STUDIES ON ANOPHELES DURANT EDWARDS

by

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INTRODUCTION

Field observations carried out by Vincke and his associates during the years 1944-1948 in the region of the Upper Katanga in the Congo brought to light the existence of endemic rodent malaria transmitted by a sylvatic mosquito - Anopheles dureni, under specific ecological conditions.

We owe to these important studies the isolation of Plasmodium berghei (Vincke & Lips, 1948) and Plasmodium vinckei (Rodhain, 1952) and our knowledge of the bionomics of their vector.

Employing methods and techniques used in epidemiological studies of human malaria, Vincke and his associates were able step-by-step to elucidate the nature of rodent malaria as an epizootic affecting certain rodents inhabiting isolated forest galleries, in which A. dureni was demonstrated to be the only natural vector of these plasmodial infections (Vincke, 1954). However, all early attempts to breed A. dureni in the laboratory, or to maintain a wild colony of this mosquito for a prolonged time under experimental conditions failed. It was generally believed at the time that this shade- and tree-loving species ("ombrophilic" and "dendrophilic") is strictly adapted to natural ecological habitat, and is too fragile to survive manipulations and prolonged transportation outside the forest galleries.

The need for an experimental mosquito vector to maintain cyclic transmissions of rodent malaria has brought about new interest in the bionomics of its natural vector, A. dureni. The work reported herewith summarizes some of the observations carried out in the field, in the forest gallery of Kisanga, and the investigations undertaken both at IRSAC laboratories in Lubumbashi and at the Department of Preventive Medicine, New York University School of Medicine.
1. **BIONOMICS OF *ANOPOHELES DURENI* EDWARDS**

Duren (1940) first recorded the presence of malaria in some villages in the Kwango province of the Democratic Republic of the Congo situated at altitudes of 1000 metres or higher. He observed that the majority of the mosquitoes captured in habitations were of a species identified later as *Anopheles dureni*. Duren found this species to be antropophilic. The breeding places of *A. dureni* in the Kwango district of the Congo were described by Henrard et al. (1944). Larvae were found frequently in a number of small, sandy, shaded and overgrown shallow rivers, with clear, slow-moving water. Larvae in large numbers were also collected among the vegetation along the edges of the rivers.

*A. dureni* as observed in the Upper Katanga region, in forest galleries near Lubumbashi, differs greatly in character of its breeding places as well as the bionomics and habits of the adult mosquitoes from those found in the Kwango district. Lips therefore designated the Katanga race as *Anopheles dureni var. millecampsi* (Lips, 1960). It is an ombrophilic, dendrophilic species closely adapted to its ecological environment of the forest galleries.

Adult *A. dureni* never leave the natural enclosure of the forest gallery. They were never found in stables or habitations. They were never seen biting man (exposed human volunteers) inside or outside their habitat. Precipitin tests performed on the blood of engorged *A. dureni* showed invariably a rodent source of their blood-meal. These studies and the subsequent demonstration that the sporozoites which these mosquitoes harbour, derived from a rodent plasmodial infection, have definitely established the zoophilic nature of *A. dureni* and its close adaptation to its sylvatic environment.

2. **CLIMATE, BREEDING AND TRANSMISSION SEASON OF RODENT MALARIA IN THE FOREST GALLERIES**

The seasonal climatic changes which take place in the highlands of Katanga greatly effect the transmission of rodent malaria. This is evidenced in the limited period of breeding of *A. dureni* and in the strongly fluctuating sporozoite indices encountered in the mosquito population of the forest galleries. These aspects were described in detail by Vincke (1954) and more recently by Yoeli (1965). We should like only briefly to summarize the findings: a high sporozoite rate in *A. dureni* is
encountered in forest galleries of Upper Katanga during December, January and
February declining rapidly by the beginning of March and disappearing during the
dry, colder eight months of the year. The high infection rate coincides with the
most intense mosquito breeding and its prevalent adult population.

In the dry season, when temperatures fall, very few adult *A. dureni* can be
detected and larval breeding is greatly reduced or kept at a standstill.

3. TEMPERATURE AND SPOROGONIC DEVELOPMENT IN *ANOPHELES DURENI*

As a result of temperature measurements in the forest gallery of Kisanga during
the breeding and transmission seasons of 1963-1964 and 1964-1965, a clearer view of
the relations between temperature and the sporogonic development of *P. berghei* in
*Anopheles dureni* has emerged (Yoeli et al., 1964). These investigations revealed
that the strains of rodent plasmodia originating from the Upper Katanga require a
relatively low temperature for their normal development in the natural mosquito
vector. Measurements of temperatures in the forest galleries at the height of
the day in the last days of December and January registered only 21-22°C. The
temperature of the tree holes was found to be not above 21°C. During the evening
hours and at night of the transmission season, the temperature descends to 18°C or
even to 16°C. From these observations, it became evident that favourable conditions
for the sporogonic development of *P. berghei* in its natural mosquito vector were
within a narrow temperature range slightly oscillating during the day and night.
These observations were the basis for subsequent work and the successful adaptation
of several *Anopheles* species to serve as experimental vectors of rodent malaria.
The early experiments by Yoeli, Most & Boné (1964) were later extended by Yoeli et al.
(1965) and by Vanderberg & Yoeli (1965, 1966), to include detailed studies on the
effect of temperature on the sporogonic cycle of rodent malaria. Vincke et al.
(1966), more recently, have demonstrated that the temperature factor which permits
normal sporogony of *P. berghei* in *A. quadrimaculatus* and *A. stephensi* acts equally
in *A. gambiae* and *A. maculipennis* var. *atroparvus*.

Recent work by Landau and her co-workers (Landau, 1965; Landau & Chabaud, 1965;
Landau & Killick-Kendrick, 1966a, 1966b) have indicated that among rodent malaria
parasites there exist species and subspecies in ecological biocenosis and geo-
graphical environments (fringes of the tropical rain forest of Central Africa), in
which an evolutionary adaptation of rodent plasmodia has taken place, which permits their sporogonic development in local vectors under more elevated temperature ranges (24-26°C). This parasite ability has been demonstrated to persist also in experimental mosquito vectors (*A. stephensi*) (personal communication from Professor Garnham and from Dr. R. S. Bray).

4. **COLLECTION AND TRANSPORTATION OF *A. DURENI***

A technique originally worked out by Dr. J. Vanderberg (in consultation with Dr. L. Rozeboom) has been successfully used for the collection and transportation of *A. dureni* from the forest galleries to the laboratory, and for subsequent shipment abroad. With some slight modifications, this technique was used by Yoeli & Boné in Katanga (Yoeli *et al*., 1964) and subsequently by Bafort in the Kamena area of the Congo (Vincke *et al*., 1966). *A. dureni*, both males and females, thin and engorged, are found during the rainy season resting on the humid, moss-covered trunks of trees in the forest galleries. They alight in the very early hours of dawn, emerging from their night shelters in tree holes, especially the stately and tall *Syzygium cordatum*. The silvery scales and wing and leg markings of these mosquitoes distinguishes them clearly from the surroundings and permits an experienced technician to catch them easily by test-tube or aspirator. Collections were usually made during the morning hours (6.30 a.m. to 10.00 a.m.). The captured *A. dureni* were immediately transferred to small collecting cages made of reinforced cardboard and mosquito netting. For the purpose of transporting *A. dureni*, only engorged females were collected because it was found that they withstood the rigours of the road and change of environment best. The total "catch" for a single morning by several experienced collectors averaged 250-350 *A. dureni*. The mosquitoes were distributed, 50 per collecting cage, and the cages deposited in a large, insulated, cooling container. Dental wicks immersed in 10% glucose solution were attached to each individual small cage. The cages were marked and the date and place of each collection underlined. The combined collections of three to four days were gathered and arranged in one cooling container, at the bottom of which a wet sponge was inserted to maintain humidity. The container was then tightly closed and sealed with adhesive tape and enveloped in a transparent, plastic bag for protection from insecticide vapours and from the sprays which are regularly applied in airplanes flying from the tropics.
Nine containers were dispatched in this manner to our laboratories in New York. The duration of the air transport varied from two to four days, and mortality among *A. dureni* also differed in the consignments and within the cages of each container. Survival rates are given below for each container (N) received:

<table>
<thead>
<tr>
<th>N</th>
<th>Survival Rate</th>
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<tbody>
<tr>
<td>1</td>
<td>93%</td>
</tr>
<tr>
<td>2</td>
<td>87%</td>
</tr>
<tr>
<td>3</td>
<td>71%</td>
</tr>
<tr>
<td>4</td>
<td>56%</td>
</tr>
<tr>
<td>5</td>
<td>78%</td>
</tr>
<tr>
<td>6</td>
<td>68%</td>
</tr>
<tr>
<td>7</td>
<td>82%</td>
</tr>
<tr>
<td>8</td>
<td>74%</td>
</tr>
<tr>
<td>9</td>
<td>71%</td>
</tr>
</tbody>
</table>

The percentage of survival for the total number of *A. dureni* dispatched (7840 *A. dureni*) was 74% on arrival at their destination.

5. ATTEMPTS TO BREED *A. DURENI* IN THE LABORATORY

A number of attempts were made to rear *A. dureni* in the laboratory, both at IRSAC in Lubumbashi, as well as in our laboratories in New York.

Oviposition of *A. dureni* has been obtained on many occasions. Spring water in enamel bowls was placed in medium sized breeding cages, the insectary lights dimmed, and a light from a blue bulb maintained to stimulate twilight conditions. Small cork rafts were floated on the water, or a stone large enough to protrude to the surface immersed in the bowl. Females harbouring mature eggs were seen alighting on the edge of the wet stone or resting on the edge of the floating raft and laying their eggs.

In water kept at temperatures of 21-22°C (resembling temperatures of the streams of the forest gallery), hatching is slow, requiring two to four days. The first instar larvae grew slowly. Finely ground “foxchow” or bread was used as larval food. It was sprinkled on the surface twice a day in very small quantities during the first days. Addition of liver extract, brain extract or yeasts to the water did not enhance growth, nor did the raising of the room temperature. It was observed that larvae survived and grew best in aerated water to which algae were added. Aeration was halted temporarily several times daily after food sprinkling to enable the larvae to take up the food particles from the surface more easily.
The period required for A. dureni development from egg to imago, at 22°C was 24-26 days. Several batches of eggs were reared in this way to adult mosquito stage. However, in spite of all efforts, copulation or fertilization of the hatched female mosquitoes did not take place under experimental conditions in the laboratory and insectary.

6. DISSECTION OF WILD A. DURENI

Dissection was carried out on many of the weak or dying A. dureni. The results of these dissections were published. The infection index of A. dureni (engorged) which were captured in Kisanga during the last days of December 1963 showed a general infection rate of 17.2%; in January 1964, the index was 15.4%; in January 1965 (combined index of three batches) 14.5%; and in February 1965, 14.4%.

The very high infection rates encountered among the captured A. dureni during the rainy season of these two years was higher than that reported by Vincke and his co-workers (Vincke, 1950, 1954; Vincke & Peters, 1953). However, one must stress the fact that all mosquitoes dissected by us were engorged when captured. This may have altered the general infection rate of the A. dureni population at the time and our figures may in fact be only the result of a selected group.

7. OBSERVATIONS ON SPOROGONY IN WILD A. DURENI AND ON CONCOMITANT MICRO-ORGANISMS FOUND DURING DISSECTIONS

The dissections of midguts of wild A. dureni revealed oocyst development in various stages. No monomorphic growth could be observed in the infected mosquitoes. Comparing this growth to experimentally infected A. dureni and other species of Anopheles, one must conclude that P. berghei tends to produce a sporogonic growth in which normal and retarded development of the parasite in the same vector take place. It is somehow different from the monomorphic development usually seen in P. gallinaceum in Aedes aegypti or P. cynomolgi in Anopheles quadrimaculatus or Anopheles stephensi. Salivary gland sporozoites were found to be infective to susceptible laboratory animals.
The glands and midguts of 14 *A. dureni* were individually dissected and injected into two to three weeks old albino rats. All the 14 animals, each injected with the sporozoites of a single mosquito, developed parasitaemias after incubation periods of three to five days. Of the 14 animals which received the contents of an infected midgut, only three became infected. During this period observations on the movements of sporozoites were also carried out.

During the process of dissection of wild *A. dureni*, nine mosquitoes were found to harbour invasive forms of a filarial worm. The filariform larvae were found emerging live from the proboscis or heads of the mosquitoes. The larvae were fixed and stained, and a study of the material is being undertaken. It is assumed that these larval forms are developmental stages of a filarial worm and that they originated from microfilariae taken up with the blood-meal obtained from thamnomys or other rodents from the forest gallery. The finding may be of interest to workers in the field of filariasis. The adaptation of a rodent filarial infection transmitted by mosquitoes would considerably facilitate research in chemotherapy and biology of filariasis.

A microsporidian parasite which infested the intestinal tract of a number of wild *A. dureni* was observed during dissection. No particular attention was given to it at the time. However, during 1965 the microsporidian spread to our laboratory colony of *A. stephensi*. It greatly interfered with the work on experimental malaria and sporozoite induced infections. For though the parasite did not seem to kill the mosquitoes, its development and spread over the midgut, Malpighian tubules and other parts of the mosquito body in overwhelming numbers interfered greatly with our work on *P. berghei* and necessitated the elimination of our breeding colony of *A. stephensi*, the complete sterilization of all insectary equipment, the painting of the walls and the beginning of a new colony from imported batches of eggs of *A. stephensi*. A detailed study of the *Microsporidium* has been undertaken at the London School of Hygiene and Tropical Medicine under the direction of Professor P. C. C. Garnham (Reynolds, 1966).
8. INFECTION OF WILD CAUGHT A. DURENI WITH S.P. 11 STRAIN OF P. BERGHEI

Thirty-eight "thin" A. dureni, 30 hours after oviposition, were allowed to engorge on a hamster infected with S.P. 11 strain of P. berghei (in its eighteenth blood transfer). At the time of the blood-meal, the parasitaemia amounted to 16% of the red blood-cells infected and gametocytes were abundant in the peripheral blood. The engorged mosquitoes were kept at 22°C and 85% relative humidity. Dissection of midguts, 80 hours after the blood-meal, showed large numbers of small, typical P. berghei oocysts containing a single curved line of fine yellow pigment. Daily dissection showed the progress of the sporogonic development in the natural vector.

On the eighth day after the infective blood-meal, oocysts contained masses of sporozoites. Sporozoites in the salivary glands were observed in small numbers on the ninth day. On the tenth, a massive invasion of the gland took place. Six A. dureni dissected on that day all contained large numbers of sporozoites. Thirty-six of the total 38 A. dureni (94%) which were dissected within 11 days showed massive oocyst development. Though the presence of a natural, earlier acquired infection of P. berghei in some of these wild A. dureni cannot be excluded, the daily dissections clearly demonstrated the progress of the rodent plasmodial infection in its natural insect host.

9. INFECTION OF LABORATORY BREED A. DURENI WITH N.K. 64 STRAIN OF P. BERGHEI

Twenty-two A. dureni, which were reared from eggs in our laboratories, were allowed to engorge on a hamster infected with N.K. 64 strain of P. berghei (on the fourth day of the patent infection). Eighteen of the mosquitoes which took the blood-meal survived 11 days. Dissection showed a 76% midgut oocyst infection and 72% salivary gland sporozoite infection.

The sporozoites obtained were found infective to mice, hamster, young albino rats and thammomys.

10. ISOLATION OF P. BERGHEI STRAINS FROM WILD A. DURENI AND THEIR MAINTENANCE IN THE LABORATORY

Sporozoites from wild caught A. dureni obtained from the glands of single mosquitoes and inoculated into a susceptible host served as parent strains and were named N.K. 64 (New York-Kisanga 1964 strains) and N.K. 65 strains. Altogether
29 strains were isolated and are kept in our laboratories in deep freeze. Some are maintained also by blood transfers and by regular cyclical transmission. Studies on clinical course, parasitaemia, and response to various drugs have shown that all the tested and studied strains from Kisanga behaved in a similar fashion. Their morphology and patterns of development in the mammalian hosts and mosquito vectors showed no marked differences. No *P. vinckei* type of infection has been isolated during this period.

**SUMMARY AND CONCLUSIONS**

Studies undertaken at Lubumbashi (Elisabethville) and at New York University School of Medicine on the bionomics of *Anopheles dureni*, the natural vector of rodent malaria in the forest galleries of Upper Katanga, revealed the close association of this mosquito species to its ecological environment. Attempts to breed *A. dureni* under laboratory conditions were only partially successful. Oviposition and larval development to adult mosquitoes were obtained in the insectary kept at 22°C. Larval development at these conditions is slow, requiring 26 days. Fertilization of *A. dureni* could not be obtained.

A number of infection experiments with wild and laboratory bred *A. dureni* were carried out. Two *Plasmodium berghei* strains have been shown to develop complete sporogony within 10-11 days. Oocyst development in the natural vector is, however, not even or monomorphic, and resembles development in experimental mosquito vectors.

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Les études entreprises à Lubumbashi (Elizabethville) et à la Faculté de Médecine de l'Université de New York sur la biologie de *Anopheles dureni*, vecteur naturel du paludisme des rongeurs dans les galeries forestières du Haut-Katanga, ont révélé des liens étroits qui unissent cette espèce de moustique à son habitat. Les essais d'élevage de *A. dureni* en laboratoire n'ont pas été entièrement couronnés de succès. L'oviposition et le développement des larves jusqu'au stade adulte ont été obtenus dans une cage maintenue à une température de 22°C. Dans de telles conditions, les larves se développent lentement (26 jours). La fécondation de *A. dureni* n'a pas été possible.

Un certain nombre d'expériences d'infection d'*A. dureni* à l'état sauvage ou élevé en laboratoire ont été effectuées. Pour deux souches de *Plasmodium berghei*, on a constaté une sporogonie complète en 10 ou 11 jours. Toutefois, le développement des oocystes chez le vecteur naturel n'est pas égal ni monomorphique et ressemble à celui qu'on observe chez les moustiques vecteurs expérimentaux.
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