A Fragment of Malaria History

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My nomination for the Henrique Aragão Medal takes me back to the distant past. About fifty years ago I was interested in the current polemic in the field of malariology – the exoerythrocytic cycle of the malaria parasite. I joined the Instituto Oswaldo Cruz in early 1939 as a research assistant at the Sege (Serviço de Estudo das Grandes Endemias), directed by Evandro Chagas and involved in investigations on endemic diseases – chiefly malaria, Chagas disease, and visceral leishmaniasis (kala azar). My first task was to examine the organs of wild animals from endemic areas of kala azar – recently discovered in Brazil – to verify the hypothesis that they could be primitive reservoirs of Leishmania chagasi.

Research on malaria was conducted chiefly in the Northeast, in highly endemic areas infested with Anopheles gambiae. Frequently colleagues from these areas met at my laboratory to correlate the results of their investigations and discuss problems that were not directly relevant to my work.

In 1940 Evandro Chagas coordinated a course in malariology, in cooperation with the Universidade do Brasil (now Universidade Federal do Rio de Janeiro), to be offered in Belém, state of Pará, at the Instituto de Patologia Experimental do Norte (now the Instituto Evandro Chagas). He entrusted me with teaching the topics “Blood cells” and “Pathology of malaria”.

Updating my knowledge I soon realized that my notion (as well as that of all protozoologists in this country) of the interaction of the plasmodium with the vertebrate host needed some revision. The possibility that another stage existed in the parasite’s development required investigation.

Emile Brumpt’s definition in the 1936 edition of his classical Précis de Parasitologie condensed the current knowledge on the Plasmodiidae:

“This is the best known family of the suborder Haemosporidea. The parasites that compose it are amoeboïd, live in the red blood cells of man and warm- and cold-blooded animals, and produce a black pigment named hemozoin. Their reproduction is by schizogyon and sporogony, as in the Coccids. All their stages of development in the vertebrate are found in the red blood cells”.

In 1900 Battista Grassi, having observed morphological differences between the nuclei of the sporozoite and of the youngest red cell trophozoite, hypothesized that an intermediate stage would occur between the two forms. Three years later, in a memorable paper on Plasmodium vivax, Fritz Schaudinn (1903) described in detail the penetration of the red cell by the sporozoite. In that paper, which for three decades stood as a classic work in malariology, he considered Grassi’s hypothesis to be improbable.

It was not until 1940 that the controversy caused by Schaudinn’s statement on the immediate fate of the sporozoite was brought to a head by several workers. These included James in England, who revived Grassi’s hypothesis, Raffaele in Italy, Kikuth and Mudrow in Germany, Hegner, Wolfson, Manwell and Goldstein in the US. It was at this point, through my lectures on malaria, that I decided to become involved in this subject.

I was finishing my lectures in Belém when an airplane crash killed Evandro Chagas. On returning to Rio de Janeiro, I found the Sege under the direction of Carlos Chagas Filho. I decided to begin working on Plasmodium gallinaceum, a parasite of Gallus gallus, for various reasons: as a source of exoerythrocytic stages recently revealed by James and Tate (1937), because a strain was available in the Institute, and the ease of its maintenance in the chicken and of transmission by Aedes aegypti.

Chicks from eggs incubated in the laboratory were inoculated with that strain in the presence of Dr Henrique Aragão. A colony of A. aegypti was established from eggs supplied by the yellow fever laboratory of the Rockefeller Foundation.

Chicks were bitten by a number of infected mosquitoes concentrated on a small area of skin, or injected subcutaneously with sporozoites from dissected salivary glands. From 6 h after inoculation to 48 h after the appearance of the first parasitized red cells the inoculated spot was excised for microscopic examination of the subcutaneous tissue stained with Giemsa. Numerous stages of the parasite – from uninucleate in the first hours to schizonts in the following days – were present in cells of the subcutaneous tissue, while inoculation of blood in normal chicks did not transmit the infection.

These results were presented at a meeting of the Brazilian Society of Biology, and I was surprised by the unfavorable reaction from prominent parasitologists. Henrique Aragão, the discoverer of the exoerythrocytic stage of Haemoproteus columbae – one of the notable achievements of Manguinhos – refuted my results, arguing that I had used “an old hen” infected by other parasites as do-
nor of material. Arthur Neiva, a pioneer in the taxonomy of triatomines and other groups of insects, and the first researcher to verify the resistance of the malaria parasite to chemotherapy, counselled me to read Schaudinn’s paper, in which there were photomicrographs confirming the penetration of the sporozoite into the erythrocyte. I responded that I was well aware of the title, text, and drawings – not photomicrographs – of Schaudinn’s paper. He alleged that the photos had appeared in a subsequent paper, and I replied that after that paper Schaudinn only published the discovery of *Treponema pallidum* and died in the following year.

This is not the place to comment on the discussion and disagreements that, for more than a year, almost impeded the publication of those results and the continuation of the investigation. Little by little the two great masters, who had their reasons to question my results, began to accept them and finally Aragão, as director of the Institute, gave me the material support that I needed. My first results (Paraense, June 1943) were published during the second world war, when scientific interchange was hampered for obvious reasons. After the war I learned that a similar investigation had been carried out by Reichenow and Mudrow (June 1943) with *Plasmodium praecox* of the canary. Two years later I was gratified to read, in a review by the great protozoologist CM Wenyon (1945): “A number of observers have attempted to trace this development with varying success. Of the earlier workers Reichenow and Mudrow in Germany, and Paraense in South America were the most successful”.

My joy was short-lived. *Science* rejected a paper with results that, in my interpretation, pointed to a new fact in the immunology of malaria, summarized more or less as follows: “One can suppose that the immunity stimulated by the erythrocytic forms does not act against the exo-erythrocytic parasites”. The refusal was based on the argument that it was strange that one and the same parasite could have different immunogenicities. My reasoning was repeated later (Paraense 1946: 187-188) but remained ignored. Nowadays any protozoologist knows that the malaria parasite, and also other parasites, induces the production of specific antibodies against not only each phase of their cycle but also against the successive stages of each phase. Hence the different approaches to the production of antimalarial vaccines, according to the stage of the parasite.

In 1942 a rumor circulated in the Institute that penicillin was being industrially produced in the United States and England, but its export was forbidden and the production techniques were kept secret: the world was involved in the second great war. Aragão, the director of the Institute, entrusted two researchers – Arêa Leão and Humberto Cardoso – with responsibility for installing a pilot penicillin plant which prepared an imperfect product, but that – we knew afterwards – was not inferior to the Anglo-American one. Soon the Institute looked like the grotto of Lourdes, with long queues of sufferers searching for the miraculous liquid. And requests for the antibiotic arrived from many neighboring countries, and even distant ones like Spain.

By that time an endemic focus of pemphigus foliaceous – the terrible “wild fire” – arose in Belo Horizonte. For want of an available specialist in the Institute, and knowing that I had attended an internship in dermatology during my medical course in Recife, Dr Aragão entrusted me with the study of the subject, chiefly regarding its etiology and the possibility of treatment with penicillin. As I was extremely motivated to continue investigating the exoerythrocytic cycle I moved my colony of *A. aegypti* and samples of avian plasmodia to a room in my house in Belo Horizonte.

The etiology of pemphigus foliaceous was extremely controversial. It was attributed to photochemical sensitization, streptococcus, staphylococcus, virus, phosphorotassic imbalance, allergy, and many other causes that rather seemed to be mere effects. My patients showed improvement of the lesions, due certainly to the elimination of associated microorganisms by penicillin. Search for a virus by intracerebral inoculation in newborn mice of material from lesions gave negative results, as well as inoculation of rhesus monkeys with blood and tissues. So, I decided not to proceed with that line of investigation. Nevertheless, I brought some patients to the hospital of the Institute and treated them with deoxycortisosterone. Several of them recovered and their skin turned heavily tanned. But my enthusiasm ceased when I read a paper in the *Archives of Dermatology and Syphilology* reporting hyaline degeneration of arterioles and death by cerebral hemorrhage in other diseases treated with that hormone.

In Belo Horizonte I continued my research on malaria. In collaboration with Santiago Americano Freire, Professor of Pharmacology at the Faculty of Medicine, we were the first to verify the curative and really prophylactic activity of a chemotherapeutic – sulfadiazine – among other less efficient sulfonamides. Our results were published (Freire & Paraense 1944) in April; later, in November of the same year, similar results were communicated by Coggeshall, Porter and Laird (1944) and Coatney and Cooper (1944) also using *P. gallinaceum*. Till then it was believed that the pathogenic action of the parasite consisted in the destruction of the red cells and its effects, chiefly anemia. Taking advantage of the activity of sulfadiazine against *P. gallinaceum* I was able to demonstrate the pathogenicity of the exoerythrocytic forms, whose multiplication in the endothelium of the encephalic capillaries always resulted in death (Paraense 1946).

At that time there was in Belo Horizonte a friendly salesman who almost everyday passed by our house offering his merchandise. Having befriended our maid, he learned about my activities, eager to pry into the laboratory. Some time later I learned that he was a dismissed employee of the Yellow Fever National Service, who dreamed of being readmitted. One day I was visited by the regional director of the Yellow Fever Service, Dr Augusto Severo, who firmly said that he had received a denunciation of a clandestine breeding of *A. aegypti* in my laboratory and that I had to stop it, since the city was free from that species. The salesman had snooped into the laboratory in my absence and immediately reported...
the news to the Yellow Fever Service. I replied that the colony of mosquitoes was known to the Superintendent of the Rockefeller Foundation, who had supplied the strain, and authorized by the Director of the Oswaldo Cruz Institute. It was up to him to control the vicinity and, if an escaped specimen were found, the colony would be immediately extinguished. Early next morning a brigade of "mata-mosquitos" (mosquito killers) rummaged through the house and put cans with water in the ceilings of all surrounding houses. Meanwhile I spent the whole day receiving and reassuring the frightened neighbors. As time went by the visits of the Yellow Fever became less frequent, while a friendly acquaintance developed between me and Severo.

I would prefer not to deal with so dangerous a mosquito, but it was easy to breed and the only efficient vector of P. gallinaceum. So I suggested that uncommon mosquitoes found by Severo's men be sent to me. Once they brought me a number of larvae that produced adult Aedes which reproduced and were somewhat easily infected. After consulting many papers and all existing classification keys I concluded that I had learned a good deal of entomology but was unable to identify the mosquito. It was really a new species – Aedes (Ochlerotatus) lepidus – which I described in collaboration with the entomologist Nelson Cerqueira, of the Rockefeller Foundation (Cerqueira & Paraense 1945), and that thereafter (Paraense 1945) replaced A. aegypti in my experiments.

During my stay in Minas Gerais I had the opportunity of isolating Plasmodium juxtanucleare, described by Versiani and Gomes (1941, 1943), in some municipalities of the western region. In 1947 I examined in Bambuí 28 hens, 6 of which (21.4%) were infected. Two years later (Paraense 1949) I found 64 infected among 300 examined (21.3%). An infection rate of 20% was found by our colleague Antoniana Kretti (1972) 25 years later. I succeeded in infecting Culex quinquefasciatus, obtaining few sporozoites but failing to transmit the parasite from the mosquito to chicken (Paraense 1944).

Whereas the exoerythrocytic forms of P. gallinaceum showed high affinity for the central nervous system, in P. juxtanucleare they concentrated especially in the spleen (Paraense 1947).

P. juxtanucleare was found in Mexican hens by Beltrán (1941) soon after Versiani and Gomes' description. Six years later Cassamagnaghi (1947) reported its presence in Uruguay. Both Versiani and Gomes and Beltrán succeeded in experimentally infecting the turkey.

Considering the wide geographical distribution of the parasite in the Neotropics it was reasonable to suppose that its natural host would be some of the native galliforms of the region, not excluding, however, birds of other orders. During my second visit to Bambuí (Paraense 1949) I extended the investigation to the adjacent forests, hunting 125 birds of 9 orders, and finding several species of Haemoproteus, besides Trypanosoma and Haemogregarina. Other species of Plasmodium were found in one woodpecker (Chrysotus melanochloros nattereri), four blackbirds (Gnorimopsar chopi chopi), four mockingbirds (Minus saturninus frater), and one cowbird (Molothrus bonariensis bonariensis).

In January 1962 a colleague from Ceylon (now Sri Lanka) informed me that he had isolated a local strain of P. juxtanucleare, and that Prof. Garnham had told him to ask me about the literature on the subject. Later, the parasite was found in four species of wild galliforms in Sri Lanka, Malaysia, Taiwan, and Tanzania. It thus became clear that, as well as P. gallinaceum, it originated in the Oriental region.

The plasmodium found in Gnorimopsar chopi chopi – pássaro preto (blackbird) in Minas Gerais – that from Bahia northward is replaced by Gnorimopsar chopi sulcirostris – graüna – was different from all the species till then recorded in Brazil. I decided not to study it immediately because I had only blood films. In March 1949 I came upon 8 specimens of that blackbird in the Municipal Market of Belo Horizonte, one of which was infected with the same parasite. It was Plasmodium circumflexum, originally described in Germany and then found in Italy, United States, Morocco, Malay Peninsula, and Argentina (Paraense 1952).

Coggeshall (1938), describing Plasmodium lophurae isolated by him from the Borneo fireback pheasant (Lophura igniti), recommended the use of chicks as experimental animals to be inoculated by rapid transfers because the infection tended to disappear. In the contemporaneous literature the longest latency of the infection in chickens had been recorded by Taliaferro and Taliaferro (1940) and Terzian (1946): up to 4 months and about 46 days, respectively. In my experiments (Paraense 1948) the infection in baby chicks survived in a latent state for an observation period of 330 days.

While I was involved in the abovementioned subjects I looked for conditions that allowed me to tackle the problem of the exoerythrocytic cycle in human malaria. It was necessary to prepare an insectary for keeping anophelines, to develop techniques for rearing a good vector such as A. darlingi, very difficult to keep in the laboratory, to produce a great number of sporozoites, and to find a way of doing experiments in humans that were ethically acceptable. At that time malaria was used in the treatment of neurosyphilis, and I was officially appointed by Dr Henrique Araçao to work in the Neurosyphilis Hospital of the University of Brazil, where I was in contact with two colleagues. In spite of the institutional support the installation of the insectary took about 2 years, and an additional year was spent in developing the technique for mosquito breeding. When everything was ready for the decisive experiments the news burst like a bombshell at the end of the International Congress of Tropical Medicine and Malaria in Washington, 1948: the first finding of exoerythrocytic forms in human malaria by a team led by Shortt, Garnham, Covell and Shute, showing that the sporozoite, instead of entering the subcutaneous cell as in the birds, enters the liver cell.

In 1966 Garnham published his classic book about Malaria Parasites and Other Haemosporidia, which I keep with a personal dedication from the author. In it all my papers on malaria are cited and discussed, except that in which the preerythrocytic stage of P. gallinaceum was detected at the site of inoculation of sporozoites. However, this paper had been abstracted by Wenyon (1945)
and considered as a pioneer in the field. Perhaps my paper, published during the Second World War, did not fall into his hands, but it was summarized by Wenyon on page 309 of the Tropical Diseases Bulletin of 1946.

I thank my colleagues Claudio Ribeiro and Hooman Momen and the national community of malariologists for the indication and approval of my name for receiving the Henrique Aragão medal. Besides being an award for which the candidate cannot apply, it strengthens my integration into the Institution where I developed my whole scientific career and to the community dedicated to the subject through which I introduced myself into the world of research.

REFERENCES


