EXPERIENTIA

Volume 40/No. 12 Pages 1305–1445 15 December 1984

The present state of malaria research

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The present state of malaria research: an historical survey

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Key words. Malaria; historical survey; parasite life history; pathology; immunity; malaria culture; experimental hosts; zoonoses.

Introduction

This multi-author review examines several aspects of malaria in which active research is being pursued today. We are still at a transitional stage in our knowledge of the subject; although the causative organism with its life cycle, the vector and its bionomics, the treatment of the disease and its parasitology, and methods for its eradication have all been discovered during the course of this century, malaria nevertheless remains a serious problem both for scientific and social reasons.

The introduction represents an attempt to describe briefly the major steps in the foregoing discoveries and some of the problems which remain. The subject of the review is *malaria research* and it is unnecessary to go back to the classical days when the Greek and Roman physicians provided the basis of the subject in describing the main clinical features of the disease.

Bruce-Chwatt and de Zulueta[9,67] have speculated on how malaria arose and spread in some parts of the world in eras long past, and in the possible relation to species of parasites and their vectors; they noted in particular its likely occurrence and decline in respect to the Ice Ages. The most recent glacial period is dated back to about 25,000 to 10,000 years ago, when the ambient temperature in Southern Europe would have been below 16 °C (the minimum necessary for sporogony of *Plasmodium vivax*) or 18 °C (minimum for *P.falciparum*). These figure refer to the temperature of the external conditions and can be a fallacious index in places where the mosquito vector spends most of its life inside human habitations and where the temperature may be much higher because of domestic fires. Such information can help to indicate what may have happened in the past and what may occur in the future.

Malaria research was carried out in the embryonic 'pre-Laveran period' and, on an entirely different scale, in the 'post-Laveran period' of the last one hundred years.

Pre-Laveran period (1650–1880)

Probably the most important discovery in this period was the demonstration early in the 17th century of the value of cinchona bark for the treatment of the disease, culminating in the investigations of Torti[60] who laid down the regimen for the treatment and cure of severe malignant malaria with preparations of the drug. About the same time, the Roman physician, Lancisi[41] described in some detail the epidemiology of 'Roman fevers' and emphasized their association with swamps. He noted that gnats (culicini) bred in the swamps and that when these insects bit man, they deposited animalculae which multiplied and provoked the fever. This speculation was nearer the truth than other theories of transmission which were prevalent in the 18th century. These included the thesis of Linnaeus[24] which he presented in 1735 for the degree of Doctor of Medicine to the University of Harderwijk. He advanced the hypothesis that the ingestion of particles of clay in drinking water led to

their lodgement in the smallest vessels of the body and thus produced the disease. Linnaeus' evidence was entirely circumstantial and in spite of his renown, the theory was soon abandoned.

Linnaeus reported the presence of black pigment in the brain and spleen, but he did not associate it definitely with malaria, nor did Schutz who in 1846 found similar pigment in the organs of patients who had died of fever. In 1847, Meckel observed pigment granules lying in 'protoplasmic masses' in the blood and spleen of patients suffering from the disease. Afanasiev made similar observations and suggested that these bodies might be the causative agents of the disease.

Of paramount importance was the isolation by Pelletier and Caventou[50] in 1820 of two alkaloids, quinine and cinchonine, from the bark of the tree, the former proving to be the more potent.

Post-Laveran period (1880–1934)

The year 1880 marks the beginning of modern malaria research when Alphonse Laveran[42] discovered the causative agent in the blood of soldiers in Algeria. He followed the clue presented by the appearance of black pigment (melanin) in the blood. When he was confronted by the phenomenon of exflagellation of parasites in fresh blood he immediately realized that a living organism lay beneath his eyes, and with simple stains he was able to demonstrate the morphology. For many years he thought that a single species was concerned, though shortly after his discovery, Golgi[28] in 1889 produced clear evidence of the existence of multiple species of malaria parasites by defining the characteristic periodicity of the fever in relation to the rupture of schizonts in the blood. Moreover, he clarified the phenomena of double tertian fever in *P. vivax* where two generations of parasites are present and of triple quartan fever in *P. malariae* infections when three generations occur. The relevance of this work to general studies in chronobiology is still not appreciated. Golgi thus differentiated 3 species while in 1922 a fourth human species *(P. ovale)* was recognized. The names have undergone bewildering changes, and not until 1954 did they receive official validation as *Plasmodium vivax, P. malariae* and *P. falciparum*. New techniques, especially biochemical taxonomy, have revealed the heterogeneity of these species, leading first to the erection of subspecies and possibly eventually to lower designations (e.g. demes, races, strains).

Very soon after Laveran's discovery, malaria parasites were found also in birds, lizards, monkeys and, to a lesser extent, in a variety of mammals. These species have provided wide scope for experimental work and have been essential for the solution of fundamental problems. Amongst many examples, the most important models included *P. gallinaceum* (isolated by E. Brumpt[10] from chickens in 1935), *P. cynomolgi* and *P. knowlesi* (from strains established at the Malaria Institute of India by Sinton and Mulligan) and *P. berghei* discovered by Vincke and Lips[62] in mosquitoes and wild rodents in Zaire forests in 1948 (see Killick-Kendrick and Peters[38]). The range of species is so large that it extends from lizards (in which the life history of the parasites is incompletely known) to the higher apes (which are too rare to permit of any extensive use). It cannot be too strongly emphasized that no animal malarias can form an exact replica of human malaria and fallacious comparisons have to be carefully avoided. Even the presence of the same parasite *(P. malariae)* in both chimpanzee and man, or the zoonotic infections of *P. cynomolgi* or *P. knowlesi* in man and monkey, does not guarantee that the behavior of the parasites in the different hosts will be identical.

The study of the hematozoa was greatly hindered by the lack of differential staining, but in 1891 Romanovsky[52] made the accidental discovery that moldy solutions of methylene blue plus eosin produced a sharp contrast in the staining of the nucleus and cytoplasmic components of the parasites; this was found to be due to the production of azures. It was not until many years later that Saal[54] in 1964 in Australia clarified the nature of the 'polychrome' stain, now in everyday use.

The next major discovery was that of MacCallum[43] in 1897, who showed that exflagellation was a sexual phenomenon involving the production of 'four' (much later shown to be eight) motile male gametes and static female gametes; he described fertilization, and metamorphosis of the zygote into the 'travelling vermicule' (ookinete). The observations were originally made on *Haemoproteus* of crows, and later identical processes were found in human malaria parasites.

In the same year (1897) and in the next, Ross[53] (inspired by Manson) made the all-important discovery that bird malaria was transmitted by the bite of culicine mosquitoes, with the final production of sporozoites in the salivary glands. He successfully transmitted the infection by mosquito bite to clean birds. Ross had also demonstrated the earlier stages of sporogony in *P. falciparum* infection of man. The Italians (Grassi et al.[29]) in 1898 elucidated the mosquito *(Anopheles)* cycle of *P. vivax* and *P. falciparum* and later of *P. malariae* in detail.

Grassi's[30] magnum opus, *Studi di un Zoologo sulla Malaria,* became the first monograph of malariology and covered the aspects of the subject as were known at the beginning of the century. Of greatest interest are his speculations on the development of the sporozoite in the human body; he thought that the structure of its nucleus was incompatible with the prevalent view that the sporozoite underwent immediate growth in the erythrocyte. He also suggested that long term relapses might be due to arrested development (see his Figure 2 in Plate 5 of the 2nd edition 1901), although he did not entirely abandon the widely held theory of parthenogenesis.

The pathology of malaria was investigated by Marchiafava and Bignami[46] in 1894 in the light of Laveran's discovery of the etiological agent of the disease. They had direct access to the mortuaries of Rome and performed many autopsies on people who had died of malaria. They showed that the cycle of *P. falciparum* in the peripheral blood largely ceases after about 24 h and continues instead in the capillaries of the internal organs. The presence of infected corpuscles gradually slows down the circulation, e.g. in the spleen and brain, and blockage occurs as the result of endothelial dam-

age. The vessels are largely blocked by corpuscles containing maturing schizonts.

Malaria research and malaria control have gone hand in hand almost from the start of the post-Laveran phase; control nearly always has a research element because the results are rarely predictable. Today, eradication of the disease in many parts of the tropics has failed and a novel approach (e.g. vaccination) to the problem must be sought. Early workers like Sir Ronald Ross, thought that all that was necessary was the application of the results of this research and to kill mosquitoes. It was quickly realized, however, that a more sophisticated policy was necessary, basically one that directed an attack on the mosquito vectors (or their avoidance by man) or that eliminated the parasite in its human phase (either by cure or prophylaxis). A search for new insecticides and drugs was thus seen to be of paramount importance.

In this brief historical survey, it is impossible to deal with the subject of malaria control and eradication in detail, but successful campaigns of historical importance are those of Gorgas on the Panama Canal and of Malcolm Watson in selected areas of Malaya; unfortunately these efforts were outnumbered by the complete failure of campaigns in West Africa and India. This approach to the situation was revolutionized later in the century by the discovery of powerful synthetic drugs and by residual insecticides (see below).

The research aspects of malaria control are excellently presented by Christophers, Covell and Sinton[13] in Bulletins of the Malaria Survey of India (1928; 1936); these have proved a stimulus to countless students and form a landmark in the history of our subject.

Later developments (1934 to the present)

The discoveries just described left many gaps which would be progressively filled in the course of the present century. Some are briefly described here.

Structure of the parasite

The introduction of electron microscopy revealed a wealth of new organelles which had been almost totally invisible under the light microscope. The method attracted much attention; the motile stages were first studied by workers at the London School of Hygiene and Tropical medicine (Garnham et al.[25] in 1960), the general picture by Aikawa[1] and his associates and by numerous other investigators who eventually used scanning microscopy. The cytological details have been studied by various workers using these methods, and of fundamental importance has been the elucidation of gametogenesis and fertilization[5,47,55].

Life history of the parasite

Grassi had suspected that a third cycle must exist; its details were increasingly clarified in the first half of the century. Aragão[3] in 1908 in Brazil produced the first evidence by finding unpigmented tissue stages of a 'malaria parasite' *(Haemoproteus columbae)* in the endothelial cells of the lungs of pigeons after they had been bitten by *Pseudolynchia* sp. and before parasites had appeared in the blood. Then in 1934 in Italy, Raffaele[51] demonstrated exoerythrocytic schizogony of *Plasmodium elongatum* in reticulo-endothelial and hemopoietic cells in the organs and bonemarrow of goldfinch. In the following years, Clay Huff[34] in the United States described in beautiful detail the complete exoerythrocytic stages of the parasite and of other avian species; he and Coulston[33] employed Maximov techiques in preparations of wing skin after inoculation of sporozoites, in order to locate the exact site of schizogony. In 1937, James and Tate[35] in England demonstrated the profuse development of *P. gallinaceum* in the endothelium of cerebral capillaries, which become completely blocked by the parasites.

In the course of the foregoing years and in the years to come, various claims were made of the discovery of the exoerythrocytic cycle in mammalian malaria parasites; even in 1982, Ph. Decourt[16] stated that he had described the liver stages of such parasites in the reticulo-endothelium as early as 1938. But the supposed stages he (and other workers, including Raffaele, van den Berghe and Missiroli) were entirely unconvincing (see Huff[32] and Bray[6]) and were totally unlike the true cycles[23] shortly to be described in the parenchyma cells of the liver, and in these cells alone.

In 1947, Garnham[22] demonstrated exoerythrocytic schizogony of *Plasmodium kochi* in the parenchyma cells of the liver of East African monkeys and, in the following years, illustrated the early stages and schizonts up to maturity of the parasite, now known as *Hepatocystis kochi* (in which the initial stages are confined to gametocytes as in *Haemoproteus* spp).

The details of the experimental work of Shortt and Garnham[56,57] were greatly influenced by the classical researches of Fairley[19] in 1946 in Cairns, Australia. The latter observer and his colleagues inoculated sporozoites of *P. vivax* and *P. falciparum* into army volunteers and proved that the incubation periods (i.e. before the blood had become invaded) are 8 days and 5½ days respectively. These periods indicated the exact time when biopsies of the liver should be undertaken. Shortt and Garnham then demonstrated the tissue stages of *P. cynomolgi* in monkeys and later (1948 and 1951), those of *P. vivax* and *p. falciparum* in man. In 1955 Garnham et al.[26], described exoerythrocytic stages of *P. ovale* in a volunteer, while in 1960, Bray[7] found the tissue stages of *P. malariae* in chimpanzees.

These observations on tissue stages of primate malaria parasites have been confirmed by many workers, particularly in the United States by Jeffery et al.[36]; Eyles[17] and the Chamblee group comprizing Coatney, Collins, Warren and Contacos[14] in 1971. Characters of the tissue stages have been determined in other species of primate parasites and also of rodents (Yoeli and Most[65]).

The exoerythrocytic cycle of many avian and fewer saurian malaria parasites has continued to be described and has always proved to be strikingly different from that which occurs in mammalian malaria – the features are so distinctive that they form one of the most important criteria of the sub-generic status of the respective parasites. Very little is known about the transmission of the malaria parasites of lizards, although Ayala[4] in 1969

discovered the sporogonic cycle of *P. mexicanum* in sandflies (*Lutzomyia* spp.) and David Young (pers. comm.) the cycle of *P. floridense* also in this insect.

Relapses

Many theories have been advanced on the causes of relapse in human malaria, especially of the long term recurrences (and delayed prepatency) associated with certain strains or subspecies of *P. vivax* (e.g. subspecies *hibernans*). The problem was to demonstrate the latent exoerythrocytic or post-sporozoite stage in the liver. Finally, in a succession of papers, Krotoski et al.[39,40] succeeded in demonstrating 'hypnozoites' in infections of *P. cynomolgi* spp. and *P. vivax*, by the use of the indirect fluorescent antibody technique (see also *Malaria Culture*).

Pathology and immunity

Taliaferro and Mulligan[59] (1937) added greatly to the original work of the Roman authors in their monograph on the histopathology of malaria with special reference to the function and origin of macrophages against the background of immunity in this disease. The subject has continued to develop enormously in the past two decades and is of particular interest in regard to the prospects of vaccination for eradication. Ideas have changed on the origin of what used to be called 'fixed lymphoid-macrophage cells' e.g. the Kupffer cells of the liver, but such cells are now thought by some workers[21] to be derived directly from monocytes of the bonemarrow and to have a 90% turnover in the liver.

Another major advance concerns the biochemical and physiological aspects of pathology, dating from Maegraith's[44] work on pathological processes in malaria and blackwater fever (1948). He showed that the intrinsic changes were the result of 'shock' following anoxemia, malnutrition of the capillary endothelium, interruption of the blood flow and tissue anoxia. This pattern is essentially similar to that of non-specific pyrogenic agents. In the course of the ensuing years, clear evidence[45] was obtained to the effect that active pharmacological substances (kinin complexes with a low molecular weight) were responsible.

The subject of immunity was considered from the earliest days, particularly in the earlier part of this century by Robert Koch, Christophers, Nicolle and Sergent (who substituted the term 'premunition' for the lay expression 'salting' of long term residents in the tropics). Immunoligical research has fluorished in the last twenty years; perhaps the most significant contributions are those of Cohen and MacGregor[15] in 1963 and later) on immunoglobulins in Gambian children and in rhesus monkeys experimentally infected with *P. knowlesi*, and of Nussenzweig and her collaborators[49] in rodent malaria. Antigenic variation in malaria parasites was reported by K. and I. Brown[8] in 1968 but its precise role is still not firmly established. Natural resistance to malaria in man has been shown to be due to various genetic traits, of which the following three examples are the most common and the best substantiated:

1) Hemoglobin S. Possession of the sickle-cell gene confers a strong resistance against *P. falciparum* malaria (Foy et al.[20], 1955).

2) Deficiency in the erythrocyte of glucose-6-phosphate dehydrogenase limits the multiplication of *P. falciparum* and reduces this infection in the community (Allison and Clyde[2]).

3) The absence of the Duffy blood group (Fy. Fy.) inhibits invasion of erythrocytes by merozoites of *P. vivax*, *P. schwetzi* and *P. cynomolgi*, but not those of *P. ovale* (or other species). The pure negroes of West Africa exhibit this character and are totally immune to benign tertian malaria, as was first demonstrated by Miller et al.[48] in 1975.

Biochemistry and isoenzymes

Biochemists in recent years have made detailed studies of the metabolism of malaria parasites, the breakdown of hemoglobin and production of hemozoin, the synthesis of the nucleic acids etc., but no single discovery stands out from any other. Homewood and Neame[31] give a useful summary of the results up to 1980. The isolation of isoenzymes and hybridization of DNA have proved to be of immense importance in the identification of closely related species, e.g. the group of *Vinckeia* present in West African rodents[11,64]. Except for a few analyses of strains *P. falciparum*, little use of these techniques has so far been applied to other species.

Malaria culture

The discovery of an efficient method of cultivating the blood stages of *P. falciparum* by Trager and Jensen[61] in 1976 was an event of major importance and has recently been applied successfully to at least five other species. Apart from providing a useful means of testing new anti-malarial drugs, and for studying the general biology of the parasite, the method offers possibilities for the production on a commercial scale of antigens for a vaccine.

Hawking had earlier (1946) shown that the EE stages of *P. relictum* could be grown in tissue culture. The latest developments are of the greatest importance and demonstrate the growth of sporozoites of *P. falciparum* and *P. vivax* in in vitro cultures of hepatoma cells. Mazier et al.[46a] succeeded in maintaining cultures for 6 or 8 days when viable EE merozoites were produced, while Hollingdale et al.[30a] revealed the presence of 2 populations of inactive hypnozoites and EE schizonts in two SE Asian strains of *P. vivax*, the former persisting unchanged at 5–6 μm up to 15 days when the EE schizonts had disappeared.

Experimental monkey hosts and zoonoses

Malaria research has suffered from the paucity of good animal models. The chimpanzee is too large, too expensive and too rare for general use – therefore the discovery in 1966 by Martin Young[65] of the susceptibility of *Aotus trivirgatus griseimembra* to *P. vivax* was of great importance, and the owl monkey has now been shown to be susceptible to *P. malariae* and to at least 8 strains

of *P. falciparum* (but not yet to *P. ovale*). Successful transmission by mosquito bite both from *Aotus* to *Aotus* and from *Aotus* to man was achieved.
The infectivity of simian species of *Plasmodium* to man was first demonstrated in nature *(P. knowlesi)* by Chin et al.[12] in 1965 and in the laboratory *(P. cynomolgi cynomolgi* and *P. c. bastianellii)* by Eyles, Coatney and Getz[18] in 1960. The facility with which accidental laboratory infections can be acquired, suggests that zoonotic infections should be not infrequent in SE Asia; surveys, however, have produced evidence to the contrary.

Chemotherapy

The article by Peters (in this issue) shows how far we have progressed from the days of Peruvian bark, and yet the demon of resistant strains of *P. falciparum* has necessitated in some cases a return to the use of its active principle – quinine. Perhaps the most interesting new – or rather re-introduced – drug is 'qinghaosu', derived from the Chinese plant, *Artemisia annua* which has been used for the treatment of fever in China for 2000 years (Jing-bo Jiang et al.[37]). The active principle is artemesine (a 'sesquiterpene lactone with a peroxy group') a substance of a totally different structure from that of the other anti-malarial compounds. Its great value is its curative effect on drug-resistant strains of *P. falciparum*. Another species of *Artemisia (A. abrotanum)* occurs in Europe where its common name is 'southernwood', and where it has been in ineffective medicinal use for other purposes for centuries.

The problem of resistance

The emergence of strains of anopheline vectors, resistant to synthetic insecticides, and of malaria parasites (particularly *P. falciparum*) resistant to most drugs have been the most disastrous events in the history of malaria. Two papers here are devoted to this problem which is far from solution. A third paper indicates the difficulty of selecting the best drugs for prophylaxis because of the great regional variation in the ever changing phenomenon of resistance.

Biological control

Early in the present century ingenious methods had been suggested for preventing mosquito vectors from biting people, which entailed either introducing lethal agents (including larvivorous fish) into the breeding places, or deflecting the adult mosquitoes from entering human habitations and feeding instead on cattle etc. Success of these and other methods has been limited, but in recent years, the introduction of pathogenic bacteria, fungi and other living agents into the vector population has given interesting results.

Malaria therapy

It is impossible to conclude even a brief account of the history of malaria research, without mentioning the, now nearly obsolete, use of malaria therapy for patients suffering from general paralysis (GPI). Wagner-Jauregg[63] introduced malaria therapy as a routine method in 1922 and it was widely applied for 50 years. Nearly 10,000 patients were treated at Horton Hospital in England; the cure rate was about 12% and improvement occurred in 20%. The study of these cases[58] and in similar large groups in the United States, USSR, Rumania, Belgium and Holland, provided unique opportunities for fundamental research of which full advantage was taken.

NOTE: Some general references are not included, but may be found in the bibliography of «Malaria parasites and other haemosporidia» by P.C.C. Garnham (1966) or in other large monographs.

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