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SECOND INTERNATIONAL CONFERENCE ON PROTOZOOLOGY¹

London, 27 July-5 August 1965

(Summary of papers on malaria)

1. Introduction

This Conference which brought to London some 600 delegates from 26 countries was held at the Imperial College of Science and Technology, Kensington, London S.W.7, United Kingdom, from July 29th to August 5th, 1965.

The National Organising Committee was composed of:

Chairman	Prof. P.C.C. Garnham, F.R.S.
Vice-Chairman	Dr C.A. Hoare, F.R.S.
Secretary-General	Dr R.S. Bray
Treasurer	Dr A.J. Duggan

An advance volume of abstracts of papers presented at the Conference has been published by the Excerpta Medica Foundation (International Congress Series No. 91) under the title "Progress in Protozoology". As the Conference dealt with all subjects of protozoology, it is understandable that parasites of medical or public health importance formed a relatively small part of the reports and discussions. Thus, out of a total of 380 papers presented only about 40 dealt with subjects of medical or veterinary interest.

During the closing plenary session of the Conference, three resolutions were adopted unanimously:

¹ Prepared by Dr L.J. Bruce-Chwatt, World Health Organization, Geneva.

(a) The first resolution approved the proposal for setting up an International Commission on Protozoology uniting the different groups of protozoologists. The objectives of such an international body and its composition were defined in the text of this resolution.

(b) The second resolution approved the proposal that the International Commission on Protozoology should provide the appropriate information service for summaries of protozoological literature.

(c) The third resolution presented by Dr Lumsden of Edinburgh and seconded by Prof. Adler of Jerusalem is of special interest to WHO and is given here in extenso.

"The Conference, at its Plenary Session on Types and Preservation of Strains of Protozoa, discussed the problems involved in the collection, maintenance, preservation, identification, standardization and nomenclature of protozoal materials for basic and applied research.

The Conference concluded that international discussion and action in these matters were urgently required, to define the protozoal materials used in research, to facilitate the supply of definitive materials to workers, and to collect, maintain and safeguard from loss, protozoal materials of special importance or significance.

The Conference, therefore, resolved:

- a) to request the appropriate international organizations to take note of these problems.
- b) in view of the special urgency of these problems in relation to the Protozoa of public health importance, to invite the World Health Organization to assist by convening a meeting for the discussion of the ways and means by which international co-operation in this field could be realized."

The next, Third International Conference on Protozoology, will take place in 1969 in Leningrad, USSR.

2. Summary of papers related to malaria

2.1 General parasitology

Corliss (Chicago, USA) stressed the importance of collection of type specimens and type culture collections in protozoology. There is need for re-organization of this task on a world-wide scale and this must be based on international co-ordination and co-operation.

George (Cambridge, UK) described the actual task of keeping large culture collections of algae and protozoa and underlined the need for a permanent set-up with a competent technical staff unaffected by short-term changes.

Goble et al. (Summit, USA) underlined the role of the sex factor in the pathogenesis of protozoan diseases. Female mice are relatively more refractory to Trypasoma cruzi, T. gambiense and T. congolense. The same was found in Leishmania donovani infection of hamsters and it appears that it also extends to Plasmodium berghei infections of mice. Hormonal factors are probably responsible for such differences in susceptibility to the protozoan infections studied.

Jeffery (Bethesda, Md, USA) gave an account of methods used for preservation of viable strains of Plasmodia by low temperature freezing. Rodent malaria parasites are easy to preserve; parasites of avian malaria are more difficult in this respect; ten species of simian malaria are being successfully kept in low temperature storage; strains of all four species of human malaria are preserved and thus far the longest period of storing a viable strain of P. falciparum is 1921 days. The importance of this technique for research on drug resistant strains of human malaria parasites is obvious.

Jirovec (Prague, Czechoslovakia) proposed a series of standard microscopic methods for description of new species of parasitic protozoa.

Laird (WHO, Geneva) gave an account of the role of WHO in research on biological control of vectors of human diseases, described the co-ordinated programme of studies, the importance of reference centres for diagnosis of pathogenic organisms of vectors, the distribution of collecting kits and the information services provided by the WHO in this field.

Lumsden (Edinburgh, UK) described the importance, possibilities and implications of long-term storage of protozoa and other organisms. He also proposed two new terms: "isolate" for a population of an organism collected for some procedure and "stabilate" for the population of an organism viably preserved on a unique occasion.

Moshkovski (Moscow, USSR) discussed some quantitative aspects of host-parasite relationship in protozoan infections. Generally, the dynamics of the parasite density in malaria infections are related to several features common to other protozoan diseases and determine the efficacy of chemotherapeutic measures. From the epidemiological point of view, the parasite density determines: (a) the degree of the probability of extension of the source of infection; (b) the probability of detection of an infected case. The relationship between the parasite density and the intensity of transmission (loimopotential) is of particular interest.

Sonneborn (Bloomington, USA) summarized the present status of genetics of protozoa and discussed the new knowledge in the field of cytoplasmic genetics related to the source and function of DNA in various organelles of protozoa.

Wijers (Amsterdam, Holland) summarized the present views on the effect of various environments on host-parasite relationship and quoted two examples of such relationship (in *Trypanosoma* and *Leishmania*) in Africa.

2.2 Primate malaria

Bruce-Chwatt and Charles (WHO, Geneva and Brazzaville) described the distribution of four species of human malaria parasites on the continent of Africa based on results of "incidental research". *P. vivax* is virtually absent in West Africa; *P. malariae* is extraordinary patchy and its true incidence has been underestimated; *P. ovale* is found predominantly in West Africa but also in some foci of East Africa. In several countries in Africa, after residual insecticide spraying, the incidence of *P. vivax* decreases faster than that of *P. falciparum*.

Sergiev and Tiburskaia (Moscow, USSR) classified all strains of P. vivax into five groups according to the incubation period of the disease and the pattern of relapses. Consideration of the ecological conditions of areas where one or another group of strains predominate was used as a basis for a hypothesis of the evolution of such strains of P. vivax from simian malaria parasites.

Warren and Bennett (Chamblee, USA) reported on the complex host-parasite relationships in simian malaria of South-East Asia. Some Anopheles strains of the same species show a high susceptibility of the infection with P. cynomolgi while others are refractory. A successful completion of the cycle depends on physiological and ecological factors and may be so specialized that it limits the parasite's distribution. Monkey malaria of South-East Asia is closely related to Anopheles leucosphyrus group.

2.3 Rodent malaria

Landau (Paris, France) investigated the receptivity of different laboratory and wild rodents to the newly discovered Plasmodium chabaudi originally found in Thammomys rutilans in Bangui (Central African Republic). The parasite is infective to white mice and splenectomized white rats, but less infective to the guinea pig than previously observed and non-infective to hamsters. Transmission through Anopheles stephensi was successful at 27°C and pre-erythrocytic schizonts were found after 52 hours.

Schroeder et al. (Urbana, USA) studied the mechanism of anaemia resulting from P. berghei and Babesia rodhaini infections in rats and concluded that the hemolytic process is due not only to the destruction of erythrocytes by the parasite but also to an auto-immune process in animals with low parasitaemia. It is possible that auto-hemagglutinins have an opsonizing effect in promoting erythrophagocytosis.

Spira and Zuckerman (Jerusalem, Israel) studied the mechanism of anaemia in rats infected by Plasmodium berghei and P. vinckei and found that splenectomy delays the onset of excessive anaemia. This finding supports the hypothesis that some of the blood loss in rodent malaria is related to an immune mechanism leading to erythrophagocytosis of uninfected cells by the R.E.S.

2.4 Bird malaria

Dissanaike et al. (Colombo, Ceylon) observed sporozoite infections in wild mosquitos Mansonia crassipes in Ceylon and by inoculation of salivary glands into chicks and canaries, isolated a strain of Plasmodium circumflexum, a strain of probably P. gallinaceum and perhaps a strain of P. lophurae. A field survey of birds of 13 different species resulted in the isolation of six strains of P. gallinaceum (from domestic fowl) and observation of frequent infection with Haemoproteus.

El-Nahal (Cairo, UAR) investigated the value of fluorescent antibody tests in infections of chickens with Plasmodium gallinaceum and indicated the fluctuations of the antibody level in relation to the time of the infection.

Freyvogel (Basel, Switzerland) described the behaviour of Plasmodium gallinaceum in Aedes aegypti and demonstrated by means of a remarkable time-laps cinematographic study the two types of motility of ookinetes of this plasmodium (also of P. cynomolgi). Active penetration of the peritrophic membrane and of the midgut epithelium of mosquitos were convincingly demonstrated.

Herman (Laurel, USA) observed a high prevalence of Plasmodium circumflexum in wild Canada geese and the attempt at passing the infection into domestic geese and other birds was successful, but domestic fowl and canaries proved to be refractory.

Hoogstraal (NAMAU, Cairo, UAR) described Plasmodium garnhami from the Egyptian hoopoe. The parasite differs morphologically from the related P. elongatum. Transmission of one strain through Culex molestus was successful.

Manwell (Syracuse, USA) isolated Plasmodium juxtannucleare from a bamboo partridge in Taiwan. This infection is common only in this bird in Taiwan. Parasites of a new species, P. formosanum, and of another one as yet undetermined have been observed.

McGhee (Athens, USA) studied erythrophagocytosis in ducklings infected with Plasmodium lophurae and found that plasma obtained from infected birds, passed through a Seitz filter and injected into normal birds, produced blood changes which suggest the presence in infected birds of a soluble antigen which stimulates the phagocytosis of erythrocytes by macrophages.

Rogge (Potsdam, Germany) carried out a survey of blood protozoa in 3700 Central European birds and found that in domestic passerine birds Haemoproteus sp. is found in 60%; Plasmodium sp. Trypanosoma, Leucocytozoon were also found.

2.5 Other blood parasites

Ball (Los Angeles, USA) reviewed critically a number of blood parasites described in reptiles and amphibia and showed the confusion existing in this field. Many of the protozoa described have not been adequately studied and some of them may be haemogregarines, others haemosporidia, still others may be related to other taxonomic groups while some may be artifacts or viral inclusions. Proper classification is difficult because of the absence of knowledge about life histories of many of these parasites. Further establishment of new genera on the basis of blood stages alone is not advisable.

Degiusti (Detroit, USA) carried out a survey of turtles (Chelonians) in USA and Canada and found a high infection rate with Haemoproteus metchnikovi which resembles H. columbae.

Ray (Calcutta, India) reported some observations on the tissue phase of Plasmodium vassali (Hepatocystis) in Indian flying squirrels and in Hepatocystis (= Plasmodium) sempiterni of Himalayan langurs.

2.6 Biochemistry, Chemotherapy and Immunology of Protozoa

Collins et al. (Chamblee, USA) studied the specificity of fluorescent antibody reaction for several plasmodia of lower primates (P. inui, shortti, brasilianum fieldi, gonderi, coatneyi, cynomolgi and knowlesi). It was found that P. brasilianum had considerable antigenic specificity; P. inui and P. shortti gave also distinct results; the highest heterologous reactions were found with P. fieldi in the tertian group of species. Some degree of cross reaction occurs also between these simian malarias and parasites of human malaria within the genus Plasmodium but the combinations of immuno-fluorescent tests are useful in differentiation of closely related species.

Cox (London, UK) produced immunity in mice to P. vinckei by controlling the infection with chloroquine. Such an immunity is long lasting but antibodies are difficult to demonstrate.

Desowitz (Singapore) reported on the results of a survey of human malaria in New Guinea using the haemagglutination test. Results were different in areas under malaria control from those obtained in uncontrolled areas of that country. The course of immune reaction in rats infected with P. berghei and monkeys infected with P. cynomolgi and P. coatneyi were also studied and it appears that this test gives a good indication of the level of specific antibodies produced by experimental infections.

Jerusalem (Nijmegen, Holland) immunized mice against P. berghei using a PAB-free diet and a homogenized antigen in Freund's adjuvant. The immunity to reinfection thus produced, while not absolute, is considerable.

Maegraith and Fletcher (Liverpool, UK) discovered in the blood of mice infected with P. berghei and monkeys infected with P. knowlesi the presence of a factor which inhibits the respiration and oxidative phosphorylation of liver cell mitochondria. Study of lipid components of the plasma indicates their significant increase in infected animals. It is likely that the toxic agent is a peptide-like compound.

Sherman (Riverside, USA) found that P. lophurae contains an enzyme, malic dehydrogenase qualitatively distinct from that of the host cell and that the quantity of this enzyme is related to the growth of the parasite.

Siddiqui and Trager (New York, USA) showed that erythrocytes of ducks infected with P. lophurae contain more folic and folinic acid than those of uninfected ducks. This might be due to an altered metabolism of the host cell rather than to synthesis by the parasite. The study of the synchronous cycle of P. lophurae indicated that a marked increase of folinic acid (but not a folic acid) is correlated with the development of the parasite from a uninucleate to multinucleate stage.

Tobie (Bethesda, USA) reported on the new method of quantitative determination of the serum macroglobulins in human malaria infections using the gel diffusion technique in which a specific antibody was incorporated into the agar plates. The

concentration of antibody was determined by measuring the diameter of the antigen-antibody precipitate. This method used in induced P. vivax and P. cynomolgi infections proved to be of great promise and was closely correlated with the results of fluorescent antibody method.

Trager (New York, USA) has shown in his work on the cultivation of the avian malaria parasite P. lophurae its dependence on the presence of adenosine triphosphate and co-enzyme A. An antimetabolite of panthotenic acid had no effect on extra-cellular parasites of P. lophurae but a clear anti-parasitic effect in vitro on plasmodia developing intracellularly. The antipanthothenate interferes with co-enzyme A synthesizing mechanism of the host erythrocytes. A similar but more striking effect was observed in in vitro culture of P. coatneyi.

Warhurst (London, UK) produced chloroquine resistance in P. berghei of chemically splenectomized mice by giving the drug in the daily diet. This was possible only when the dosage of the drug was 0.01% - 0.015% base in the diet. Lower degree of selection (0.005% chloroquine base) did not produce resistance. A hypothesis is advanced that chloroquine acts by stabilizing the lysosomes of malaria parasites. A potentiation of promethazine and chloroquine on the malaria parasites was described.

Weiss (Michigan, USA) has immunized mice with a strain of P. berghei which became non-invasive to mice after several passages in tissue culture containing hamster serum. Quite high degree of immunity obtained in mice persisted for about 4 months and even longer.

Zuckerman et al. (Jerusalem, Israel) showed that active immunization of rats with a product obtained from specially processed P. berghei was possible and the acquired immunity thus obtained was considerable as evidenced by lower mortality rate, shorter period of patency and lower level of parasitaemia. In another paper Zuckerman and her colleagues (Jerusalem, Israel) demonstrated the presence of antibody to P. berghei in serum of immunized rats by the technique of double diffusion in agar employing as antigen cell-free plasmodial antigen. The number of precipitation lines varied between one and five (or more). The number of lines was roughly proportional to the number of immunizing inoculations.

2.7 General biochemistry and physiology

Ebringer et al. (Bratislava, Czechoslovakia) reported some promising work on new antibiotics active against Trypanosoma cruzi, Toxoplasma gondii, Leishmania sp. Screening for antiprotozoan substances requires different methods from those used in microbiology. Screening for activity against plasmodia is now being planned.

The purpose of the WHO/Mal series of documents is threefold:

- (a) to acquaint WHO staff, national institutes and individual research or public health workers with the changing trends of malaria research and the progress of malaria eradication by means of summaries of some relevant problems;
- (b) to distribute to the groups mentioned above those field reports and other communications which are of particular interest but which would not normally be printed in any WHO publications;
- (c) to make available to interested readers some papers which will eventually appear in print but which, on account of their immediate interest or importance, deserve to be known without undue delay.

It should be noted that the summaries of unpublished work often represent preliminary reports of investigations and therefore such findings are subject to possible revision at a later date.

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