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HEALTH ORGANISATION.

MALARIA COMMISSION.

The Secretary of the Malaria Commission has the honour to communicate herewith an extract from a

Report on a Third Visit to Roumania for
the Study of Malaria

by

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This visit was part of the programme of co-operation in experimental malaria which has been in operation since 1933 under the auspices of the Health Organisation between the Horton Laboratory and the various Roumanian laboratories.

The object of this last study tour was:

- (1) To study some immunological problems in relation to infections with P.ovale and with P.vivax (strain Madagascar);
- (2) To ascertain whether a Roumanian strain of P.falciparum, after numerous passages in a non-immune population (at Horton) over a period of four years showed any increased virulence when tested again upon Roumanian subjects;
- (3) To investigate in the field some problems connected with the varieties of A. maculipennis in Northern Roumania.

In 1933, Col. S. P. JAMES and I toured in Roumania and in addition to carrying out a series of laboratory experiments, we visited many of the field stations in Northern Roumania and Bessarabia as well as the malaria station at Gurbanesti. A second visit was made by me in 1935 and a third visit in June of this year.

I. EXPERIMENTS WITH PLASMODIUM OVALE.

In view of the resistance to infection recorded among many patients inoculated with malaria in Roumania, it was decided to try whether this resistance occurred also in the case of P. ovale, a species which, so far as known, does not occur in Roumania. Infected material was therefore taken by me on my trip to that country.

The strain of P.ovale used was one kindly supplied by Professor WARRINGTON YORKE from the Liverpool School of Tropical Medicine in May 1936. It originated from a patient recently returned from Nigeria.

It is interesting to note that this is the third strain of P.ovale we have used at Horton; that all three strains have returned their morphological characters after both mosquito and blood transmission. Clinically all the patients infected by each of these three strains have shown the same classical picture. No cross immunity could be established against either the Madagascar or a Roumanian strain of P.vivax. The results obtained confirm the identity of P.ovale as a separate species of Plasmodium.

From the time of obtaining the strain, until it was taken to Roumania, 34 patients have been inoculated at the (G.P.I.) Malaria Therapy Centre at Horton Hospital under the direction of Dr. W. D. NICOL and his colleague, Dr. HUTTON. Of the above 34 patients, 12 were infected by direct blood inoculation involving three direct passages. This was followed by 7 further direct passages from a patient who was infected by the bites of mosquitoes. A third series consisted of 13 direct passages also arising from a mosquito infected patient. Of the 34 patients infected, 27 had spontaneous recoveries. The remainder were apparently radically cured following one or two days treatment with 1 gramme of quinine.

Three batches of mosquitoes (Anopheles maculipennis, var atroparvus), have been infected with this strain. The strain of P.ovale was not productive of large numbers of gametocytes and, as was the case with two previous strains which we have used at this centre, the complete development of the parasite in the insect host took a longer time than is usually the case with either P.vivax or P.falciparum under identical conditions.

Experiments in Roumania. As mosquitoes infected with P.ovale were not available at the time of my departure, 200 ccs. of defibrinated blood was taken in a bottle on the forenoon of 21st June. This was packed with ice in a Thermos flask and the ice renewed each day during the journey.

On arrival at Bucharest on the evening of the 23rd the blood was heated to 37 C. immediately before using it, and seven patients were inoculated both intravenously and intramuscularly. Six of the patients have never, as far as is known, had malaria before. All six of them have been living in the City of Bucharest for many years, a locality where malaria transmission is absent; each patient showed negative "Henry's reaction" before infection, and no parasites were found following protein shock produced by milk. One patient, who had been infected previously with P.vivax and with P.knowlesi, was also inoculated.

On the 12th day following the inoculations, all seven patients showed typical P.ovale parasites in their peripheral blood, and the clinical picture produced was similar to that observed in non-immune patients in England.

The results of these experiments show that the type of patient inoculated possesses no high degree of immunity

to infection with the strain of P. ovale used. This is interesting in view of the fact that a large percentage of Roumanian patients have been found resistant to infection with other species of Plasmodium.

II. EXPERIMENTS WITH P. VIVAX (Madagascar strain).

This strain is now classical and has probably been studied more than any other known strain of P. vivax. Originally it was obtained from a lascar on a ship coming from Madagascar who, on arrival at the London Docks, had parasites in his blood. This was in April 1925. For two years previous to 1925 Col. JAMES and I had been working with an Indian strain of P. vivax but it was not producing many gametocytes. Whether the Madagascar strain is really indigenous to Madagascar is not known. Its usefulness as a therapeutic agent in the treatment of G.P.I. is unquestionable. It is vigorous but not too dangerous and usually a fairly strong G.P.I. patient is able to tolerate ten to fourteen peaks of fever moderately well. This strain has also been sent to many countries abroad and has been used in most of the malaria laboratories of Europe for the treatment of general paresis, including Austria, France, Italy, Germany, Holland, Roumania and Spain.

The large number of gametocytes which are usually produced by this strain makes it especially useful in research work connected with the cycle of the Plasmodium in the mosquito and its transmission by this insect. Between 1925 and 1933, ninety-six batches of A. maculipennis (var. atroparvus) were infected at Horton, comprising a total of over 25,000 insects.

During the years between 1933 and 1935, it was decided to investigate the value of another strain of P. vivax for work on induced malaria. A strain from Roumania was used (the "Apostol" strain). It was found however that, as with the Indian strain previously used, this strain was unsatisfactory because it did not produce a sufficient number of gametocytes for our purpose.

The Madagascar strain was therefore re-introduced in 1935, when we obtained it through the kindness of Professor SWELLENGREBEL who had maintained it in Holland during the interval. Since then this Madagascar strain has been in constant use and a further seventeen batches of mosquitoes have been infected making a total of over 27,000 insects in a period of about 12 years.

Experiments in Roumania. A batch of heavily infected mosquitoes were carried to Roumania in a Barraud's box. As noted later, these withstood the journey well. During my present visit to Roumania Dr. CHELARESCU VIERU infected seven patients with these mosquitoes in the hospital of Professor BALLIF. Two patients were infected directly by mosquito bites and the others by the inoculation intravenously of a known number of sporozoites obtained from the salivary glands of the infected mosquitoes.

The results of these infections will be published later in a separate paper.

Professor CIUCA wishes now to establish this strain at one or more of the Roumanian research centres and to study it in all its aspects.

III. EXPERIMENTS WITH PLASMODIUM FALCIPARUM.

The strain of P. Falciparum in use at Horton was obtained from Roumania about 4 years ago. Professor CIUCA has also worked with this strain for several years in Professor BALLIFF's hospital at Jasey and he reports that many of the local patients are quite immune, at least clinically. Since it was received at Horton, the strain has been maintained by passage through a long series of non-immune patients by both blood and mosquito transmission. We were anxious to know whether this had resulted in any changes in either its vitality or its toxicity, and considered that some indication might be obtained if the strain was re-introduced into Roumania and tested on the type of patient used by Professor CIUCA.

In our laboratory at Horton we have worked with a number of P. falciparum strains during the last ten years. Patients have been infected with strains from Rome, Sardinia, India, and East and West Africa. The Roumanian strain is still in use here. We have found it moderately severe and in the opinion of Drs. NICOL and HUTTON, it is clinically dangerous in primary cases, usually markedly so about the sixth to seventh day of fever.*

The patient on whom the mosquitoes were fed showed numerous ripe gametocytes (800 male and 2000 females per c.mm.). About a fortnight before my departure for Roumania the insects were given several infecting feeds and in many of those dissected over 800 oocysts were counted. This gives some idea of the severity of the infection obtained. Sporozoites were present in the salivary glands before leaving Horton. They were carried by me to Roumania in a Barraud's cage.

An experiment of causal Prophylaxis. It was not found possible to give the infected insects a blood meal on the day of my departure, so it was considered necessary to give them a meal during the journey if a large number were to survive in a condition suitable for transmission work.

It has been found that infected insects given a fruit meal do not bite so readily as those which have only fed on blood, so I decided to feed them on myself during the journey. As the insects were already infective, I took prophylactic doses of atabrin, of which the effects are recorded below.

* The danger period usually corresponds with the occurrence of a high parasitic prevalence in the peripheral blood (about 20 ring forms of the parasite per field of the thin film) accompanied by the appearance of larger and older forms of the parasite. The appearance of such forms is always considered by us to be an indication that treatment is urgently needed.

The details were as follow:-

21. 6. 37. Left Horton.
22. 6. 37. Most of the batch having digested their previous blood meal they were given the opportunity to feed on my arm. After 20 minutes ten were gorged with blood. Immediately after they had fed I took 0.3 grms. atebtrin.
23. 6. 37. 10 a.m. Again applied cage to arm and six more fed. Took 0.3 grms. atebtrin.
24. 6. 37. 6 p.m. Took 0.3 grms. atebtrin.
25. 6. 37. 10 p.m. Took 0.3 grms. atebtrin.
26. 6. 37. Forgot to take atebtrin.
27. 6. 37. Midnight. Took 0.3 grms. atebtrin.
28. 6. 37. Forgot to take atebtrin..
29. 6. 37. 1 p.m. Took 0.3 grms. atebtrin.
30. 6. 37. 6 p.m. Took 0.3 grms. atebtrin.

As regards the clinical symptoms: 3.7.37. Severe headache with pains in joints. Thick films taken and examined by Professor Ciuca, Dr. Badenski and myself failed to show parasites. Temperature normal. 4.7.37. Films again examined but no parasites found. Headache and pains in joints continued. Temperature normal. In the evening, quinine grains 15 (= 1 gramme). 5.7.37. Feeling well again.

Whether or not the clinical symptoms were really malarial in origin I do not know, but the headache and pains in the joints resembled closely the kind of pains experienced on numerous occasions during attacks of malaria which I have experienced periodically during the past twenty years as the result of repeated laboratory infections. Although I have had infections with P.vivax many times and with P. ovale once, I have only twice been infected with P. falciparum - this Roumanian strain - and on each occasion the attack has been treated almost as soon as it began. It is therefore improbable that I am immune to P.falciparum.

My impression from the symptoms was that I had acquired an infection, but there is no definite proof of this. It seems possible that, on account of the very large dosage of sporozoites which must have been inoculated at the time of biting, the quantity of atebtrin taken was insufficient to cope with such a heavy inoculum. From the known slow rate of excretion of atebtrin, it is probable that a considerable amount of this drug was still circulating in my body when the symptoms developed. This may have been sufficient to keep the parasites at such a low level that they could not be detected before routine treatment was started.

Experiments in Roumania: On arrival at Jassy four patients were infected by injecting known numbers of living sporozoites by the method described in a recent paper, (Shute, 1937),* and two by the bites of infected mosquitoes.

It is not possible to include in this report the results and conclusions of the experimental infections. It is hoped that Professor CIUCA and his colleagues will report them later when they have completed their studies and summarized the results.

* "A Technique for the Inoculation of Known Numbers of Sporozoites as an aid to Malaria Research." P.G. SHUTE. Annals of Tropical Medicine and Parasitology. Vol.31. April 8th, 1937.

IV. FIELD INVESTIGATIONS.

During my stay in Roumania Professor CIUCA kindly arranged for me to visit some of the villages where malaria is endemic. In 1933 and 1935 I was afforded the opportunity of working in Osoi and Tomesti.

A. Factors influencing the relative prevalence of the races of *A. maculipennis* in Roumania.

On my first visit in May 1933 *Anopheles maculipennis* - (sensu lat) were very numerous, the cattle sheds in the villages were swarming with adult insects. Large numbers of females with ripe ovaries were collected and put to lay eggs. These adults were collected from human and animal habitations in villages of Osoi and Tomesti, and still further north in Bessarabia. Several hundreds of batches of eggs were laid and examined, but only two races of this species of *Anopheles* were found; - typicus and messeae. The batches of eggs examined showed the adults to be 90% typicus and 10% messeae but no atroparvus. This finding was, at least to me, rather surprising, because malaria is endemic all over the area and yet, as far as could be seen, only two races of *Anopheles maculipennis* were present, at least in large numbers and neither of them are usually considered to be of much importance in the spread of indigenous malaria. We did, however, succeed in finding many adults of the variety atroparvus (identified from eggs) in animal houses in the suburbs of Jassy.

During the present visit the picture was greatly changed. The variety atroparvus was now found in fairly large numbers in Osoi, Tomesti and Ospriseni. Numerous batches of eggs were examined, the results showing an adult distribution as follows:-

<u>Typicus</u>	58%
<u>Messeae</u>	9%
<u>Atroparvus</u>	33%

It is not easy to decide whether there has been a change in the relative prevalence of the different varieties of *A. maculipennis* during the last few years but, based on actual findings this would seem to be the case. The collections were made at the same season of the year on both occasions, namely, during the month of June. It is perhaps significant that in June 1933, the rainfall had been very heavy whereas this year (1937) actual drought exists. This is reflected in the numbers of adult insects present in the districts. Compared with 1933 *A. maculipennis* were relatively few in numbers although still numerous. If atroparvus have always been present in the above districts but in only small numbers relative to the other races, and if the majority of breeding grounds are more suitable for typicus than they are for atroparvus, then a dry season may reduce the actual numbers of typicus but not atroparvus. On the other hand, if all the varieties present are breeding in the same waters, a possible explanation may be found by studying the length of life of the adults of these varieties.

This particular branch of ecology has interested me for some years past. As is well known, the whole of malaria transmission work at our laboratory at Horton is by the agency of the variety atroparvus. Our experience here is that atroparvus in the adult stage are relatively long-lived insects under laboratory conditions; in a recent experiment* I showed that some individuals survived for seventy days and laid seventeen separate batches of eggs having taken over 30 separate blood meals. In 1931 Dr. VIERU-CHELARESCU spent nearly a year in our laboratory learning the technique of mosquito-malaria transmission. She was surprised when, repeating the same kind of work on her return to Jassy, 90% of her mosquitoes died within a week. She wrote to me about this problem on several occasions but despite all the suggestions made the death rate among her insects was always very high. When at the Socola hospital in 1933, we carried out a series of feeding experiments with the local maculipennis. It was then found that typicus failed to survive for more than a few days under the laboratory conditions in use, whereas atroparvus lived for several weeks. This result was confirmed later.

During my tours abroad in various countries in Europe I have always carried infected atroparvus with me from Horton. Providing a blood meal had been available when necessary, no difficulty has been experienced in keeping the insects alive for several weeks. For example, over one hundred atroparvus were taken from Epsom to Roumania during the present tour and on arrival at Bucharest only two of the insects had died, i.e. after 60 hours. Such had not been the case when attempts were made to bring typicus to England. In 1933 and 1935 several hundreds of typicus were fed and put into Barraud's cages, but on arrival in England only about ten had survived the journey; even those which had had a blood meal died.

It would therefore seem to be the case that, at least in the laboratory, the adults of our English strain of atroparvus outlive by many weeks the variety typicus and probably messeae also. Should this be the case in nature, it may be of some importance. Mosquitoes of the variety atroparvus are fairly plentiful in the villages around Jassy this year compared with 1933 but, owing to the drought, very little active breeding is taking place. If, as is suspected, typicus are short-lived insects in nature, then the diminution of suitable breeding grounds would tend to cause a decrease in their numbers because of the short life of the adults plus the lack of freshly hatched insects. Atroparvus adults, being longer-lived, may be fairly numerous for a considerable time after their breeding grounds have dried up, whereas typicus adults being short-lived would show a rapid decrease in numbers within a week or so following the drying up of their breeding places.

If subsequent investigation is able to confirm the suggestion that the adult life of the female typicus in nature is short compared with that of atroparvus, then it

* SHUTE (1936) A study of laboratory bred A. maculipennis var. atroparvus, with special reference to egg laying. Ann. Trop. Med. & Par. Vol. XXX. No. 1. April 8, 1936.

will add to the importance of the latter race as a carrier of malaria. This is especially the case if it is found that a large percentage of typicus fail to survive long enough for the complete development of the malaria parasite in them.

B. The sleeping habits of the population in relation to the transmission of malaria.

It was interesting to learn that the peasants sleep in the court-yards outside their houses during the summer months, beginning in May and extending well into October if the weather remains fine and warm. In rainy weather during the summer it appears that the peasants do not retire into their houses but sleep under the shelter of the verandahs. As the peasants sleep in the open-air from May until October it follows that for the whole of the malaria transmission season the houses are, for the most part, unoccupied at night, and therefore in these villages the interior of houses is not the chief places where the people become infected. Dr. CORNELLSON had already pointed this out in one of his papers which included a series of photographs showing the beds prepared for the night in the open air. Even when the peasants are suffering from an attack of fever they often lie on their beds on the grass in the court-yards and remain there until the attack is over. I saw one little girl on her bed on the ground in the open air and having a rigor. Her temperature was 103.F. and P.vivax gametocytes were present in her blood in fairly large numbers.

As A.maculipennis is the only species of Anopheles present, it is reasonable to suppose that for the transmission of malaria they must attack man in the open-air. Our knowledge of the habits of A.maculipennis and its varieties from sun-set to dawn is still incomplete.* If they readily attack man in the open during darkness, and at dawn seek the shelter of buildings, infected insects may be present in almost any type of building which is suitable as a resting place. It was interesting to observe that blood-gorged A.maculipennis were present in fair numbers in many of the chicken houses. On dissecting and examining the blood clots in the stomachs of these mosquitoes, not one specimen showed nucleated blood corpuscles. Obviously then the mosquitoes had fed some hours previously on mammalian blood and had retreated to the chicken houses for shelter. The number of infected insects may be just as high or even higher in the animal houses as in the houses of the peasants. It should be remembered that during most of the day, and particularly at night, it must be a little difficult for mosquitoes to enter the human habitations even if they wished to do so, as the doors may be, and usually are, closed, whereas the animal houses adjoining are easy to enter because one side is usually open. Therefore, those insects which bite the patients in the open-air would seek the same kind of shelter after feeding, and in most cases would come to rest in one of the animal houses. Should this be the case, then more infected mosquitoes may be found in the animal houses than in the human dwellings.

* Reference may be made to the work of S. & E. de BUEN in Spain, in document CH/Malaria/205 (1933). Note of the Secretary of the Commission.

I collected the few specimens which could be found in the houses where some of the children were having fever but none showed malarial infection. In one house in Osoi, I was only able to collect 10 female mosquitoes in the house but about one hundred in the cow shed adjoining it. In the evening each insect was put aside to lay its eggs. During the following days each was examined and when eggs were found the variety was determined and the insect dissected for oocysts and sporozoites. Thirty three batches of eggs were laid, nineteen were typicus; three were messeae and eleven atroparvus. One positive insect was found; it had 5 oocysts approximately 7-8 days old and easily recognised as those of P.falciparum; the insect was a specimen of messeae. It is interesting to record that this infected insect was collected from a cattle shed. It is evident, therefore, that further studies of the ecology of A.maculipennis and its varieties in Roumania are needed. Special attention might be directed to the length of life of the different races and their feeding habits in relation to the transmission of malaria.

REMARKS.

It was pleasing to see the large amount of malaria research which is in progress. At Socola Hospital in Jassy there is a team of workers under the direction of Professors CIUCA and BALLIF. Dr. CHELARESCU-VIERU is in charge of the laboratory and all are actively engaged in malaria research. Most of the villages around Jassy are malarious and therefore many of the G.P.I. patients who are admitted to Socola Hospital for malaria treatment show varying degrees of immunity to one or more of the indigenous species or strains of Plasmodium. Therefore the introduction of outside strains is useful and desirable for therapeutic purposes; it also offers a good opportunity for studying immunological effects and other problems.

At Bucharest Mental Hospital, Dr. BADENSKI is in charge of the laboratory and, as both he and Dr. CHELARESCU-VIERU of the Socola Hospital spent nearly a year studying at our laboratory at Horton, close co-operation exists between the three laboratories and, it is hoped, will continue in the future as it has done in the past.

The laboratory technique employed in the three laboratories is identical, and Dr. BADENSKI, Dr. CHELARESCU-VIERU, and myself keep each other informed of any improvements or alterations which may from time to time occur.

I did not, of course, see very much field work but it did seem to me that there is much to be done in ecological studies. The dispensaries at Osoi and Tomesti are well equipped and a number of workers assist Dr. ALEXA who is in charge. A new addition to the team is the recent appointment of Mr. UNGUREANU, a biologist. It is Professor Ciuca's intention that Mr. UNGUREANU shall devote himself to ecological studies of A.maculipennis. We were able to formulate a small programme, which included:-

- (1) Studying the length of life of the female adults of those varieties of A.maculipennis which are present in his area.
- (2) To try to find out to what extent peasants are bitten when sleeping in the open air; I suggested that tethered cattle in the court-yards, (particularly cream coloured cows,) could be observed nightly as an initial experiment.
- (3) Blood-filled adults are numerous by day under the edges of the bamboo roofs at the angle where the roof protrudes from the outer wall; (the "eaves").

It would be interesting to find what percentage of these resting adults contained human blood.

There are numerous field experiments connected with the life history and habits of A.maculipennis which, when completed, should throw some light on the epidemiology of malaria in Roumania. The appointment of Mr. UNGUREANU at Osoi, and Miss IONESCU at Bucharest, should form a useful connecting link between dispensaries, laboratories and the field.