

Hygiene. — *On the so-called "daily turnover" of the Anopheline population in resting-places and its bearing on the evaluation of the Anopheline incidence, to test the effect of antilarval measures* ¹⁾. By N. H. SWELLENGREBEL and W. H. DOORNBOS. (From the Institute of tropical hygiene, Amsterdam.) (Communicated by Prof. W. A. P. SCHÜFFNER.)

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1. Object of the investigation.

During the antilarval operations around the town of Medemblik (N. Holland, Netherlands) in 1927 and 1928, regular anopheline counts were made in a number of restingplaces within and outside the area under control, in order to obtain a measurement of the results. In the ordinary stables (the majority of them containing pigs) the mosquitoes were killed and counted once a fortnight; in the "experimental stables", specially constructed to facilitate observation, with 4 pigs to each stable, the Anopheles were counted (without catching them) twice a week. In the "shelters", i.e. open, uninhabited outhouses, the Anopheles were caught and counted daily.

If the data, collected in this fashion, are to be relied on, it is essential to make sure that the regular catching of Anopheles in the ordinary stables and shelters, does not make the subsequent findings differ from what they would have been if no catching had been going on. In other words: we should be certain that our method of measurement does not alter the thing we want to measure. Are we sure of this? The answer to this question seems to be definitely affirmative.

ROUBAUD (1920) states that the anopheline population (*A. maculipennis*) of a resting-place with an abundant foodsupply, completely repairs its losses within 24 hours, each time one catches all Anopheles there, so much so, that the catching seems to effect no change at all in this population. Every host attracts each night approximately the same number of ever changing mosquitoes.

BARBER and HAYNE (1924) likewise observed the complete change occurring each night in the population (*A. quadrimaculatus*) of a resting-place. They term this change "*the daily turnover*". Like ROUBAUD they

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

found that in resting-places where *Anopheles* had been destroyed or removed, their numbers were as large as ever on the succeeding day.

In MISSIROLI and HACKETT's (1927) observations the "turnover" is not so complete. Still, a stable where all *A. maculipennis* had been caught, became replenished after 3—6 days. Seventy-five pct. of the anopheline population leaves the stable each night.

SCHÜFFNER and HYLKEMA (1921) had arrived at a similar figure (69%) for *A. ludlowi* some years before, but, according to them, this change is due to the *Anopheles* being destroyed.

These observations are confirmed in three ways.

Firstly, by experiments to release stained mosquitoes in a stable and to ascertain how many of them may be recaptured on the following days. ROUBAUD released 2000 and did not recapture a single one after 10 days. BARBER and HAYNE, out of 4875 stained mosquitoes, recaptured 6.7, 1.1, 0.2, 0.5, 0.2, 0.02% of the original number released, on the 1st—6th day respectively. GRASSI (1920) recovered 0.9, 0.6, 0.2, 0.2, 0.15, 0.06, 0.02, 0.02, 0.04% on the 6th—14th day respectively, out of a total number of 5297 stained *A. maculipennis*¹⁾. DAVIS (1926) released 274 *A. argyritarsis* in human dwellings and recaptured 3.3%, but only on the 1st and 2nd day.

Secondly, by the high mortality of *Anopheles* compelled, by confinement, to remain in the same stable, although food and water are both available. ROUBAUD records the survival of one or two *Anopheles* out of each lot of 20, after a confinement of 10 days. BARBER and HAYNE found only one or two, out of 1100—2000, living after 6 days. NICHOLLS' (1912) mosquitoes, however, (*A. albimanus*) kept alive much longer in confinement: up to the 31st day.

Thirdly, by ascertaining the incidence of infection and the age of the oocysts among mosquitoes in houses and stables. It is taken for granted that: 1^o. If the infected mosquitoes remain in the same resting-place for a long time, they will show a high proportion of mature infections and house-infections will greatly outnumber stable-infections. 2^o. If they frequently change their abode, infected *Anopheles* will only show young oocysts in the house where they became infected and the insect carriers will be as numerous in stables as in houses or even more so. GRASSI (1920) found only 3 *A. maculipennis* with mature oocysts among 15 infected ones in prisoners barracks in Italy and SELLA (1920) noted a higher incidence of summer-infection among *A. maculipennis* in stables than in houses of the same locality. SCHÜFFNER and HYLKEMA (1922) recorded 61% of carriers of very young zygotes among infected *A. ludlowi* in houses. Still the rate of infection there was 10 times as high as in stables. The reduction of the advanced stages of infection is explained by the mortality among the mosquitoes. KLIGLER and LIEBMAN (1928), in Palestina, observed the same difference of houses and stables for *A. elutus* during the summer. But, the contrary obtains for *A. superpictus* during the autumn.

There is, consequently, strong, although not unequivocal, evidence forthcoming in favour of the universal occurrence of the "daily turnover". This would relieve us of the difficulty stated before, as it implies that repeated catches have no influence on the anopheline incidence. But some doubts occurred to us regarding the general applicability of the observations recorded here, because we were well aware that confinement is by no means fatal to all *Anopheles*.

A. maculipennis in the vicinity of Amsterdam, confined in large cages, fed daily on human blood and provided with water, survived, on an average, for a maximum of

¹⁾ He does not accept the "turnover" as an explanation of the failure to find stained *Anopheles* at a later date. According to him they have all died within that time.

16 days in June and for 34 days in August (DE BUCK, SWELLENGREBEL and SCHOUTE 1927). It is true that BARBER and HAYNE try to deal with this objection by the argument that the prolonged life in cages, as compared with that in stables, is due to the greater humidity and the absence of natural enemies. But this argument does not meet our case, because we could raise at will the mortality of *Anopheles* in our cages up to the level observed by these authors (maximum life of 4 days in June, and 9 days in August), simply by selecting as an object of observation, mosquitoes from a region different from that which provided us with the long-lived *Anopheles* mentioned above.

The longevity in confinement varies, accordingly, with the different anopheline populations kept under the same environmental conditions. May not this apply likewise to the phenomenon of the "daily turnover"? To answer this question was the object of the present investigation.

2. *Restingplaces where the "daily turnover" occurs and others where it is absent.*

We have already mentioned three types of restingplaces, but there actually are only two of them viz. the "*sheltertype*" and the "*stabletype*". In the first the male *Anopheles* are, at least, the equals of the females in number; their incidence, moreover, is not influenced by the distance from the breedingplaces. As a rule this type is limited to the localities we have termed "shelters", but occasionally we find a stable among them. In the second type the females outnumber the males by, at least, 2 to 1. The relative number of the males increases on the approach to the breeding-places. This type is limited to inhabited localities, usually to stables.

As an instance, we found in the town of Medemblik, i.e. the protected centre of the area under control (July 30th—Sept. 30th), 15 % of males in "stables" and 63 % in "shelters". Outside the boundary of this area (the same period) 25 % of males in "stables" and 54 % in "shelters".

Examining representatives of both types at short intervals, without interfering in any way with their anopheline population, simply by counting the mosquitoes as they are resting on walls and ceiling, we noticed a marked difference between them.

10. *In the "stables" the anopheline numbers exhibit but little daily variations, in the "shelters" there are considerable ups and downs.* Diagram N°. 1 shows this difference in two experimental stables, one of which belongs to the sheltertype; but the same condition obtains in the actual shelters (Diagram N°. 1 and Table I).

The first and second column in Table I show another difference between the two experimental stables. Although they are identical as to external and internal structure and outfit, inhabitants, situation in respect of breedingplaces and of inhabited localities, the four pigs in the first stable attract each night almost twelve times as many *Anopheles* as those in the second. In this last one, *Anopheles* are conspicuously below the average of the three neighbouring experimental stables. But in another group of those stables, we have observed one, showing an average daily number of *Anopheles* more than thrice the average of the other three. We therefore believe that "the constancy with which a given host is surrounded by *Anopheles* each night in approximately the same numbers" refers to observations which should not be taken as expressing a general law.

TABLE I (see: Diagram N^o. 1).

Variation in the daily number of female Anopheles in an ordinary experimental stable and in one belonging to the "sheltertype". Both are identical in respect of construction, size, number of inhabitants (4 pigs). The third column shows the findings in three uninhabited shelters.

Date	<i>Ordinary stable:</i> Percent. of males; 27 % average daily number of females: 1253	<i>Stable "sheltertype":</i> Percent. of males; 54 % average daily number of females: 106	<i>Shelters.</i> Percent. of males; 63 % average daily number of females: 58
	Daily number of females, expressed as a percentage of the above average		
Aug. 1	152	123	22
4	148	18	15
8	129	12	207
11	154	241	150
15	181	17	279
18	173	64	196
22	133	25	84
25	139	185	177
29	133	53	390
Sept. 1	136	123	59
5	128	207	112
8	105	140	107
12	87	122	24
15	54	128	5
19	—	98	52
22	18	20	5
26	12	113	3
29	10	100	0
Oct. 3	14	123	15
5	15	39	0

Note: In this table and the next one, we mention the females only, because the first restingplace differs too much from the other two (table I) or from the third one (table II) in respect of the relative number of males, to justify a comparison of the total number of Anopheles. A separate daily record of the males, would have materially complicated these tables, without any change in the facts they convey in their present form.

20. A "stable", from which all *Anopheles* have been removed, requires about 9 days to repair its losses; a "shelter" performs this feat within 24

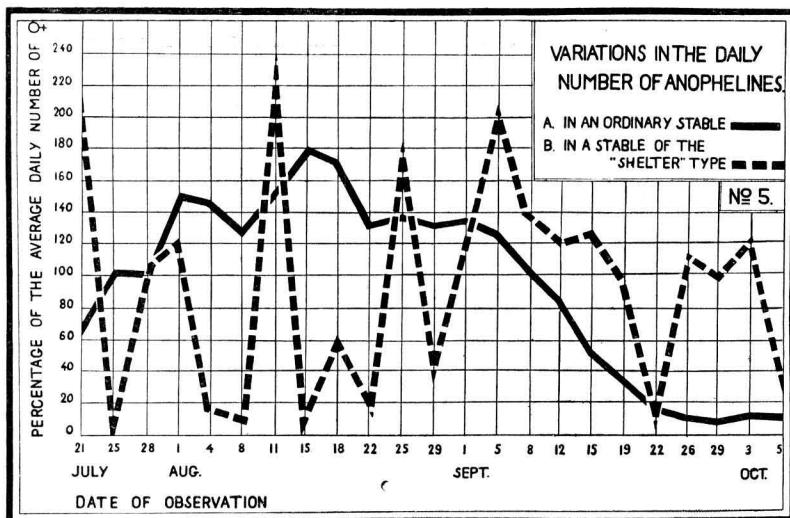


Fig. 1.

hours, often even to the point of over-compensation (Diagram N°. 2 and Table II).

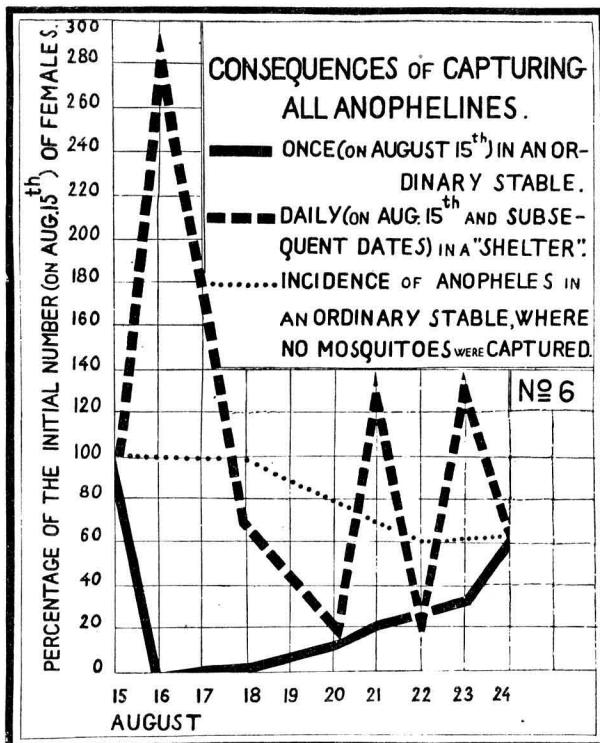


Fig. 2.

TABLE II (see: Diagram N°. 2).

Showing the consequences of removing all Anopheles; a. from a stable;
b. from an uninhabited shelter.

Date (August)	a. Experimental stable; All Anopheles removed on Aug. 15 th		Control. Three neighbouring exper- imental stables: not interfered with		b. Uninhabited shelter: Anopheles removed daily	
	Number of females	Percent. of number on Aug. 15 th	Number of females	Percent. of number on Aug. 15 th	Number of females	Percent. of number on Aug. 15 th
15	4590	100	9180	100	162	100
16	0	0			475	293
17	146	3			294	181
18	174	4	9011	98	114	70
20	632	14			37	23
21	1099	24			226	139
22	1275	28	5542	60	49	30
23	1569	34			226	139
24	2861	62	5680	62	112	69

Note. 1st. In the first stable („a“) the number of females was equal to 62% of the original on Aug. 24th, and did not increase anymore after that date. The same applies to the three control stables. So we take it, that the first one, on Aug. 24th, contained as many mosquitoes as might have been expected, if not interfered with on the 15th.

2nd. The note at the foot of table I also applies to this table.

Here we find the unmistakable effect of the "daily turnover" as described by ROUBAUD and BARBER & HAYNE. Our observations completely confirm theirs. But at the same time they show that this phenomenon is only the expression of one of the (probably many) ways in which Anopheles behave in their restingplaces. The "daily turnover" is an extreme variant of a number of possibilities, ranging from the conditions in which a complete change in the anopheline population occurs every night, through those experienced by MISSIROLI and HACKETT (repopulation of an emptied stable completed in 3—6 days) and by ourselves (repopulation completed after 9 days), up to conditions approaching more or less those, observed at a time when the sexual activity of Anopheles has come to an end and they settle down, prior to hibernation or semihibernation.

This last-named condition does not belong to the breeding season and so it lies outside the scope of this paper. But it is evident that removing Anopheles from a stable, where

the mosquitoes have finished their sexual activity, becomes absolutely fatal to the subsequent collection of reliable data. For such a stable often remains empty, even when catching was done in early autumn only, when the weather is quite warm still and the mosquitoes feeding actively (without however developing their eggs). At such a time one is apt to forget the fundamental change which has occurred in the life of the mosquitoes. We almost committed a gross mistake, while observing in early autumn the scanty numbers of Anopheles in the stables just outside the area under control. We took this as an indication of the effect of our antilarval measures extending further than the boundary of this area. Only after observing a similar reduction in stables, sufficiently far away (5 km.) to render this influence imperceptible, it occurred to us, that this reduction of the mosquitoes in early autumn was due to continuing the practice of killing them each fortnight, at a time sexual activity had come to a close.

The coincidence of : 1^o. the variability of the daily numbers, 2^o. the large quantity of males, 3^o. the rapid repair of the losses consequent upon the removal of the total anopheline population, is not merely accidental, if explained on the assumption that the newly hatched Anopheles (which show a proportion of 50 % of males approximately) gather provisionally in the restingplaces of the "sheltertype" (taking a blood-meal or not, according to circumstances), pending further distribution among more permanent settlements.

3. Experiments with stained mosquitoes.

In addition to the above observations, we have carried out experiments in stables, in order to determine the length of time stained mosquitoes remain there. For this purpose all Anopheles were captured in four experimental stables, stained and released, each lot in its own stable¹⁾. Out of :

1950	stained females,	588	were recaptured after one day	= 30 %.
1820	" "	659	" " two days	= 36 %.
1650	" "	45	" " five "	= 3 %.
2240	" "	11	" " ten "	= 0.5 %.

For the purpose of the present investigation this result is more satisfactory than the preceding one, which showed that a stable, where Anopheles have been removed, requires 9 days to reestablish normal conditions. For this experiment suggests that this process goes on at a

¹⁾ In other words: each time a separate stable was reserved to ascertain the number of stained mosquitoes among the total population present in that stable on the 2nd, 5th and 10th day after the release. Other investigators determined this number in one single stable, on various dates following upon the release of the marked mosquitoes. They did so, by examining a sample only of the mosquitoes present on each one of these dates. It is evident that this difference in method will tend to raise our figures in comparison with theirs.

quicker rate, as only little of the original stock remains after 5 days¹⁾. But we hesitate to rely on this kind of evidence so long as we do not know the effect of the staining-process on the subsequent movements of the mosquitoes subjected to it. It may inhibit their flight, but it may also render them restless ; and in that case an experiment like this is very inconclusive.

4. *Evidence regarding the occurrence of the "daily turnover" in human dwellings.*

For our purpose, this point is less important, because we never used the Anopheline incidence in houses as a gauge of the effect of our antilarval work.

Owing to the small numbers of Anopheles in human habitations and the impossibility to perform experiments (the inhabitants do not tolerate them), the evidence adduced here is necessarily circumstantial. It is based on a comparison of houses and stables, with regard to : a. the incidence of males; b. the percentage of females with human blood; c. the percentage of infected females.

a. *The relative number of males.* — In the vicinity of Amsterdam this number, observed over a period of 9 years, during the months of June—August, is higher in houses (16%) than in stables (8%). If the coincidence of a high number of males and a short stay in the same place applies to other cases, as well as to those mentioned before, this difference points to Anopheles staying longer in stables than in houses. But the percentage of males in the latter is too low to suggest the occurrence of a "daily turnover".

b. *Percentage of Anopheles with human blood in houses.* — The proportion of the Anopheline incidence in houses and stables is about 1 : 200 in the vicinity of Amsterdam, the average number per house is ten approximately. Assuming that all mosquitoes, which are found of a morning in a given house and which contain recently ingested blood, can only have bitten the inhabitants of that particular house and ought, therefore to be excluded from the present consideration, the odds against mosquitoes with "black blood" in their stomachs (i.e. an almost digested bloodmeal taken at some earlier night) having entered this house, after leaving some other human habitation and not some stable, are as 200 : 1 in rural areas. If a "daily turnover" occurs, mosquitoes with "black blood" ought to show the human precipitin test very rarely, thrice in 200 approximately, allowing for an incidence of 0.7% of engorged mosquitoes, with human blood, in stables. As a matter of fact, 69% of these mosquitoes were found to contain human blood²⁾. So the majority of them must have stayed at least two nights in the same house.

c. *Infected mosquitoes in houses.* — Infected mosquitoes are extremely rare in houses from April—August (SWELLENGREBEL 1922, 1924). This fact suggests that, in summer, they remain less than four days (i.e. the average time necessary for young zygotes to grow to a size observable in ordinary routine-examination³⁾) in the house, where they

¹⁾ It is not even certain, that the stained mosquitoes, found after 5 and 10 days, actually stayed there all the time and did not return to the same spot after some flight, actuated by the (hypothetical) "homing instinct".

²⁾ Eliminating the reactions with negative result, this figure increases to 89%. Anopheles with recently ingested blood show the human precipitin-reaction in 86% of the cases (88% without the negatives). This suggests that the mosquitoes remain in the house where they took their blood-meal, during the time it takes to digest it (3—4 days under the prevailing conditions).

³⁾ This average was established experimentally, under optimal conditions as to temperature. SCHÜFFNER and HYLKEMA (1921) arrived at the same figure, working with *A. ludlowi* in the tropics. So our figure is probably too low.

acquired their infection. That we do not recover them in stables is no wonder, considering the enormous numbers of mosquitoes, among which they are hiding there. During the autumn and winter, when sexual activity has stopped, and mosquitoes remain where they are, we find 5–6% of infected Anopheles in houses of malarious localities, with a gradual increase of the proportion of mature infections, from 44% in October and 61% in November, to 87% in December. During the same period they are very rare in stables (0.16%). These conditions are the exact opposite of those in Palestine, described by KLIGLER and LIEBMAN (1928) where infected *A. elutus* are confined to houses in summer but migrate to stables prior to hibernation.

The circumstantial evidence summarised here does not, as a whole, support the view, that the "daily turnover" is of common occurrence among *A. maculipennis*, visiting houses in this country. Although the relative number of males suggests that the length of time they stay in houses is inferior to that in stables, it is longer than one day and may be approximately set down at three or four.

5. Summary.

We take the safer course, to disregard the experiments with stained mosquitoes altogether, and to accept the evidence that the consequences of catching Anopheles in an ordinary stable extend over a period of nine days. In that case our method of testing the effect of antilarval measures (by comparing the anopheline density, within the protected centre of the area subjected to these operations, with that in the region outside this area) needs not be discredited. For daily catches were carried out in the "shelters" only, and these showed the unmistakable symptoms of the "daily turnover". In the stables the catches were either limited to once a fortnight or we did without them altogether.

But with a view to the custom in experimental stations, to rely on daily catches of mosquitoes, in order to establish the local anopheline density or to check the results of antilarval measures, it seems worth while to consider the results of the present inquiry. To prevent the observations becoming unreliable as a consequence of their being repeated too often, it is essential to ascertain : 1^o. the type of the various restingplaces selected as the objects of the routine observations, with regard to the occurrence of the "daily turnover"; 2^o. the physiological status of the Anopheline population concerned in the investigation, whether sexually active, still feeding after the close of sexual activity, or completely inactive.

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