HISTORICAL REVIEW

A Fragment of Malaria History

W Lobato Paraense

Departamento de Malacologia, Instituto Oswaldo Cruz-Fiocruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

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.

Émile 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 amoeboid, 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 schizogony and sporogony, as in the Coccids. *All their stages of development in the vertebrate are found in the red blood cells*".

Fax: +55-21-2280.5840. E-mail: paraense@ioc.fiocruz.br

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 mosquitos 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 exoerythrocytic 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, phosphoropotassic 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 deoxycorticosterone. 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 mosquitos 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 Krettli (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 (*Chrysoptilus melanochloros nattereri*), four blackbirds (*Gnorimopsar chopi chopi*), four mockingbirds (*Mimus 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* – *graúna* – 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 Aragão 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

Beltrán E 1941. Hallazgo de *Plasmodium juxtanucleare* Versiani y Furtado em gallinas de Chiapas. *Rev Inst Salubr Enferm Trop 2*: 353-354.

Brumpt E 1936. *Précis de Parasitologie*, 5th ed., Masson et Cie., Paris, 2139 pp.

Cassamagnaghi A 1947. Malaria en las aves del Uruguay. *Bol Mens Direct Ganad 29*: 105-129.

Cerqueira NL, Paraense WL 1945. Uma nova espécie de *Aedes*, transmissora de *Plasmodium gallinaceum*. *Mem Inst Oswaldo Cruz 42*: 11-15.

Coatney GR, Cooper WC 1944. The prophylactic effect of sulfadiazine and sulfaguanidine against mosquito-borne *Plasmodium gallinaceum* infection in the domestic fowl (preliminary report). *Publ Hlth Reps 59*: 1455-1458.

Coggeshall LT 1938. *Plasmodium lophurae*, a new species of malaria parasite pathogenic for the domestic fowl. *Amer J Hyg 27*: 615-618.

Coggeshall LT, Porter RJ, Laird RL 1944. Prophylactic and curative effects of certain sulfonamide compounds on exo-erythrocytic stages in *Plasmodium gallinaceum* malaria. *Proc Soc Exper Biol Med 57*: 286-292.

Freire SA,Paraense WL 1944.The prophylactic and curative action of sulfadiazine (2-sulfanilamide-pyrimidine), sulfapyridine (2-sulfanilamide-pyridine) and sulfanilamide (p.aminobenzo-sulfonamide) on the erythro and exo-erythrocytic cycles of *Plasmodium gallinaceum* (therapeutic and parasitological aspects). *Rev Brasil Biol 4*: 27-48.

Garnham PCC 1966. *Malaria Parasites and Other Haemosporidia*, Blackwell Scientific Publications, Oxford, 1114 pp.

Grassi GB 1900. *Studi di uno Zoologo sulla Malaria*. *Mem Rend Accad Lincei 53*: 299-502, Rome.

James SP, Tate P 1937. New knowledge of the life-cycle of malaria parasites. *Nature 139*: 545.

Krettli AU 1972. *Plasmodium juxtanucleare* in the State of Minas Gerais, Brazil. Studies on its prevalence and some aspects of its biology. *Rev Inst Med Trop S Paulo 14*: 235-245.

Mohan RN, Manwell RD 1969. *Plasmodium juxtanucleare* in African partridges (*Francolinus* sp.). *J Parasitol 55*: 543.

Paraense WL 1943. Aspectos parasitários observados no local inoculado com esporozoítos de *Plasmodium gallinaceum*. *Mem Inst Oswaldo Cruz 38*: 353-359. (English summary: 357-359).

Paraense WL 1944. Infecção experimental do *Culex quinquefasciatus* pelo *Plasmodium juxtanucleare*. *Mem Inst Oswaldo Cruz 41*: 535-540. (English summary: 540).

Paraense WL 1945. A transmissão de *Plasmodium gallinaceum* pelo *Aedes* (*Ochlerotatus*) *lepidus*. *Mem Inst Oswaldo Cruz 42*: 81-84. (English summary: 83).

Paraense WL 1946. Ação patogênica das formas exoeritrocitárias do *Plasmodium gallinaceum*. 1. Investigações preliminares. 2. Prova terapêutica da ação patogênica. *Mem Inst Oswaldo Cruz 44*: 147-192. (English summary: 185-192).

Paraense WL 1947. Observações preliminares sobre o ciclo exoeritrocitário do *Plasmodium juxtanucleare* Versiani e Gomes, 1941. *Mem Inst Oswaldo Cruz 45*: 813-823. (English version: 820-823).

Paraense WL 1948. A latência parasitêmica na infecção do *Gallus gallus* pelo *Plasmodium lophurae*. *Mem Inst Oswaldo Cruz 46*: 73-87. (English version: 81-87).

Paraense WL 1949. Um inquérito sobre a ocorrência do *Plasmodium juxtanucleare* em Bambuí (Estado de Minas Gerais). *Mem Inst Oswaldo Cruz 47*: 355-359. (English version: 361-365).

Paraense WL 1952. Observações sobre uma amostra brasileira do *Plasmodium circumflexum*. *Mem Inst Oswaldo Cruz 50*: 211-227. (English version : 229-241).

Reichenow E, Mudrow L 1943. Der Entwicklungsgang von *Plasmodium praecox* im Vögelskörper. *Deut Tropenmed Zeitsch 47*: 289-299.

Schaudinn F 1903. Studien über krankheitserregende Protozoen. II. *Plasmodium vivax* (Grassi & Feletti), der Erreger des Tertianfiebers beim Menschen. *Arb Kaiserl Gesundheits 19*: 169-250.

Taliaferro WH, Taliaferro LG 1940. Active and passive immunity in chicken against *Plasmodium lophurae*. *J Infect Dis 66*: 153-165.

Terzian LA 1946. The effect of splenectomy on avian malaria infections. *J Infect Dis 79*: 215-220.

Versiani W, Gomes BF 1941. Sobre um novo hematozoário da galinha – *Plasmodium juxtanucleare* n. sp. (Nota prévia). *Rev Brasil Biol 1*: 231-233.

Versiani W, Gomes BF 1943. "Plasmodium juxtanucleare", parasita da galinha domestica. (Notas adicionais). *Rev Brasil Biol 3*: 113-117.

Wenyon CM 1945. Tropical medicine in war and peace. *Trans R Soc Trop Med Hyg 39*: 177-194.