbers of monkeys from Central America are decreasing but this began long before there was any demand for these species for research. One cause for the decrease appears to be due to the destruction of the forests in which the monkeys live. Also, epizootics of disease, such as yellow fever, at times has almost wiped out populations of certain monkeys, such as the howler (*Alouatta*). The possibility that other epizootic diseases can create similar devastating effects indicates an area of needed investigations.

The second, and very important factor, in considering the supply of monkeys is the quality of the research animal. When captured in the jungle, these animals have been exposed to an unknown, but obviously very large, number of infectious agents and trauma. This would result in a varied and equally unknown immunologic profile. It is logical that some of this feral preconditioning may affect adversely the tests undertaken with these specimens in the laboratory.

There is inadequate knowledge at present for the breeding of these animals under controlled conditions. Of the Western Hemisphere monkeys, only a few can be bred in captivity. For some of much importance at present, no breeding is possible. In fact, so little is known of the husbandry that it is difficult to maintain some of the animals in captivity for very long. The lack of husbandry knowledge and of ability to breed in captivity indicate areas in which tremendous effort is required.

With the recent burst of information showing that these small monkeys are good models for the study of human diseases, it appears patent that there is at present a need, and that the need will rapidly increase in the future, for the establishment of breeding colonies to supply some of the research needs. In addition to leading to a higher quality of experimental subjects, this would perhaps aid in the preservation of the species, should the numbers taken for biomedical research show to have any importance in this area.

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