

spores may be found in the vicinity, and other stages (sporonts and trophozoites) are less often encountered. The Malpighian tubules may be greatly distended by the parasite, which probably first develops therein, breaks out into the haemocoel and invades the fat-body—auto-infection apparently occurring on a large scale. Sometimes, even the acini of the salivary glands may contain spores.

The pansporoblasts (see figure above) measure about 15μ and contain 32 or more sporoblasts. The ovoid spore itself measures about 5μ in length, more than double the length given by Weiser^d for *P. culicis*. Smears fixed in methyl alcohol and stained with Giemsa's stain reveal the different stages of development.

Although the Malpighian tubules are often intensely parasitized, and the fat-body is converted into a mass of microsporidian material, the mosquito shows little or no evidence of the infection and may live for many weeks.

The chief purpose of this note is to draw attention to at least three potential difficulties arising from the presence of this microsporidian in laboratory colonies of mosquitoes or even in nature. These are as follows:

1. Pansporoblasts lying on the wall of the mid-gut have a superficial resemblance to the oocysts of *Plasmodium*. They may be differentiated from the latter by the presence of discrete sporoblasts or spores; and also by the absence of the characteristic hyaline appearance of the malaria oocyst.

2. In experimental malaria work, where microsporidian-infected mosquitoes are employed as the vector, there may be interference with the normal development of the oocyst and sporozoites.

3. Comparisons of the biological characters of mosquitos (egg-laying, size, etc). might be vitiated if the presence of the microsporidian were unsuspected.

^d Weiser, J. (1947) *Acta Soc. Sci. nat. (Morav.)*, 18, 1

Aspects of Malaria Vector Research in Africa

An address delivered by BOTHA DE MEILLON, D.Sc., Ph.D., Chief of the Department of Entomology, South African Institute for Medical Research, Johannesburg, at the Second African Malaria Conference, Lagos, Nigeria, November-December 1955

In the early days of malaria control, *Anopheles funestus* presented a problem which may now be largely forgotten. It was known to be strongly exophilic in some parts and not in others. Where it was exophilic it was never found infected. Later, and before the situation really caused much worry, a study of larval and egg characters revealed the whole position and it was found that what we had called *funestus* consisted of at least four forms, namely, *funestus funestus*, *funestus confusus*, *funestus lesoni* and *funestus rivulorum*. Only the first one was endophilous, endophagous, anthropophilous and a vector. The others were harmless.

Nevertheless, two instances of *funestus funestus* "sine malaria" are known, one in Zululand and the other in Swaziland. In both these regions there is a sudden rise of altitude over otherwise flat country. Larvae of the typical form giving rise to typical adults occur but there is no malaria, and adult mosquitos are not found in habitations. This mystery remains unexplained. With the gradual elimination, and in some places extermination, of *funestus funestus* by residual insecticides, the species is now becoming of minor importance and the study of its behaviour is largely academic.

The position with regard to *A. gambiae*, however, may be said to be charged with explosive. The question of the possible existence of biological races is not new. The first time their existence was seriously suggested was by Hackett in 1936.

From the earliest days of the study of mosquito biology it became evident from the reports of biologists that there were apparent differences in the behaviour of the same species in different places. The main reason for this appeared to be climate or environment, the latter, of course, including the water in which the immature stages are to be found. The first real evidence of this was produced by Haddow in 1945, in the forests of Uganda, where it was shown that the biting activity of *gambiae* was related to temperature and saturation deficiency. Since then Haddow and his colleagues have come to believe that the biting behaviour of mosquitos is not actively conditioned by microclimate but that the biting impulse is inhibited by unfavourable conditions. However this may be, the fact remains that environment is still important, whether it plays a part in stimulating or in inhibiting biting. Here I should like to draw attention to the interesting work of Terzian & Stahler with *quadrimaculatus*. They reared larvae under identical conditions except that in one set the larvae were overcrowded. It was found that in the overcrowded set the length of life was prolonged in the larval stage, pupae were smaller, the biting rate of adults was erratic and they were less inclined to bite. Here we have mosquitos in which the only variable was larval environment and yet this produced a difference in adult behaviour. Smith in Tanganyika has shown that there is a change in resting position and deviation to cattle with season.

Gillies' penetrating study, which has cleared up so much of the fog surrounding exophily in *gambiae*, shows very clearly how behaviour is affected by environment.

An interesting aspect that has arisen from this and more recent work in Uganda on the biting cycle is that it appears that different sections of the mosquito population—of the same species—come to bite at different times. The mosquito must apparently find itself in a certain physiological state before it will actively seek a meal and the whole population does not necessarily find itself in this same state at the same time. With recent advances in the estimation of the age and survival rate of female anophelines by members of the Ross Institute, and as a result of Gillies' work on the recognition of age-groups within a population of *gambiae* in East Africa, a study of the movements of sporozoite-infected anophelines may well yield dividends.

The question of the existence of biological races was again revived in a very definite manner by Holstein in French West Africa in 1949. He followed

this up with further work and many came to believe that the matter was settled once and for all. It was left to workers in East Africa to show that so far the maxillary index cannot be used as evidence in support of the existence of biological races in *gambiae*. They have shown that the index is dependent on the larval breeding places—a most surprising discovery. Eggs from multidentate females caught at Kihurio when transferred to Amani and reared under crowded and cooler conditions gave rise to paucidentate females. Multidentate females apparently arise when the immature stages are reared in waters rich in organic matter. Campbell in West Africa has shown that waters rich in chlorophyll produce multidentate whereas those poor in this substance yield paucidentate *gambiae*.

Further, data so far collected on differences in resting places and biting habits do not provide evidence for the existence of biological races. The East African workers rightly point out how careful one must be before assessing mosquito behaviour. In the Union of South Africa, for instance, one can capture large numbers of *gambiae* from outdoor haunts in the Kruger National Park and certain parts of South West Africa; precipitin tests will show 100% zoophilism. One might find certain morphological differences and infer that these identify a zoophilic race. However, all one has to do is put up a tent and sleep in it to collect large numbers of *gambiae* full of human blood in the morning. The adults are zoophilic only because these game parks have no human inhabitants; when human bait is supplied they go for it without hesitation.

It would, of course, be convenient to be able to prove the existence of biological races. However, as we have seen, attempts to do so have not been successful; nevertheless, different patterns of behaviour do appear to exist, even if we cannot answer the very important question whether they are genetically fixed or determined solely by environment. It is highly important that these patterns be fully investigated as they lie at the very basis of an understanding of control. The distribution of *gambiae* is not continuous, hence it is most likely that patterns of behaviour will not be the same everywhere—quite apart from environmental influences. It is therefore obvious that work on vector behaviour must continue in as many areas as possible.

There is little doubt that significant strides are being made in our knowledge of the bionomics of the vectors. It is necessary to know not only what their normal behaviour is, but also whether this can be modified by residual insecticides. Taking the *gambiae* population left after the insecticidal campaigns in Swaziland and Mauritius as an example, a number of questions arise. Is this considerable remnant an ordinary endophilic-anthropophilic strain which has been chased out of doors and forced to become zoophilic, or did the original population consist of two parts of which the one has been eliminated and the other, quite or relatively harmless, left? More important still, is such a process reversible or not? Will the *gambiae* that for one reason or another live outside now revert to endophily, endophagy and anthropophily if insecticidal work is stopped? These are important questions; they call for intensive work before, during, and after insecticidal campaigns.

One of the things we have to thank residual insecticides for is that they have served to expose our ignorance of vector biology and behaviour. Research into such subjects as movement in and out of huts, size of outdoor population, outdoor biting, endophilism, age determination of vectors, sources of blood meals, etc., has received a tremendous stimulus from the use of the residual insecticides. When I trifled with these subjects in the early 'thirties they were regarded as highly academic; today they are of such practical importance that one wonders sometimes at our ignorance. It is only recently, for instance, that Gillies in Tanganyika has shown that 95% of *gambiae* and *funestus* which have fed are to be found indoors and that at certain times many more gravid *funestus* occur indoors than *gambiae*. Incidentally, this exposure of a higher proportion of gravid females to insecticide may possibly account, in part, for the relative ease with which *funestus* can be controlled. Again, it is only within the last year or so that an attempt has been made to find out what percentage of vectors must be killed before control is achieved, or that it has been shown why it is not necessary to produce a 100% kill in order to control the disease, or how markedly the epidemiology of malaria in Africa differs from that in other parts of the world. This goes to show how new the work is and how much remains to be done. As far as the immature stages are concerned, it is only necessary to point out that from the Mediterranean there is definite evidence of a change in anopheline fauna following larvae control; this has shown in a rather startling and unsuspected manner how effective inter-specific competition is in nature—a subject we know very little about.

One might well ask why, when so many campaigns have been successful, more is not known? The answer, of course, is that really we do not know why they were successful. How is it that malaria carried by *gambiae* has been relatively easily controlled in the Transvaal and Natal when elsewhere it presents such a hurdle? Is it because the mud from which the huts are made is less absorbent than elsewhere; that *gambiae* is more endophilic and endophagous and less anthropophilic; that climate and environmental conditions in South Africa are not really optimum for the vectors and that a slight disturbance interferes with their existence or their power to transmit their habits; that the extremely rapid industrial and agricultural expansion during and after the war has in some way upset the balance; that the disease in any case had a precarious existence? We do not know the answers to any of these questions and we probably never shall. These same questions may be asked about Swaziland and Mauritius. Plainly, the fact remains that the past cannot teach us all we want to know, hence the demand for further work in all territories under the great variety of conditions which the African continent has to offer.

Muirhead-Thomson in recent work has cleared the air somewhat regarding the salt-water breeding on the East coast of Africa. Such breeding probably occurs all along the coast as it is known as far down as Natal. It was known that the Natal larvae did not show a *melas* type of pecten. At Zanzibar, Muirhead-Thomson found the same thing and further showed that according to other characteristics this salt-water form was not *melas*. He further found it to be a vector, although, at Zanzibar, of minor impor-

tance compared with typical *gambiae*. The status of the salt-water form has not been finalized but it may well turn out to be the first distinct race of *gambiae* to be discovered.

The secondary vectors must not be forgotten. Many of them are obligatory exophiles; others are endophilic in some places. In the presence of the primary vectors they may be of little importance but when the latter have been controlled the picture may change and they should be watched very carefully.

Conclusion. Resistance to insecticides is a subject that concerns us very much. It is a biological problem which is important not only because of the purely physiological processes that enable it to arise but also because of the way it is reflected in the changed behaviour of the vectors, now referred to as "behaviouristic resistance". Physiological resistance, which results from the ability of the insect to metabolize the insecticide and render it harmless, is unfortunately inherited and determined by a complex polygenic system. Furthermore, it has been shown in work with experimentally produced resistant *Drosophila* that there is no reversion to susceptibility after three years of breeding in absence of contact with the insecticide. More important still is the phenomenon of "cross-resistance", whereby an insect which has been rendered resistant to one insecticide shows resistance to others to which it has not been exposed. If an insect becomes resistant to a second insecticide, exposure again to the first will show that its original resistance has been enhanced.

If vectors could be exterminated with residuals all would be well, but evidence that this can be done is not conclusive. It appears then that we have to look forward to synthesizing more and more insecticides to which vectors become more and more resistant either physiologically or behaviouristically. And until we know more the latter may well prove to be a sword of Damocles.

For these reasons, and also because of mounting costs, it seems that we cannot go on forever relying on insecticides. The final and permanent solution of the malaria problem, as indeed in all other vector-borne diseases, lies in ecological control, which simply means changing the environment so that man is happy, and the parasite and the vector unhappy, and the least this calls for is renewed, intensive and relentless research into vector biology.

Malaria is a living dynamic phenomenon; there is nothing static about it. The central figure in the problem, man himself, is changing by the day, not only in himself but in his ecology. The antimalarial drugs are bringing about a change in the parasite and there is evidence that modern insecticides can influence the behaviour of the vector. It has truly been said that no biological phenomenon is likely to have a simple explanation. As every biologist knows, change and adaptability lie at the very core of living systems and it is this which has secured and guaranteed their existence through aeons of time.