Pathology. — *On anophelism without malaria around Amsterdam.* (From the Institute of Tropical Hygiene, section of the Royal Colonial Institute at Amsterdam.) By N. H. SWELLENGREBEL, A. DE BUCK and E. SCHOUTE. (Communicated by Prof. W. A. P. SCHÜFFNER.)

(Communicated at the meeting of December 18, 1926).

Although we have no map showing the distribution of *Anopheles maculipennis* throughout the whole of the Netherlands, still we know that this mosquito is numerous in localities where malaria is rare or absent and was so likewise in the past. Perhaps this may be explained in this way. We do not contest that the presence of anopheles is a condition absolutely necessary for the existence of malaria in a given locality. But as this condition is fulfilled almost everywhere, the presence or absence of malaria does no longer depend on it, but is determined by wholly different factors. Still we should likewise consider the possibility of different biological races of *A. maculipennis* existing in malarious and non-malarious regions. It stands to reason that one's views regarding the practical means to deal with malaria cannot fail to be influenced by these widely divergent opinions, and it is of the utmost importance, to have the question answered which of them is the correct one.

An investigation to test the validity of the second opinion is the subject of this paper. It is limited moreover to a small region enclosing the southern part of the province of North-Holland and the adjacent portions of South-Holland and Utrecht. We are convinced that such fundamental problems can be solved only by numerous local investigations.

We have chosen this particular field because it is intersected by a line of demarcation, dividing it in two regions I and II, N⁰ I to the north where malaria is endemic, N⁰. II to the south where it is absent or extremely rare. The accompanying map shows the distribution of malaria as far as this was to be ascertained by the annual returns collected by the Malaria Commission in North-Holland.

A. maculipennis is numerous in both regions but more so in I than in II. Still there are so many in II, as compared with notorious malarious regions in Europe, where this mosquito is the local vector, that we cannot use this particular quantitative difference between I and II as an explanation of the absence of malaria in II.

The question to be answered is, whether there exists any difference between *A. maculipennis* in I and II. Only such differences are important which bear on the capability of anopheles to transmit malaria.

Consequently they should be biological. Morphological differences cannot interest us unless they show a correlation with biological ones. Still we commenced by looking for the existence of the former because they are easier to detect and to measure and because VAN THIEL's [1]) investigations already pointed in that direction.

It is quite out of the question to determine their origin (whether from region I or region II) by examining a single specimen or even a few ones. And still there is a difference. This became apparent by measuring the length of the thorax, the abdomen and the wing [2]) and by counting the number of maxillary teeth, of hibernating [3]) anopheles from a number of stations situated in both regions (likewise from elsewhere in the Netherlands and from other European countries).

For the sake of brevity we only consider the length of the wing, which we will indicate by the symbol "W." [4]). For the same reason we omit to mention separately [5]) the result of the measurement of more than 7000 mosquitoes (from 35 stations in region I, 18 in region II, 7 in Friesland and Zeeland and 8 outside the Netherlands) except for the particulars shown in the map.

These measurements show that the average length of the mosquitoes from II exceeds that from I.

The large Anopheles from II show an average W. above 122. At least 17 % of them, and usually 22—40 %, are „long wings" having a W. above 129. Among the small Anophelines in region I we make a distinction between the smallest type (either W. below 119 with 1—6 % long wings, or W. 119 but then with less than 3 % long wings) and the intermediate type (W. 119—121 and 3—16 %, usually 7—11 % long wings). As a rule (not without exceptions) this last type is to be found on the boundary between I and II. The results of the measurements, in the stations harbouring the large, intermediate and smallest types, taken together, yield the following average figures:

I.	Large anopheles W. = 125.53	Difference between	I	and	III :	9.21 ± 0.29
II.	Intermediate „ „ = 120.36	„ „	I	„	II :	5.71 ± 0.29
III.	Smallest „ „ = 116.32	„ „	II	„	III :	4.04 ± 0.27

All these differences are, consequently, valid because they exceed thrice the average error. In Friesland we examined Bergum, Tietjerk, Leeuwarden, Nieuwe Biltzijl and Bolsward and we found the large type in the first two, the smallest one in the last two and an intermediate condition in Leeuwarden.

1) Arch. f. Schiffs u. Trop. Hyg. Beihefte, XXX, 1926, 70.

2) Length of the wing: from the anterior margin of the alula to the apex of the wing. The length is expressed in units of 41.7 micra each.

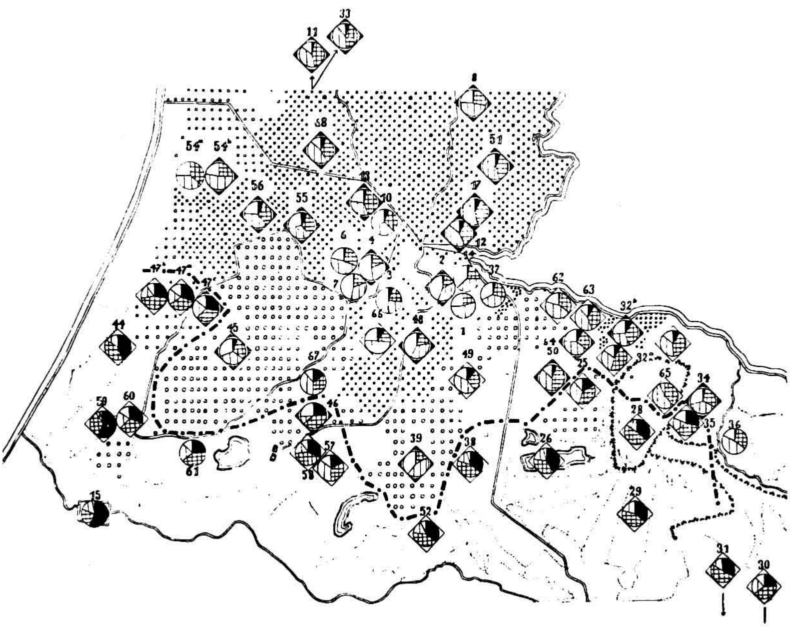
3) Hibernating, because they are all, approximately, of the same age and generation. In summer, old and young ones are intermixed. For a comparative investigation it is necessary to start from a homogeneous material.

4) No objection can be raised against this limitation as the length of the wing and of the body show a high positive correlation, from $+ 0.804 \pm 0.015$ till $+ 0.820 \pm 0.010$.

5) For these and other particulars omitted here, we refer to our detailed account to be published elsewhere and to the thesis of one of us. (A. DE BUCK, Acad. Proefschrift, Amsterdam, 14 Dec. 1926).

As is shown in the map, the stations with large and small Anopheles arc not mixed up together but show a distinct segregation, forming two separate

EXPLANATION OF THE MAP OF THE SURROUNDINGS OF AMSTERDAM.
SCALE 1 : 400.000.



The circles give the situation of the catching stations. The sectors indicate:

black: $^0/_0$ anopheles with a W. above 129
squares: „ „ „ „ of 122—129
vertical lines: „ „ „ „ „ 115—121
white: „ „ „ „ under 115.

The angles of the squares enclosing the circles indicate the chlorine contents of the water in the breedingplaces:

black: 1000 mgr. Cl. p. Litre or more
squares: 500—999 „ „ „ „
white: under 500 „ „ „ „

A. Area with black dots: approximately 20 $^0/_{00}$ or more of malaria.
B. Area with white dots: approximately 1—5 $^0/_{00}$ of malaria.
C. In the remaining areas the incidence of malaria is less than 1 $^0/_{00}$ or 0.
N^0. 11 and 33 are situated in an area like A, N^0. 30 and 31 in an area like C

groups; the small ones to the north, the large ones to the south, in such a way that the bulk of the former are situated in the malarious region I and the majority of the latter in the non-malarious region II, in consequence of which these regions can be practically identified with the two harbouring the different forms of *A. maculipennis.*

But there are important exceptions outside our field of observation e. g. in South Beveland (Goes) and the adjacent parts of North-Brabant (Woensdrecht), where small anopheles occur in the absence of malaria. The coexistence of large anopheles and malaria we infer from the fact of mosquitoes we received from Oltenia (Rumenia), Prizren (Macedonia) [1]) and Moscou, belonging to this last type.

All this holds true for the hibernating generation. In summer the situation changes of aspect: After the hatching of the first summer generation, the females in region I attain the length of the hibernating ones in region II (e.g. W. from 115 to 126). But in region II they have likewise increased in size (e.g. W. from 128 to 132) in consequence of which the difference between the two remains. Next autumn there was again a decrease in size, without however attaining the figures of the preceding winter (e.g. W. = 120 instead of 115 or 131 instead of 128).

Are these two types (the large one from region II and the small one from region I) to be regarded as local modifications, brought into existence by varying external circumstances, or are they to be taken as two hereditary varieties? [2]) Before endeavouring to answer this question we wish to refer to a very old theory stating that mixing of salt and fresh water favours the occurrence of severe marshfevers. This theory has been recently recalled to life by: 1^0. GRASSI [3]) asserting that *A. maculipennis* bred in brackish water is of a more robust disposition than if bred in fresh water and therefore better able to transmit malaria; 2^0. ALESSANDRINI [4]) whose opinion is quite inverse: the mosquitoes are larger and more robust in the fresh and clear water of the ricefields and this enables them to resist malarial infection, 3^0. VAN DER HOEVEN [5]) who holds a similar opinin when ascribing to brackish water an influence rendering Anopheles, bred in it, weaker and of smaller size and more liable to become infected with malaria; 4^0. VAN THIEL [6]) who sustains the same view by his findings of large mosquitoes from the freshwater around Leiden and of small ones from the brackish breedingplaces of Nieuwendam and Bolsward. Large or small mosquitoes, whether they be dangerous or innocuous, they are all considered to be bred under the influence of external circumstances.

Our findings would seem to corroborate this last named view. In the lowlying landreclamations of N.-Holland there are small Anophelines, in the higher districts of Gooi, S.-Holland and Utrecht the large ones occur. In Friesland we observe similar conditions. In localities where the districts, inhabited by small Anophelines, run like a wedge into those where the long ones are prevalent, one finds particularly lowlying areas (Mijdrecht, Haarlemmermeer). The chlorine contents of the breeding places near to

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1) By the kindness of Messrs ZOTA, SFARCIC and MARZINOWSKI.

2) Or as two populations with a different hereditary composition.

3) Rendic. Re. Ac. Lincei, 1922, XXXI, 535.

4) La risicoltura etc. d'Italia. Roma 1925. ROUBAUD and SERGENT likewise find large anopheles in regions without malaria.

5) Cited after van THIEL, loc. cit.

our catching-stations was, as a rule, high in region I (54 % above 1000 mgr. Cl. per Litre and 17 % below 200 mgr.) and low in region II (none above 1000 and 76 % below 200 mgr. Cl. per L.) which is likewise shown in the map [1]).

But we had to modify our opinion when we measured Anopheles hatched from pupae which we caught in breeding places showing a widely divergent chlorine contents, because these Anopheles did not differ much with regard to their size. The W. of anopheles hatched from pupae caught in water with:

330—225 m.gr. Cl. p. L.	was 123	in 40	females and	113	in 30	males,
4515—2955 „ „ „ „	„ 125	„ 34	„ „	107	„ 32	„ ,
3620—1685 „ „ „ „	„ 124	„ 30	„ „	106	„ 29	„ .

One might even be tempted to assert that a higher chlorine contents increases the size, of the females at least. A similar negative result attended our breeding experiments in water from the "Zuiderzee" (3000 mgr. Cl. p. L.) and in tapwater with 0.25 % NaCl, which hatched 279 females with a W. (108.6) equal to that of 450 bred in fresh water (108.5), the males being even slightly longer (276 with W. = 98.1 against 287 with W. = 95.0).

Breeding at higher temperature [2]) (22°—25° against 17°—19°) caused a better marked difference in size. Bred at:

22°—25°, 118 females showed a W. of 101.9; 110 males a W. of 86.2
17°—19°, 134 „ „ „ „ „ 106.6; 211 „ „ „ „ 91.7.

But this cannot help us to explain the difference in size of the mosquitoes from Santpoort and Sassenheim or from Diemen and Maartensdijk!

Still it would be an error to suppose that the size of the mosquitoes is independent from external conditions. But neither the chlorine contents nor the temperature are directly concerned, but the food is. In our breeding experiments we fed the larvae with green unicellular algae, which we scratched from the bark of trees ("tree-algae"). This diet suited them in so far that they pupated and that adults did hatch; but these were subnormal in size (W. of the females 100—113 against 126—130). Even almost mature larvae, fed on this diet during the last 2—5 days of their larval existence only produced undersized adults, whereas pupae caught in nature and allowed to hatch in the laboratory did not suffer in this way. This distinctly points to the influence of the food. This opinion was

[1]) We find it difficult to imagine that the brackish water influences anopheles unfavourably: In region I the average number of larvae per dip was 2,2, against 0,7 in region II. Within certain limits this number increased with increasing chlorine contents, an observation already recorded by SWELLENGREBEL (Ned. Tijdsch. v. Geneesk. 1922, 2nd. half. p. 350—359).

[2]) In accordance with MARTINI's view (Arch. f. Sch. u. Trop. Hyg. XXVI, 1922 and XXVIII, 1924) that *A. maculipennis* is larger in the cool Northern Europe than in the hotter Southern Europe.

confirmed when we tried to breed larvae without tree-algae, by simply adding numerous waterplants *(Elodea canadensis)*. We failed in this, unless we added the algae, except in those cases where the vegetation began to die. For then a rich growth of *Chilomonas paramaecium* occurred and this flagellate seems to be particularly suitable as food, for the larvae bred on this diet produced almost normal adults.

Supposing that the difference in size between the mosquitoes in region I and II is caused by a difference in natural diet [1]), we should succeed in rearing adults of the same average size when starting our breeding experiments with eggs deposited by two lots of adults, the large ones caught in a station of region II, the small ones from region I, on condition that temperature, P_H, chlorine contents (and other circumstances relating to the water) and food supply are the same in both sets of experiments. But this proved not to be the case. Bred under identical external conditions the eggs of mosquitoes from region II produced larger adults, than these from region I. Eggs from :

region I yielded 131 females with W. = 101.8 and 88 males with W. = 87.7
„ II „ 241 „ „ W. = 112.6 „ 159 „ „ W. = 98.4.

Consequently we believe that the difference in size between the mosquitoes of both regions is not only caused by a dissimilarity of external conditions, but likewise by a difference of the hereditary constitution of the anopheline populations in both regions.

We have now to face the question whether these morphological differences are correlated with biological ones, which may influence the ability of anopheles to transmit malaria. The most obvious difference : dissimilarity in infectability, could not be detected: the mosquitoes of both regions showed the same degree (13 % with *Plasmodium vivax*) of infectability [2]).

But we found two other biological differences which are of importance in this respect:

1⁰. We compared the inclination of mosquitoes from both regions to take human blood [3]). In June and September those from region II were

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[1]) Which is a likely supposition, because the vegetation of the breedingplaces of both regions is somewhat different (more *Elodea* and *Hydrocharis* in II and more *Potamogeton pectinatus* and *Myriophyllum* in I). Perhaps the chlorine contents influences the larvae indirecty by modifying the microflora and -fauna.

[2]) In a number of experiments conducted with 224 *A. maculipennis*, 131 from region I (17 infected) and 93 from region II (12 infected), each pair of experiments being performed with an identical gamete carrier.

[3]) In large wooden cages, with glass and wire-gauze walls and a hole to pass the human arm through. Feeding experiments were performed during the day-time and at night, but here we only take the latter into account. It was curious to see how this difference in voracity was almost completely obscured if the mosquitoes were kept in small glass jars covered by gauze and were allowed to feed by applying this gauze on to the human skin. In June the mosquitoes from region II were nearly as ready to feed in this way as those from region I, whereas they almost completely refused to do so in the large cages.

much less voracious than the mosquitoes from region I, but in August there was little difference between the two. Experiments, conducted with many hundreds of mosquitoes, showed the following percentages of anopheles taking human blood:

in June	19.4 %	for region	I	and	1.9 %	for region	II,
„ August	25.1 %	„ „	I	„	21.9 %	„ „	II,
„ September	25.2 %	„ „	I	„	8.5 %	„ „	II,
„ October	37.0 %	„ „	I	„	0 %	„ „	II.

2⁰. When determining the "blood number" (i.e. the percentage of females with blood in their stomachs) of stable mosquitoes in region I and II, this number was found to be at its maximum (87 and 77 % respectively) in June. In region I it did not decrease much till November (29 %). In region II this occurred as early as September and in October it became 0. The "fat-number" (i.e. percentage of females with a well-developed fat-body) was high in region II since the beginning of autumn (sometimes above 90 %), but it was low in region I (1—20 %) [1]).

To use GRASSI's terminology [2]), we have found "semihibernation" in region I and a complete hibernation in region II. Moreover it appears that the lack of appetite in our experiments, shown by the mosquitoes of region II in autumn, is not a sign of a desinclination to take human blood, but to take blood of any kind. But the same absence of appetite in June has quite a different meaning as it occurs at a time when the "bloodnumber" indicates a maximum of voracity with regard to animal blood. Here there is reason to assume a desinclination limited to human blood [3]).

In which way do these biological differences, between anopheles of region I and II, influence their ability to transmit malaria? To answer this question it should first be remembered, that the annual malaria epidemic in N.-Holland occurs in spring, with a maximum in May or June. According to KORTEWEG's [4]) and HONIG's [5]) investigations, primary cases occur as early as February and these authors hold the view that many of the spring-cases are a consequence of an infection incurred in the preceding autumn. This view is supported by our observations [6]) showing that the

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1) With regard to the „bloodnumber" in region I, our experience since 1920 allows us to consider the statement mentioned here as a general rule. But our statements regarding the „bloodnumber" in region II and the „fatnumber" in both regions should not be taken this way until our present findings are confirmed by a more extensive examination.

2) Ann. d'Igiena 1923, XXXII, 438.

3) We make this statement with some reserve, because the anopheles from region I likewise show in June an appetite for human blood which is less than in the following months, whereas our experience teaches us that in human habitations the bloodnumber usually reaches its maximum in June, just as it does in stables.

4) Geneesk. Bladen, 22nd Series, No. 1, 1920.

5) Studie over de malaria te Nieuwendam en omgeving. Amsterdam 1922, 79 pp.

6) SWELLENGREBEL, Ned. Tijdschr. v. Geneesk. 1924, 2nd half, p. 750—763.

majority of anopheline infections are found in autumn, infections which they can transmit to man because bloodsucking in winter is never wholly discontinued. The complete hibernation of anopheles in region II prevents it from taking part in these winter infections. But perhaps the malaria cases in May and June are caused by infections incurred in spring ? Even granted that this may be so, the anopheles of region II cannot take a share in it because of their desinclination towards human blood at that time of the year. The fact that later on they become less fastidious may cause them to incur a malaria infection in early autumn. But complete hibernation, setting in at that time, prevents them from transmitting it to man and even if they keep it through the whole length of the ensuing winter, they will spill it next spring into the blood of some animal.

The results of our continued investigations will have to decide whether or not this is the true explanation of "anophelism without malaria" in our field of observation. Even if they should completely confirm our present statements, we shall not be tempted to make them serve as a general explanation of this phenomenon. For we are convinced that by the term, mentioned above, quite a variety of phenomena is denoted with widely divergent causes. A long series of local investigations should precede any attempt to give a general explanation of this complex of phenomena.