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MALARIA AT THE RIO CONGRESSES

A review of papers on malaria presented at the  
Seventh International Congresses of Tropical Medicine and Malaria  
Rio de Janeiro, September 1963

ANNEX II

ENVOI

This Annex II contains eight additional abstracts of papers which were presented at the Rio Congresses but the text of which appeared neither in the printed volume of abstracts nor in the WHO/Mal/417 document or its Annex I.

Out of the total number of 166 papers on malaria presented to the Rio Congresses, 143 abstracts were given in the document WHO/Mal/417 and its two annexes of which this one concludes the issue.<sup>1</sup> An index of authors has been prepared for easy reference.

The Editor of this series wishes to thank the authors who transmitted to the World Health Organization their papers or the relevant abstracts.

It can be said that the sum total of the scientific papers submitted to the Rio Congresses represents a true cross-section of global aspects of malaria research and malaria eradication at the present time.

In releasing the WHO/Mal/417 document and its two annexes, it is felt that the World Health Organization complies with one of its aims, namely the wide dissemination of valuable scientific information, so ably collected and admirably brought together by the organizers of the Seventh International Congresses of Tropical Medicine and Malaria in Rio de Janeiro.

CORRIGENDUM

The title and authors of the abstract which appeared under the number 2.39 on page 3 of Annex I to WHO/Mal/417 should read as follows:

Susceptibility and resistance to residual insecticides of *Anopheles*  
vectors in Romania after 16 years of employment of DDT and BHC -  
M. Ciuca, G. Lupasco, M. Duport, M. Sandulesco, A. Cristesco,  
I. Cambiesco and I. Sandesco (Bucarest, Romania)

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<sup>1</sup> The printed volume of abstracts prepared by the Secretariat of the Rio Congresses contains 114 abstracts of papers.

1.19 Immunofluorescent studies of exo-erythrocytic stages of malaria parasites-  
R. L. Ingram (Bethesda, Maryland, USA)

Tissue stages of Plasmodium gallinaceum and P. cynomolgi were stained by the immunofluorescent method. In all instances sera were obtained from animals which had recovered from an infection with the specific organism and were subsequently challenged several times with live forms of the parasites. The sera were fractionated by the addition of ammonium sulfate to 40 per cent. saturation. After dialysis, the sera were conjugated with fluorescein isothiocyanate essentially according to the method of Coons & Kaplan.

Liver tissue was removed from monkeys which were infected by intravenous inoculation with sporozoites of P. cynomolgi bastianelii. Brain tissue was obtained from chicks which were infected with sporozoites of P. gallinaceum by intramuscular inoculation. The tissue specimens were fixed with Carnoy's fixative, prepared for histological examination by standard procedures and stained with the conjugated sera.

The usual criteria of specificity for immunofluorescent staining were applied. Fluorescent objects were located precisely; then, the slides were washed with phosphate buffered saline (PBS) and restained with Giemsa. This procedure permitted us to see one-, two- and three-day-old exo-erythrocytic forms of P. cynomolgi bastianelii for the first time. By this method, the one-day-old and most of the two-day-old stages contained a single nucleus surrounded by a ring of cytoplasm. The parasites almost invariably were seen next to the nucleus of hepatic parenchymal cells. Three-day-old forms contained six to eight nuclei; individually, the parasites looked like the one-day-old forms, e.g. a nucleus centrally located within a clear area. There was no problem in identifying tissue stages of P. gallinaceum.

1.20 Submicroscopical aspects of the exo-erythrocytic forms of Plasmodium gallinaceum in tissue cultures<sup>1</sup> - H. Meyer and M. de Oliveira Musacchio (Rio de Janeiro, Brazil)

In previous work we described the fine structure of the exo-erythrocytic forms of Plasmodium gallinaceum in thin sections of infected tissue cultures and reported that the fine structure undergoes great changes during the various phases of the cycle. These investigations were resumed with the intention of studying these transformations in more detail. In the present paper, the fine structure is shown of the young merozoites which had just entered a new tissue cell. We wanted to investigate the manner in which the parasite takes up material from the host, whether this is done by the same intracellular phagotrophy which Rudwiska & Trager had demonstrated in the erythrocytic forms of several malaria species, or by diffusion processes through the surface membrane, as in so many other intracellular parasites.

For this purpose, only very restricted areas of tissue cultures were used, which had been observed in vivo under the optical microscope, and where a rupture of a parasitized cell and the liberation of new merozoites had been observed. Such a region was marked, and after fixation of the whole culture (2-24 hours later), only the marked region was cut out, embedded and sectioned for electron microscopy.

In electron micrographs from recently penetrated merozoites in tissue cells, the following features have been observed:

The fine, electron-dense granulation of the parasite's cytoplasm is well preserved but tends to become less electron-dense after some time. Mitochondria, which had not been found with certainty in the future merozoites in the rosette formations at the end of the intracellular cycle, are very prominent now in the cytoplasm of the parasite. The large nucleus shows its double membrane in the beginning only. In many nuclei an irregular dark area is seen at one side.

The very electron-dense oval body which had been previously described by us in the future merozoites at the distal pole in the rosette formations has disappeared. In some small schizonts it has been observed in direct contact with the nucleus. Its significance and its nature are unknown so far and will be investigated further.

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<sup>1</sup> Amended title

In these young, initial forms no other than their own, finely granulated cytoplasm has been found; no pinching off or engulfing of host material like in the erythrocytic forms has been seen. The double surface membrane of the parasite is in intimate contact with the host's cytoplasm. It is believed that these exo-erythrocytic forms of the parasite take up material through their membranes like many other intracellular parasites.

(The paper in its full text will be published in the Journal of Protozoology.)

1.22 Observations on the gametogenesis of *P. berghei* - I. H. Vincke and G. Michiels  
(Antwerp, Belgium)

A fresh strain of *P. berghei* has been kept under observation for two years. When attempts at successive passages were made in adult white mice exflagellation was not obtained after the 16th passage. In suckling rats and white mice this was prolonged to the 20th and 25th passages respectively. If the number of passages is reduced when transmitting the plasmodium from rats in the metacritic period numerous extraflagellations can still be obtained after two years.

3.16 Clinical and physiological responses to infection with the B strain of Plasmodium cynomolgi and Plasmodium vivax in normal volunteers -  
S. F. Kuvin, H. K. Beye, F. Stohlman, jr, P. G. Contacos and  
G. Robert Coatney (Bethesda, Maryland, United States of America)<sup>1</sup>

The object of this study was to define, as reasonably as possible, the clinical manifestations and pathological processes of simian malaria in man and contrast them briefly with vivax infections.

Clinical and physiological observations in inmate volunteers infected with the B strain of P. cynomolgi and the comparison of these results with volunteers infected with P. vivax have been performed.

Five inmate volunteers were experimentally infected by sporozoite inoculation with the B strain of P. cynomolgi. The course of their infection was contrasted with that of two volunteers infected with the Venezuelan strain of P. vivax.

The group of patients infected with the B strain of P. cynomolgi demonstrated a prepatent period of 9-15 days, an incubation period of 10-17 days, with the duration of their febrile illness lasting 8-12 days. The maximum parasite density in this group was 1100 parasites/mm<sup>3</sup>.

The patients infected with P. vivax demonstrated a prepatent period of 14 days, with an incubation period of 15 days. The duration of their febrile illness was 19 days, with the maximum parasite density controlled to 16 770/mm<sup>3</sup>.

Both groups of patients exhibited tertian fever patterns, but with the fever rising higher and lasting longer in the P. vivax infected patients. Headache, abdominal pain, and hepatosplenomegaly were prominent in the B strain P. cynomolgi infections. Generalized malaise, anorexia, weight loss, and shaking chills were pronounced in the P. vivax infected volunteers.

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<sup>1</sup> Amended title and list of authors

Significant laboratory findings in both groups of patients were anaemia, leucopenia, thrombocytopenia, and elevated erythrocyte sedimentation rate, and shortened survival time of labelled red blood cells. The group infected with P. vivax demonstrated a moderately severe anaemia with a depressed reticulocyte response in comparison to the group infected with the B strain of P. cynomolgi.

Other positive findings were hypoalbuminaemia and hyperglobulinaemia in both infections and a fall in the excretion of 17-hydroxycorticosteroid excretion in three of the five volunteers infected with the B strain of P. cynomolgi.

4.24 Role of chemotherapy in the control and eradication of malaria<sup>1</sup> -  
J. Schneider (Paris, France)

Chemotherapy permits one to obtain easily the individual protection against clinical effects of malaria. This action, often called "control", should be distinguished from a true prophylaxis. "Control" can be applied to large communities and may lead, at times, to a disappearance of the disease particularly in areas where the transmission is seasonal and/or of short duration. The problem is different when one considers eradication of malaria in endemic or even holo-endemic areas. In theory eradication could be achieved by chemotherapy alone if the disease did not behave like a zoonosis; action limited to the human reservoir alone is bound to be a failure. Various drugs (schizontocides, gametocytocides, sporontocides) and their combinations are used. Field trials carried out in Africa are a good example. The main cause of failures is the short action of drugs that can be used nowadays; their fortnightly administration to the whole population is insufficient while a weekly administration gives satisfactory results but this frequency is hardly possible in practice.

To remedy this situation antimalarials have been used incorporated into common salt but analysis of this method showed some advantages and also imperfections and there is the risk of drug resistance.

Research is necessary to discover drugs with longer duration of action. One might conclude that in the present circumstances chemotherapy (drug administration, medicated salt and drug association) must be used concurrently with imagocidal measures by insecticides whenever these measures used alone have not been successful.

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<sup>1</sup> A slightly abbreviated translation of this abstract appeared in WHO/Mal/417; the present version reconstitutes the complete text.

5.13 Refractory malaria in Western Venezuela<sup>1</sup> - A. Gabaldon, L. Guerrero and G. Garcia-Martin (Maracay, Aragua, Venezuela)

Western Venezuela is not a uniform region geographically, climatologically, or ecologically, and this reflects on the epidemiology of malaria. In the northern part (Sector 1), A. albimanus predominates, but A. darlingi is also found. In the intermediate part (Sector 2), the vectors are A. darlingi and A. nuñez-tovari, with areas where A. darlingi has been the only vector (Sector 2-a), areas where both vectors are present (Sector 2-b) and areas where A. nuñez-tovari is the only vector (Sector 2-c). In the southern part (Sector 3), too, A. nuñez-tovari is the only vector.

In Sector 1, malaria was highly endemic; spleen and parasite rates dropped (to normal) after five years of spraying, and eradication was achieved. In Sector 2-a, malaria was very stable. It has been eradicated after five years of spraying, but the spleen rates remain much above normal levels; it is of interest to point out this fact because it permits a retrospective diagnosis to be made of the malaria condition in a region and we know that the time necessary to reach eradication depends on the degree of endemicity. In Sectors 2-c and 3, malaria was also very stable. In deforested zones, malaria has been eradicated with DDT spraying every three months. In zones where villages were near to the forest, transmission persisted in spite of DDT, indicating that exophily of A. nuñez-tovari varies in importance according to the ecological environment. Here, chemotherapy has been able to achieve eradication in areas with small numbers of imported cases, but in areas bordering Colombia (Sectors 2-c and 3) eradication has been impossible owing to the importation of cases.

Weekly peridomestic space sprayings with lindane have been carried out and this has produced a considerable reduction in the density and longevity of the vector, resulting in the interruption of transmission. These space sprayings have been less expensive than chemotherapy and have been more effective among population groups which are not under some form of discipline.

(Full text of this paper will be published in Revista Venezolana de Sanidad y Asistencia Social.)

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<sup>1</sup> Amended title and list of authors

6.23 Special technical problems in malaria eradication in Latin America -  
R. W. Babione (PAHO/WHO, Washington, United States of America)

Problem areas in the Americas have been found usually to be due to a combination of factors. While double resistance (to both DDT and dieldrin) is the most important single factor, DDT usually continues to exert considerable suppression of transmission, although not sufficient to halt it entirely, even in the presence of resistant vectors. This may be due to irritability, repellency, or merely to a low or moderate level of resistance.

There has been very little recent change in the levels of physiological resistance of anophelines to insecticides, except for a few small localities in El Salvador, Guatemala and Nicaragua, where land was recently converted to cultivation of cotton or rice.

Irritability may be either present or absent in susceptible vectors, and likewise in resistant ones. Like resistance, it may be either partial or total in any population. When vectors are susceptible, irritability favours their survival. A. albimanus, long resistant to both DDT and dieldrin in parts of Nicaragua, has become non-irritable in some districts, and this strain shows no effects of any kind even on fresh applications of DDT. A. pseudopunctipennis is susceptible to and not irritated by DDT in South America, and programmes are progressing well in those countries. In western Mexico, the same species is irritated, does not rest in sprayed houses, and constitutes a stubborn problem. Dieldrin has been found to be non-irritating to both A. albimanus and A. pseudopunctipennis and a change to this insecticide is under trial where the vectors are irritated by DDT and susceptible to dieldrin.

The remaining technical problem which is causing some difficulty with DDT, and much more with malathion and other newer insecticides proposed as replacements for DDT, is the problem of sorption presented by certain types of mud walls. Because most of the other substitutes for DDT also are rapidly lost on sorptive mud walls, the possibility of applying an inexpensive sealer to such walls before spraying is now being studied.

Movement of people is a serious problem in many areas with persistent transmission. Total eradication in the whole region under the influence of migration is the best answer.

One solution to the problem of persistence of transmission is to change to an alternate insecticide where insecticide resistance is the cause, but satisfactory substitutes for dieldrin and DDT have not yet been found. Two other methods of attack are being applied in problem areas as rapidly as funds and personnel allow: mass drug treatment and anti-larval measures.

Mass drug administration programmes using the combined tablet of chloroquine (or amodiaquine) plus primaquine were expanded during 1962 as a result of a field trial in El Salvador in 1961. Pilot drug projects were also initiated in 1962 in Mexico (80 000 persons), Guatemala (14 000 persons) and Nicaragua (6000 persons), and much valuable experience was gained. In Costa Rica a mass drug treatment campaign is being undertaken to clean up the final foci in six problem areas of the Pacific coastal region, with about 30 000 people. Venezuela has had extensive experience with various mass drug treatment programmes since 1957 in the western states bordering on Colombia. It appears that these have been transiently successful, but re-importation of malaria from Colombia has usually prevented lasting benefits.

Chloroquinized salt programmes are a special form of mass drug administration, not often usable. The Amazon Valley chloroquinized salt programme was terminated in December 1961. It is clear that several factors contributed to its failure - "leaching" of chloroquine, some chemical deterioration and in some areas tolerance to chloroquine of P. falciparum.

In British Guiana in January 1961, the Government began adding 0.4 per cent. chloroquine to all salt destined for the interior. The results were excellent in two districts and very poor in a third one which was close to the Brazilian border, and received much non-medicated salt from Brazil, as well as importation of chloroquine-tolerant strains of P. falciparum.

Larviciding operations have proved a very valuable adjunct in the city of Guayaquil, Ecuador, and transmission has been halted and the programme is about to be terminated. Larviciding by various means has been tried in Managua, Nicaragua, with good but not completely successful results as yet. It is considered that aircraft dispersal of larvicides will be more effective and economical.

In overcoming problem areas, the malariologist must be ready to use any combination of means of attack that promises the most rapid reduction of transmission for the least money, using supplementary attack methods only to the extent necessary in place and time to achieve eradication. This selection requires more epidemiological knowledge of individual localities and sound judgement than do routine spraying operations. Fortunately, problem areas involve smaller areas and smaller numbers of people than those areas which respond reasonably well to simple residual spraying.

6.24 Lessons learned in the final stages of a malaria eradication programme -  
Wan-I. Ch'en (Ch'ao-chow, Ping-tung, Taiwan)

In Taiwan extensive residual insecticide house spraying, at the dose of 2 g technical DDT per m<sup>2</sup> carried out once a year from 1953 through 1957, was able to interrupt transmission of malaria and brought down the over-all parasite-rate among pre-school children from 9.74 to 0.06 per cent. and the annual average number of A. minimus (the main vector) per house from over several hundreds to below one. During the subsequent malaria surveillance operations, the annual number of microscopically confirmed malaria cases was reduced from 461 in 1959 to 98 in 1962, a rate of only 0.009 per thousand population per annum. Generally speaking, malaria in Taiwan is gradually disappearing.

The organization of the campaign has had two interesting features. Both in the attack and consolidation phase, executive responsibility has gradually been handed over to the general public health service. The surveillance mechanism has been stratified; it has been applied much more intensely in the areas where transmission is most likely.

Malaria eradication in Taiwan by the end of 1964 depends on the existing services carrying out the following plan, and on no new indigenous cases occurring within the period. First, the radical treatment of all remaining cases of malaria, especially those among migrating people, must be accomplished before the vector population regains the critical level. Undue optimism and relaxation of the public and the related agencies, created by the apparent success achieved so far, must be controlled. Passive detection measures require intensification and active ones modification to avoid the laxity caused by the frequent negative results due to disappearing malaria. Secondly, measures must be developed to prevent or to cure instantly imported malaria infections. These include quarantine measures and official international arrangements to minimize the importation of malaria. The last, but by no means the least important, is the organization of a maintenance phase scheme to strengthen the regular health services to cope with emergencies by timely spraying, mass drug treatment, etc.

Arrangements are being made to ensure that the staff of the malaria service have prospects of satisfactory employment when the eradication of malaria has been achieved.

With very few residual malaria infections, low density and limited distribution of vector mosquitos (which are still very sensitive to DDT), a developing health education plan, unflinching loyalty of the service personnel, Government's determination to eradicate malaria, and continued international support, the prospect of malaria eradication in Taiwan is bright.

Critical review of the past achievement has indicated that malaria in Taiwan might have been eradicated much earlier, if the objective of the programme had been set for time-limited eradication from the very beginning.

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

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