Pathology.— *On anophelism without malaria around Amsterdam.* (Third communication.) *On the food of adult Anopheles maculipennis in malarious and non-malarious regions* [1]). By N. H. SWELLENGREBEL, A. DE BUCK and E. SCHOUTE. (From the Intitute of tropical hygiene, Amsterdam.) (Communicated by Prof. W. SCHÜFFNER.)

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1. *Introduction.* As a rule, Anopheles in stables outnumber, to a greater or lesser extent, their congeners in houses. The average number of Anopheles settled in human dwellings (*"domestic incidence"*), proportional to that in stables (*"stabular incidence"*), yields a ratio: the *"stabular attraction"*, which greatly varies, according to the species (race or strain) concerned, even among the principal vectors of numerous highly malarious localities. Under certain conditions, it enables us to estimate the probability of an Anopheles, which left a house for the sake of oviposition, to alight in some human habitation on its return flight. But it is not sufficient to evaluate the chances of malarial transmission by this Anopheles, unless it has been ascertained that it takes food within the house [2]).

The percentage of engorged specimens, among the female Anopheles settled in houses (*"domestic blood-rate"*) supplies the missing evidence, provided these females did not take their meal in some other place. To make sure of this point, it is necessary to ascertain what kind of blood they ingested (KING and BULL's precipitin test, 1923), so as to make a distinction between the *"human blood-rate"* and the *"animal blood-rate"* which form together the domestic blood-rate, mentioned above.

Finally, if the average number of females per house is known by the domestic incidence and the percentage of males, the human blood-rate allows of establishing the *"effective incidence"*, i.e. the average number of females per house containing human blood.

The human blood-rate and effective incidence increase or decrease in proportion to the anopheline species concerned: 1°. feeding more or less exclusively on blood; 2°. being more or less adapted to a human environment with regard to its choice of either food or shelter 3°. remaining for a longer or shorter time in a particular resting-place as a consequence of either sedentary habits or "homing instinct".

Consequently, it is a mistake to explain a high human bloodrate by "anthropophilism",

[1]) These investigations have been carried out under the auspices and with the financial support of the International Health Division of the Rockefeller Foundation.

[2]) This needs not be the case, as Anopheles seek their various resting-places in view of obtaining: 1° food and 2° shelter. Localities affording the latter only, may be more attractive than others, apparently offering both: In Medemblik (N.-Holland, Netherl.) we found an average of 2 Anopheles per house and no less than 148 per uninhabited shed.

and the contrary condition by "zoophilism", unless it has been ascertained that the other factors, enumerated here, are of no consequence. The term "blood feeding habits" is hardly warranted, considering that the observations admit of an explanation in which these habits need not enter at all. Considering, however, that all these forms of behaviour are in favour of malarial transmission, it follows that the human bloot-rate and the effective incidence yield an indication of some value to estimate the comparative importance of an anopheline strain as a malarial vector. Of course the rate of infection in nature is a much more reliable one. In the present instance it is of no use, as the long-winged paucidentate strain of *A. maculipennis* occurs in habitats, where it is not, for the moment, in a position to become infected.

2. *Object of the present investigation.* During our studies on the racial differentiation of the anopheline fauna inhabiting the Netherlands (1927, 1928), we found the short-winged multidentate, and the long-winged paucidentate strains of *A. maculipennis* (prevalent in the malarious and non-malarious regions respectively) to differ in certain biological

TABLE N⁰. 1.
Analysis of the contents of the stomach A. maculipennis.

Period of investigation	Number examined		Percentage of females showing the precipitin reaction with serum against:			
			Human blood		Animal blood 3)	
	malarious regions	non-malarious regions	malarious regions	non-malarions regions	malarious regions	non-malarious regions
A. In human habitations						
March — April	39	19	5 %	26 %	95 %	75 %
June 1st — July 22nd	202	125	81 „	66 „	19 „	34 „
July 23d 1) — Aug. 15th	147	164	95 „	64 „	5 „	36 „
Sept. 1st — Nov. 23d	173	— 2)	54 „	—	46 „	—
Total	561	308	71 %	63 %	29 %	37 %
B. In stables						
Total	126	301	2 %	0.3 %	98 %	99.7 %

1) Date on which Anopheles with hypertrophic adipose body appeared for the first time in non-malarious regions. About the same time the steep annual rise of the anopheline incidence sets in.

2) None examined, because in non-malarious regions Anopheles in houses do no longer feed in autumn.

3) Including: Pigs, horses, cattle, sheep and negative reactions with freshly ingested (red) blood. Negative reactions with black blood have been discarded. Excluding all negative reactions, the percentages of engorged females with human blood, during the four periods mentioned above, become: 8 %, 83 %, 98 % and 66 % for the malarious regions; 42 %, 70 %, 68 % for the non-malarious regions.

characters. These characters prevent transmission in non-malarious regions, during the period of sexual inactivity of Anopheles (September-March), whereas they greatly favour it in malarious regions. But the evidence is insufficient to prove the inferiority, as a carrier during the height of the malarial season (May, June), of the strain prevalent in non-malarious regions. We expected the determination of the kind of food, used by the females of both strains, to supply us with the missing evidence, by showing that Anopheles in non-malarious regions rarely contain human blood in houses, whereas they commonly do so in malarious regions.

3. *Evidence supplied by precipitin test.* Table N⁰. 1 is far from fulfilling these expectations, even during the most favourable period (August). Considering that MISSIROLI and HACKETT (1927) found (in summer) 44 % of engorged *A. maculipennis*, in houses, to contain human blood in malarious regions and 23 % in non-malarious ones (a ratio of 1.9 : 1), our figures of 95 % in the former and 64 % in the latter are not only unexpectedly high [1]), but their difference (ratio of 1.5 : 1) is much less conspicuous.

The difference however, becomes well-marked (Table N⁰. 2) by taking

TABLE N⁰. 2.

Number of female Anopheles, containing human blood, found in each house.

Period of investigation	Domestic incidence [1])				Domestic blood-rate [2])		Human blood-rate [3])		Effective incidence [4])	
	malarious regions		non-malarious regions		malarious regions	non-malarious regions	malarious regions	non-malarious regions	malarious regions	non-malarious regions
	total	females	total	females						
March — April	7.0	7.0	13.8	13.8	38 %	4 %	1.9 %	1 %	0.13	0.14
June 1st — July 22nd	4.0	2.6	3.7	2.9	60 „	34 „	49 „	22 „	1.27	0.64
July 23d — Aug. 31st	13.7	11.3	9.9	8.6	58 „	27 „	55 „	17 „	6.21	1.46
Sept. 1st — Nov. 23d	23	23	70	70	11 „	0.04 „	6 „	0	1.38	0

[1]) i.e. the average number of Anopheles per house, based on the examination of 1022 houses with 13245 anoph.

[2]) i.e. the percentage of female Anopheles in houses, containing blood of any kind.

[3]) i.e. the percentage of female Anopheles in houses, containing human blood.

[4]) i.e. the average number of females per house, containing human blood.

account of the domestic blood-rate and the domestic incidence (females). The former allows of an estimation of the percentage containing human

[1]) In houses of malarious regions KING and BULL (1923) observed a maximum of 55 % (*A. quadrimaculatus*) ; IVANIC (1926) : 49 % (*A. maculipennis*) ; DAVIS and SHANNON (1928) : 48 % (*A. pseudopunctipennis*) ; KLIGLER and LIEBMAN (1928) : 69 % (*A. elutus, sergenti, pseudopictus*). But WALCH and SARDJITO (1928) record higher figures (100 %) in *A. umbrosus* and *A. bancrofti* var. *pseudobarbirostris*.

blood, among all females found in houses (*human blood-rate*) ; the latter supplies information on the average number per house of females carrying human blood (*effective incidence*).

4. *Seasonal variation :*

a. late summer. In July and August the domestic blood-rate is more than twice [1]), the domestic incidence (females) 1.3 times as high in malarious as in non-malarious regions, and so the human blood-rate in the former (55 %) and the latter (17 %) are widely divergent (ratio of 3.2 : 1). So is the effective incidence, which amounts to 6 in malarious and 1.5 in non-malarious areas (ratio of 4 : 1).

b. early summer. But in June and the first half of July the difference is much less marked, because at that time the domestic incidence (females) does not differ at all in both regions. This causes a decrease to 2.2 and 2.0 of the ratios between the human-blood-rates and between the effective incidences in malarious and non-malarious regions.

Consequently the differences between the Anopheles of these regions are best marked in late summer, when the malarial incidence is already decreasing, and much less so in June, when this incidence is at its highest point or only just beyond it.

c. autumn. During the autumn a comparison of this kind is not possible, because Anopheles in non-malarious regions do not feed. The domestic incidence (females) in malarious areas is higher than in August ; nevertheless the effective incidence is much reduced, owing to the decrease of the domestic blood-rate and the comparatively low percentage of engorged females with human blood (54 %, against 95 % in August). And still, this is the season infected Anopheles are most numerous, i.e. when the contact between mosquito and man is most intimate.

5. *Length of wing of Anopheles with human and animal blood.* During the months of June-August, the length of the wing was 4.963 mm. in 291 specimens of Anopheles with a human blood-rate of 49 % in June and 55 % in August. It was 5.256 mm. in 292 specimens with a human blood-rate of 22 % in June and 17 % in August. In both groups the Anopheles with human blood were shorter winged than those containing animal blood, viz. 4.721 and 5.087 mm. in the first group ; 5.212 and 5.296 mm. in the second group ; 5.046 and 5.256 mm. in both combined.

Quite apart from the interpretation of these morphological differences, the fact that females, having fed on human blood are shorter winged than the others, suggests that some obstacle exists, preventing these groups of Anopheles from mixing freely.

[1]) This is partly due to Anopheles in non-malarious regions showing the first symptoms of hibernation at the end of July, by a hypertrophy of the adipose body among certain individuals (about 21 %), which do no longer take blood. If they are discarded when establishing the human blood-rate, this rate increases from 17 % to 22 %, i.e. to the figure observed in June.

TABLE N⁰. 3.

Comparison of the human blood-rate [1]) and the effective incidence [2])
in groups of houses with numerous and little Anopheles.

Specified observations	Malarious regions June—August		Non-malarious regions June—August	
	34 houses with less than 12 Anopheles per house	18 houses with more than 11 Anopheles per house	27 houses with less than 12 Anopheles per house	27 houses with more than 11 Anopheles per house
Relative number of males	15.0 %	31.0 %	9.0 %	17.0 %
Domestic incidence, total	3.0 „	52.0 „	4.7 „	41.0 „
„ „ , females	2.6 „	35.0 „	4.3 „	33.0 „
„ blood-rate	78.0 „	56.0 „	60.0 „	30.0 „
Human blood-rate [1])	73.0 „	49.0 „	51.0 „	17.0 „
Effective incidence [2])	1.9 „	17.0 „	2.2 „	5.8 „

[1]) Percentage of females in houses containing human blood.
[2]) Average number per house of females with human blood.

6. *Inverse relation between domestic incidence and human blood-rate.* By arranging the houses examined according to the number of Anopheles in each one, and collecting them in lots of 20 each, the percentage of engorged females containing human blood was found to be 87—100 % in the lots with less than 12 and 70—76 % in those with more than 11 Anopheles per house. Accordingly, the material collected in summer was rearranged with regard to the houses containing less than 12 or more than 11 Anopheles. From this arrangement four groups resulted and the human blood-rate was made out for each of them, as shown in table N⁰. 3.

The result confirms KING and BULL's (1923) observation that an inverse relation exists between the abundance of Anopheles inside houses and the proportion feeding on man (human bloodrate). But, moreover, we observe a marked difference between malarious and non-malarious regions, the phenomenon in the latter being the more distinct : the ratio between the human blood-rate coinciding with a domestic incidence (females) of 3—4, and that accompanied by a domestic incidence of 33—35, being 1.5 : 1 in malarious and 3.0 : 1 in non-malarious regions. In houses with the lower domestic incidence, the effective incidence is almost the same in malarious (1.9) and non-malarious (2.2) regions. But in those with the higher one the effective incidence in the former regions (17) is the threefold of that in the latter (5.8). Consequently, the epidemiological significance of houses with numerous Anopheles in malarious regions is far superior to that in the others, where the houses evidently serve to a larger extent as simple shelters for unfed or animal-fed mosquitoes.

Still, the importance of this difference should not be overrated as it holds for the 2nd half of July and August only, houses with numerous Anopheles being rare in June and the 1st half of July.

7. *Influence of the degree of digestion of the blood.* The blood in the mosquito's stomach shows various stages of digestion, which naturally segregate into two principal groups. In the first one only little blood is left; it is either completely black or mixed with a little red. In the second the full stomach distends the abdomen, the blood-clot is wholly or largely red.

In houses within the malarious regions, the percentage of engorged females with human blood (excluding all negative reactions), is almost equal in both groups (87 % and 90 %), showing that only very few leave the house, where they took their meal, before digestion is well advanced. In non-malarious regions this percentage is higher among the recently fed ones than among those with black blood (84 % and 60 %), showing that many of them leave before digestion is over.

If we had chanced to examine females with recently ingested blood only, we would have failed to detect the full extent of the difference in the human blood-rate of Anopheles from malarious and non-malarious regions.

TABLE No. 4.

A comparison of the principal data recorded in Table 1 and 2, with similar observations in Italy (MISSIROLI and HACKETT, 1927).

Specified observations	*Netherlands* July 23d—Aug. 15th		*Italy* July—Sept.	
	Anopheles maculipennis			
	malarious regions	non-malarious regions	malarious regions	non-malarious regions
Domestic incidence-females (average number of female Anopheles per house)	11.3	8.6	46.0	9.6
Domestic incidence-total (average number of Anopheles per house)	13.7	9.9	48.0	12.1
Domestic blood-rate (percentage engorged females in houses)	58 %	27 %	43 %	28 %
Human blood-rate (percentage females with human blood in houses)	55 „	17 „	19 „	6 „
Effective incidence (average number per house of females containing human blood)	6.2	1.5	8.8	0.8
Stabular incidence-total (average number of Anopheles per stable)	2472	936	122	849
Stabular attraction (ratio between total stabular and domestic incidence)	180	94	2.5	70

Hence we conclude that the differences observed are less dependent on preferential feeding habits than on a more or less prolonged resting-stage after each meal. The contents of the preceding paragraph lends support to an analogous conclusion, as it shows that our results would have been quite different, if we had examined nothing but houses with a scanty anopheline population.

8. *Comparison of conditions in Italy and the Netherlands.* Table 4 compares our observations with similar ones in Italy as recorded by MISSIROLI and HACKETT (1927).

a. Malarious regions. The domestic and stabular incidence offer the most striking differences. In Italy the former is the fourfold of that in our country, whereas the latter in the Netherlands is about 200 times as high as in Italy. Hence, the stabular attraction in the former country even surpasses that of the non-malarious areas in the latter. The considerable superiority in domestic incidence is, however, largely compensated by the human blood-rate in the Netherlands being near the threefold of that in Italy. As a consequence, the effective incidences in the two countries differ much less (6 and 9) than might have been expected.

b. Non-malarious regions. The comparison of these regions in Italy and the Netherlands reveals a marked similarity, disturbed only by the lower human blood-rate in the former. It is noteworthy that the stabular attraction in both, although 28—36 times as great as in the malarious regions in Italy, is only one half to two fifths of that observed in the malarious areas of the Netherlands.

9. *Summary.* The differences, mentioned here, between the strains of Anopheles in malarious and non-malarious regions may be summarized as follows:

1. Owing to the higher figures, in malarious regions, for: *a.* the domestic blood-rate, *b.* the percentage of engorged females with human blood, *c.* the domestic incidence, the human blood-rate of malarious regions is the treble and their effective incidence the fourfold of that in non-malarious areas, during the 2nd half of July and in August.

2. These differences vary considerably in extent, according to the season, being well-marked in late summer only, much less so, or even absent, in early summer.

3. In autumn (at the time of the highest incidence of infected Anopheles in houses) the effective incidence in malarious regions sinks to a level it attained in June, owing to the considerable increase of the animal blood-rate.

4. These differences are most marked when comparing the records of houses with a numerous anopheline population.

5. Among other factors, responsible for their existence, one is operative in malarious regions by keeping the majority of the females within the houses until the digestion of the blood is far advanced, whereas in non-malarious areas many of them leave at an earlier moment. Another one acts by the precocious development of the adipose body of Anopheles in non-malarious regions reducing the domestic blood-rate. Whether true preferential feeding habits enter among these factors cannot be infered from the contents of this paper. But our feeding experiments (1927—1928) suggest the existence of such preferences for human or animal blood. Another fact pointing in the same direction is

the inverse relation between the domestic and the stabular blood-rates in malarious and non-malarious regions. In respect of the former we have seen already that the malarious regions are in the advantage (60 % against 34 % ; both in early summer) ; with regard to the latter the non-malarious regions are, although in a slight degree (85 % against 77 % ; both in early summer).

10. *Conclusion.* If the acme of the malarial season occurred in late summer (as it actually did in former times), the differences, summarized here, might be admitted as an explanation of anophelism without malaria in the Netherlands. But, at present, the malarial incidence attains its annual maximum during a period, when these differences are of little consequence. So, we fail to perceive how they can affect, to any appreciable extent, the epidemiology of malaria in this country.

At best, the conditions obtaining in malarious areas (late summer) might counteract, to some extent, the powerful stabular attraction, maintaining, in this way, a limited stock of parasite carriers, to fill the gap between the top of the annual epidemic in early summer and the time Anopheles are in the best position to transmit malaria (i.e. in autumn). But, as autumnal transmission by the strain of Anopheles in non-malarious regions is impossible anyhow, this does not help us to prove the inferiority of this strain to transmit malaria during the period of its highest incidence.

Consequently, the existence of this inferiority cannot be upheld. The strain of Anopheles in non-malarious regions is as likely to act as a vector during the malarial season as the one prevalent in malarious areas. The obvious fact that the former is not operative in this way, implies that the latter can be neither. There is no transmission during the malarial season but only after it : perhaps in late summer, at any rate in autumn.

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