WILLIS (1952) and RAHM (1958) proved that the antennae are the sites of these sense organs.

In order to continue this work and in view of the importance of female anopheline mosquitoes for the transmission of malaria, a study of the morpho-histology, distribution and quantitative record of the sense organs in the antennae of *Anopheles maculipennis atroparvus* (v. THIEL), has been carried out by the author and will be described in the present paper. The study was followed by experiments designed to identify the possible chemo-, hygro- and thermo-receptors by cutting the antennae at different levels and exposing the operated mosquitoes to an air stream loaded with the different factors. The behaviour of the amputated mosquitoes was recorded quantitatively and compared with the distribution and number of the different types of sense organs in the antennal segments.

The author was also anxious to study the sense organs, as well as their distribution and number, in the antenna of the male mosquito in order to obtain a clear picture of the differences in both sexes.

II. Previous Investigations of the Role Played by the Host and the Female Mosquito in the Attraction.

In discussing the attraction of the female mosquito to the host we must remember that the process is twofold. On the one hand, the host emits from his body the necessary stimuli, and on the other, the mosquito receives these stimuli through its sense organs and is directed by the latter towards their source.

A. The host.

It is a known fact that the female mosquito does not approach and attack the host as an act of volition—in a search for food, for example—but because it responds to a number of factors introduced by the host into the environment, each of which might be a stimulus that the mosquito makes use of. These factors can be divided into: 1. Optical factors. 2. Physical factors (heat, moisture, etc.). 3. Chemical factors (carbon dioxide and other volatile substances which we may call "odours").

1. Optical factors.

As regards guiding the mosquito to the host, optical factors are not of primary importance either in Anopheles, which is a nocturnal insect, or even in Aedes, which is active in the daytime. It has been proved that mosquitoes are capable of finding man in total darkness. ROTH (1951), by covering the eyes of female *Aedes aegypti* with a layer of shellac rendered opaque with carbon dioxide, found that the eyes are not necessary for locating the host. The experiments of WILLIS (1947) showed that female *Aedes aegypti* and *Anopheles quadrimaculatus* will orient themselves towards an air stream loaded with human body odour in the dark chamber of an olfactometer.

2. Physical factors.

Heat: Attempts have long been made to find the causes which excite the insects to attack. HOWLETT (1910) was one of the first workers in this field. He found that if a test tube filled with hot water is put close to a cage containing

female Aedes, it attracts them and incites them to probe the gauze of their cage. HOWLETT believed that temperature was the dominant factor in provoking the attacks of mosquitoes.

Differences in temperature have a considerable effect on the behaviour of mosquitoes. RUDOLFS (1922), by raising the temperature of a tube containing female Aedes, noticed that this caused a considerable change in their behaviour. They were most active at about human body temperature and became less active with each degree of additional heat until death resulted at 45-46°C. When an actual blood supplier was used, BROWN (1958) found that warm-skinned Caucasian males attracted on the average 30% more than cool-skinned Caucasian males. Even when one of the hands of an individual was artificially cooled to 22°C and compared to the other hand at its normal temperature of 30°C, the cooled hand was found to be much less attractive than the normal one.

Heat was thoroughly investigated by PETERSON and BROWN (1951). They found that the mosquitoes are attracted when they are reached by a convective heat stream but do not respond to heat radiation.

Moisture: REUTER (1936) studied the part played by this factor on *Aedes aegypti* and *Anopheles atroparvus*. He found that a warm moist strip of filter-paper is attractive, while a moist paper at room temperature attracts no mosquitoes at all. On the other hand, PARKER (1953) mentioned that a moist surface (i.e. moisture alone) exerts a quite definite attraction on female Aedes. His findings contradict those of REUTER, as well as CHRISTOPHERS (1947), who also observed that a moist surface alone (at room temperature) exerted no influence on Aedes.

Nevertheless, PARKER explained that the difference in results between his experiments and CHRISTOPHERS' lies in the fact that whereas CHRISTOPHERS' experiments were carried out at an ambient temperature of 25°C and a relative humidity of 80-90%, his experiments were done at 28°C and a relative humidity of 50-70%. He suggested that behaviour reactions of this type depend on the precise climatic conditions to which the insect is subjected. Beside the part played by the temperature and humidity of the milieu on the behaviour of mosquitoes, HAUFE (1955) showed that the atmospheric pressure takes a part as well in the process (see page 34).

BROWN and co-workers (1951) showed that a slightly heated, moist current of air (85% R.H.), introduced in an olfactometer, attracted 3-5 times as many Aedes mosquitoes as a dry one (15% R. H.) at the same temperature, and that a moistened billiard ball was approximately five times as attractive as one with a dry surface. These results were obtained in an experimental room with a relative humidity of 36-65%. But when the relative humidity was raised, the attractivity was reduced, even being reversed at very high room humidities.

BROWN (1951) also noted the importance of the part played by the temperature of the milieu in his field experiments on the effect of moisture. Moisture increased the attractiveness of a warm body two to four times when the air temperature exceeded 15.5°C, while at cooler temperatures, surface moisture decreased in attractiveness.

Water vapour is not attractive at all concentrations. ROTH and WILLIS (1952), working on the hygroreceptors in *Aedes aegypti*, noticed that when the females were given a choice between 0% and 100% R. H., their behaviour was very erratic; the insects were almost continually in flight. This erratic behaviour was thought to be due to the fact that the mosquitoes were attempting to avoid both 0% and 100% R. H. When presented with a choice between 75% and 100% R. H., they chose dry conditions, and when presented with a choice between 0% and 75% R. H., they chose wet conditions.

3. Chemical factors.

The volatile chemical emanations of which the body odour is made up play the chief part in orienting the mosquito from far away to the source of stimuli, while temperature and moisture are no longer of any importance. Flying mosquitoes normally travel upwind, but they take off very much more readily when the wind carries human emanations than when no such stimuli are present. Important observations had been reported by HADDOW (1942). Washed and unwashed native children were placed in similar huts, with all their clothes and personal belongings removed; the boys slept naked in clean blankets. Records of the number of mosquitoes caught both during the day and at night indicated that the unwashed children were more attractive to *Anopheles gambiae, Anopheles funestus* and *Anopheles pharoensis*. HADDOW also found that when dirty native clothes were put alternately on successive nights in 1 of 2 empty huts, they were attractive to *Anopheles gambiae* and *Anopheles funestus*.

WILLIS (1954) succeeded in constructing an insect olfactometer for testing the behaviour of mosquitoes. By means of this apparatus he reached the conclusion that it was only the odour in the "arm air" which attracted *Aedes aegypti* and *Anopheles quadrimaculatus*. His experiments were made by passing an air current over a human arm, held inside a cylinder, and then led to a cage containing the mosquitoes. The degree of attraction was expressed quantitatively as compared to that of control currents of air which had not been passed over the skin. The test and control air currents were similar in temperature, humidity, light, sound and velocity. WILLIS also added that carbon dioxide which is excreted in small quantities through the skin, is not the chemical factor that attracts mosquitoes. In addition, the results obtained by RAHM (1956-57) using a different kind of technique on *Aedes aegypti* parallel those of WILLIS concerning the importance of the host odour in the attraction of mosquitoes and the unimportance of carbon dioxide in the process.

The results of these two authors are supported by LAARMAN's experiments (1955) on *Anopheles maculipennis atroparvus*. LAARMAN concluded that the diffusion of odour from epithelia of the lung, skin and mouth is an important element in the attractiveness of the host. But with his type of technique, LAARMAN, unlike WILLIS and RAHM, found that carbon dioxide plays a part in the attractiveness.

We are not going to discuss here in detail the possible effect of carbon dioside alone as in our experiments no attempts have been made to distinguish between the effect of body odour and that of carbon dioxide on the attractiveness.

Some years ago SCHAERFENBERG and KUPKA (1951), working together with O. BALLAUS, reported that they had succeeded in separating, from ox blood, a substance called "Blutduftstoff", which is very volatile and exercises a strong attraction on *Culex pipiens* and *Stomoxys calcitrans*. This substance, when diluted 1/2,000 times in water, was 5 times more attractive than pure water. Later SCHAERFENBERG and KUPKA (1959) demonstrated the chemical nature of this volatile substance. It is a mixture of cysteine, cystine, alanine, glutamic acid, lactic acid, methylamine, dimethylamine, trimethylamine and ammonia. They concluded that the attraction of the insects to the host increases with rises in temperature and in the concentration of the substance from 0.05 to 0.1%. From this they inferred that flying blood-sucking insects would follow a trace of the host smell, carried by the air, until they reach the host itself, where the concentration of the volatile odour is at its maximum.

Recently BROWN and CARMICHAEL (1961) have shown that lysine present in the blood plasma is the most attractive component for culicine mosquitoes; alanine also exerts attraction, but not so strongly.

B. The female mosquito.

The sense organs in the female mosquito which receive the different host stimuli and direct the female mosquito to the source of these stimuli are located in the antennae. ROTH (1951) found that bilaterally antennectomized *Aedes aegypti* and *Anopheles quadrimaculatus* do not react with any sense of direction to an arm placed in their cage. Similar results were obtained when he used temperature as the only stimulus. ROTH added that since in using the arm as a stimulus one is dealing not only with chemical but thermal stimuli as well, it is not possible to conclude that the antennae function as "distance chemoreceptors", although this is highly probable. RAHM (1958), working with *Aedes aegypti,* showed by the type of select experiments that the antennae are the directional chemoreceptors. His female mosquitoes with different antennal segments missing, could not differentiate between a human hand and a moistened warm bottle (artificial arm).

Finally, the experiments of ROTH and WILLIS (1952) on the behaviour of Aedes mosquitoes towards airborne vapour have shown that the antennae are also the sites of the water-vapour sense.

There is still one point which we should mention, and which plays an important part in the attraction of mosquitoes to the stimuli—viz., the physiological condition of the individual mosquito. This factor can lead to differences in results between experiments of the same type. On this point VAN THIEL (1937) remarked that it was not strange that out of 100 unselected Aedes only a few reacted to carbon dioxide, while in experiments, where mosquitoes were caught on the arm and therefore were in a state of "reaction-preparedness", they reacted more violently.

LAARMAN (1955) wrote about the importance of the physiological condition of the mosquito: "This question may become particularly important when it is found that, at a given moment, certain stimuli do, and at another moment, do not lead to responses".

The writer himself has noticed that female *Anopheles maculipennis atroparvus,* though reared in the same cage and being approximately of the same age, do not all react in the same way. When the hand was put into the cage, it was found that the time required for the mosquitoes to start reacting varied from one individual to another in spite of the fact that they were all in the same state of hunger.

The above discussion can be summarized as follows:

1. Temperature, moisture and the volatile substances (odours) emanating from the body are important factors in the attraction of mosquitoes.

2. The degree of temperature and humidity, to which the mosquitoes are exposed, has a considerable effect on the intensity of their reaction.

3. The attraction of heat and moisture depends on the ambient temperature and relative humidity.

4. The physiological condition of the female mosquito has an effect on its behaviour towards the attracting factors.

5. The antennae of the female mosquito are the sites of the chemo-, hygro- and thermo-receptors which receive the various factors from a distance and guide the mosquito to the host.