Conclusion:

Clinical observations suggest the factor of auto-intoxication in catatonia. Therefore a series of experiments have been begun in our laboratory concerning the possibility of the production of catatonia through auto-intoxication in experimental animals.

Some of these experiments with ligatures of the intestine produced pronounced positive results.

We may remember, that in human pathology the hypothesis of enterogenesis in dementia praecox, has been proposed (f.e. by BUSCAINO) ¹).

Since experimental catatonia can be produced in many ways, it may not be concluded from the above, that human catatonia results, in every instance from intestinal-intoxication. Therefore, the other biological possibilities of experimental catatonia must be investigated. Such experiments are now in the process of investigation.


Medicine. — On the seasonal longevity of Anopheles maculipennis in Holland with reference to their ability to act as malarial vectors.

By A. DE BUCK and N. H. SWELLENGREBEL. (From SWELLENGREBEL's Laboratory in the Institute of Tropical Hygiene [Director Prof. Dr. W. A. P. SCHÜFFNER] of the Royal Colonial Institute at Amsterdam). (Communicated by Prof. W. A. P. SCHÜFFNER).

(Communicated at the meeting of February 23, 1935.)

1. Introduction.

JAMES, NICOL and SHUTE ¹) have shown that the rate of survival in batches of infected anopheles (i.e. the percentage still alive on the day sporozoites appear in the salivary glands) varies with the month in which they have been infected. The rate of survival is high (about 50%) from August till November, dropping to about 30% in December and January, still lower (about 20%) in February and March, to attain the lowest level (less than 10%) in April and May. In June and July it starts rising again but not above the level reached in February and March. According to these data the year can be divided into two seasons, one is favourable to malarialtransmission: August to November, the other is not: December

to July, the second one including the greater portion of the period of
sexual activity of the mosquitoes. This sexual activity, endangering life
by the ovulation and parturition it entails, seriously impedes malarial
transmission by mosquitoes in the laboratory and still more so in nature,
where anopheles are threatened by the dangers consequent upon the long
flights they are forced to take for the sake of oviposition. Not until the
moment sexual activity is over and they find an undisturbed shelter in
human dwellings, anopheles attain that particular position JAMES had
postulated as the one indispensable for a truly effective malarial trans-
mission.

In Holland JAMES’ results were particularly interesting, not only because
they agree with observations collected there but also because they have
opened vistas of an entirely new interpretation of these observations.

We knew already as a broad rule that anopheles (A. maculipennis var. atroparvus)
in Holland are mostly found malaria-infected in September—December, a period prac-
tically coinciding with JAMES’ first (favourable) season. Infected mosquitoes are rare
during the time the malarial season reaches its acme, viz. in May—July, i.e. a time
included within JAMES’ second (unfavourable) season. In agreement with ROUBAUD’s 2)
views, we interpreted these observations by assuming that infected anopheles are just
as numerous in summer as in autumn but that they cannot be found in summer in
human dwellings because: 1. anopheles do not stay there as they have always to
leave them for the sake of oviposition; 2. infected anopheles are speedily lost in the
inhabited stables which attract them much more than human dwellings. At their return
from the breedingplaces. This “stabular attraction” is no longer available in autumn
as it influences none but anopheles on their returnflight from the breedingplaces. In
autumn there are no such flights as oviposition has stopped although blood-feeding
continues. Anopheles stay where they happen to be when the process of ovulation
comes to a close: in the stables where they are numerous but harmless; in human
dwellings where they are comparatively rare but where one single specimen is of more
value to malarialtransmission than a hundred in summer.

JAMES’ observations admitted of a different interpretation of the observ-
ations made in Holland: It is not their being lost in stables, which
renders it difficult to find infected anopheles in summer, but their being
actually rare because of the increased mortality at that time of the year.

2. Longevity of infected anopheles caught in nature and infected
in the laboratory.

Table No. 1 and the black columns of the diagrams No. 1 and 2 are
showing month by month: a. the number of anopheles infected by causing
them to bite a carrier of benign tertian gametes on a certain day of that
month; b. the number surviving on the day (usually the 10th—12th after
the infecting meal) the first specimen of each separate batch of mosquitoes
was found harbouring salivary sporozoites; c. the number surviving
4 weeks after the infecting meal. We have added this third column for

TABLE I. Anopheles maculipennis var. atroparvus caught in nature experimentally infected with benign tertian malaria.

Rate of survival.

<table>
<thead>
<tr>
<th>Month</th>
<th>Rate of survival at the time salivary infection had become established</th>
<th>Rate of survival four weeks after infecting meal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total of anopheles biting gamete carrier 1)</td>
<td>Total of anopheles biting gamete carrier 2)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>Percent (rate of survival)</td>
</tr>
<tr>
<td>Jan.</td>
<td>420</td>
<td>265</td>
</tr>
<tr>
<td>Feb.</td>
<td>433</td>
<td>287</td>
</tr>
<tr>
<td>March</td>
<td>496</td>
<td>291</td>
</tr>
<tr>
<td>Apr.</td>
<td>849</td>
<td>374</td>
</tr>
<tr>
<td>May</td>
<td>190</td>
<td>45</td>
</tr>
<tr>
<td>June</td>
<td>207</td>
<td>106</td>
</tr>
<tr>
<td>July</td>
<td>328</td>
<td>190</td>
</tr>
<tr>
<td>Aug.</td>
<td>607</td>
<td>389</td>
</tr>
<tr>
<td>Sept.</td>
<td>251</td>
<td>202</td>
</tr>
<tr>
<td>Oct.</td>
<td>354</td>
<td>276</td>
</tr>
<tr>
<td>Nov.</td>
<td>635</td>
<td>481</td>
</tr>
<tr>
<td>Dec.</td>
<td>352</td>
<td>270</td>
</tr>
</tbody>
</table>

1) Not including mosquitoes sacrificed to ascertain the presence of salivary infection.
2) This total is less than that in the first column because a number of mosquitoes had been sacrificed for various reasons after salivary infection had become established.

practical considerations, arguing that sporozoites in Holland take a longer time in maturing under natural conditions, than they do in our “tropical chamber” at 80° F. and, moreover, that it is no use for anopheles to have their salivary glands invaded by sporozoites unless some additional time is allowed for their transmitting the infection.

The anopheles referred to in Table I, II and Diagram No. 1 and 2, were A. maculipennis var. atroparvus. They had been allowed to bite gamete-carriers once, rarely twice or more times, after which they were transferred to the “tropical chamber” (temperature 80°, relative humidity 90%), where each batch of mosquitoes was kept separate in cages of 2.4 cub. ft. and fed on sugar-water. By the time sporozoites might be expected to make their appearance in the salivary glands, a few mosquitoes were sacrificed out of each batch. When salivary infection was detected on a certain day (usually the 10th—12th), the rate of survival was computed for that particular day, after which the cages were transferred to an unheated room (temperature 60°—75°
in summer, 50°—52° in winter, 37°—39° during frosts). Feeding on sugar-water was continued unless the mosquitoes were put into requisition to infect a patient. The rate of survival was established a second time 4 weeks after the infecting meal, i.e. 16—18 days after salivary infection had first been detected and anopheles had been transferred from tropical climate to one natural to Holland.

Not all anopheles surviving on the 10th—12th day were found infected. Their sporozoite-rate was 72% as established by the dissection of 2042 specimens. Within the period of March—October this rate was 68%.

On the whole these results well agree with James’. It is true our rates of survival are all higher than his, the minimum is not so low and the rate of survival in Sept.—Oct. is not so much above that during the beginning of the year. As a consequence the favourable and unfavourable seasons are less distinctly separated. This, however, is true only of the rates of survival computed at the time salivary infections make their appearance. The rates established on the 28th day after the infecting meal (and they are the ones which are of really practical importance) are in complete agreement with James’ figures: a favourable season from September till December (rate of survival 59%), an unfavourable one from April till August (rate of survival 15%) joined by a period of transition in January—March (rate of survival 32%). The sporozoite-rates of anopheles infected within these three periods are about the same, viz. 74%, 70% and 71% respectively. So the summer mosquitoes are indifferent malaria vectors not because they are bad carriers but because they are too short-lived.

3. Longevity of infected anopheles bred in the laboratory.

Keeping alive anopheles caught in nature (in stables around Amsterdam) was found to be most difficult during the final stages of the life of the hibernating mosquitoes, when they leave their wintershelters to deposit their ova and, then, to die. In May, for instance, the rate of survival was 24% only on the day salivary sporozoites appeared and as low as 8% on the 28th day. So we resolved to try whether laboratory-bred anopheles would do better and they answered our purpose so well that we continued their use during the whole of the unfavourable period.

In March—April and in September—October we bred our anopheles in the tropical chamber, in May—August in an unheated room. The sporozoite-rate, established in 477 specimens, was found to be 63% which is about the same as in anopheles caught in nature and infected during the same months.

Table II and diagrams 1 and 2 (white columns) show rates of survival on the first appearance of salivary infection two to three times higher in laboratory-bred mosquitoes infected in April and May than in the hibernating generation caught in stables and infected during the same period. In June and July the superiority of laboratory-bred anopheles
TABLE II. Anopheles maculipennis var. atroparvus bred in the laboratory experimentally infected with benign tertian malaria.

Rate of survival.

<table>
<thead>
<tr>
<th>Month</th>
<th>Rate of survival at the time salivary infection had become established</th>
<th>Rate of survival four weeks after infecting meal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total of anopholes biting gamete carrier 1)</td>
<td>Percent (rate of survival)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Mrch.</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>Apr.</td>
<td>78</td>
<td>77</td>
</tr>
<tr>
<td>May</td>
<td>233</td>
<td>154</td>
</tr>
<tr>
<td>June</td>
<td>219</td>
<td>165</td>
</tr>
<tr>
<td>July</td>
<td>162</td>
<td>139</td>
</tr>
<tr>
<td>Aug.</td>
<td>62</td>
<td>44</td>
</tr>
<tr>
<td>Sept.</td>
<td>352</td>
<td>335</td>
</tr>
<tr>
<td>Oct.</td>
<td>61</td>
<td>52</td>
</tr>
</tbody>
</table>

1) See note 1) Table I.
2) See note 2) Table I.

over wild ones becomes less marked, and in August—October there is little difference between the two groups.

The superiority of laboratory-bred anopheles is even more marked and extends over a longer period when measured by the rate of survival after 28 days: in April—May and in July—August it is three to five times greater than in wild anopheles and it is not till September—October that the two groups become almost equal in this respect.

Here it is no longer the revived hibernating wild generation only which is inferior to the laboratory-bred anopheles infected simultaneously, but likewise the wild summer generation as far down as August, i.e. anopheles weakened by the wear and tear of an outdoor life during the period of sexual activity, to which they had been exposed previous to their being infected with malaria and from which the laboratory-bred generation had been protected.

It may be noticed that the laboratory-bred anopheles show a drop in their rate of survival over 3 successive months (April—June). As the numbers of laboratory-bred anopheles we worked with are necessarily much smaller than those of the wild anopheles, we are not sure whether this drop in spring and early summer has any more significance than the ones observed in August and October. If it has, it evidently cannot be
Diagram No. 1.

Monthly rate of survival at the moment sporozoites make their appearance in the salivary glands of:

a. Anopheles caught in stables (black columns).

b. Laboratory-bred Anopheles (white columns).

Note: The black columns stand in front of the white ones, hiding the basal portion of the latter.
explained — as it was in wild anopheles in April and May — by the debility of the rearguard of the hibernating generation. For the laboratory-bred anopheles in those months are equal to the first summer generation. The change in breeding conditions occurring in May (from tropical chamber to unheated room) may be partly responsible for this lessened rate of survival of laboratory-bred anopheles.

Diagram No. 2.
Monthly rate of survival four weeks after the infecting meal of:

a. Anopheles caught in stables (black columns).

b. Laboratory-bred Anopheles (white columns).

Note: The black columns stand in front of the white ones, hiding the basal portion of the latter.
4. Longevity of heavily and slightly infected anopheles.

In the foregoing no account has been taken of the degree of infection of anopheles as estimated by the number of oocysts on the stomach. In the following account, referring to anopheles caught in nature only, we are going to mend this defect by considering separately the longevity of anopheles suffering from:

1. Heavy infections: i.e. batches with 93% of the mosquitoes infected, 91% of these with more than 20 oocysts, 80% with 100 oocysts or over, average per infected mosquito: 205 oocysts.

2. Slight infections: i.e. batches with 31% of the mosquitoes infected, 18% of these with more than 20 oocysts, none over 80, average per infected mosquito: 12 oocysts.

As the mosquitoes were infected for practical purposes (treatment of G. P. I.) we had to be careful not to sacrifice too many of them for the sake of assessing the degree of infection. So the number of mosquitoes examined in this way is necessarily much smaller than the group dealt with in the preceding paragraphs. For that reason their seasonal arrangement had to be in groups comprising three months instead of one.

The result of this arrangement is shown in Table III and Diagram 3.

**TABLE III. Anopheles maculipennis var. atroparvus caught in stables infected with benign tertian malaria.**

<table>
<thead>
<tr>
<th>Three-monthly period</th>
<th>Rate of survival at the time salivary infection had become established. (see Diagram 3 A)</th>
<th>Rate of survival four weeks after infecting meal. (see Diagram 3 B)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total of anopheles biting gamete carrier</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>Anopheles still alive on the day salivary infection was first detected</td>
<td></td>
</tr>
<tr>
<td>Ja.—Mr.</td>
<td>137 105</td>
<td>77%</td>
</tr>
<tr>
<td>Ap.—Jn.</td>
<td>128 46</td>
<td>36%</td>
</tr>
<tr>
<td>Jl.—Sp.</td>
<td>114 72</td>
<td>63%</td>
</tr>
<tr>
<td>Oc.—Dc.</td>
<td>388 303</td>
<td>78%</td>
</tr>
<tr>
<td>Total</td>
<td>767 526</td>
<td>68%</td>
</tr>
</tbody>
</table>

A. Heavily infected anopheles.
TABLE III. (Continued).

B. Slightly infected anopheles.

<table>
<thead>
<tr>
<th></th>
<th>Ja.—Mr.</th>
<th>Ap.—Jn.</th>
<th>Jr.—Sp.</th>
<th>Oc.—Dr.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>896</td>
<td>583</td>
<td>457</td>
<td>624</td>
<td>2560</td>
</tr>
<tr>
<td></td>
<td>545</td>
<td>193</td>
<td>346</td>
<td>435</td>
<td>1519</td>
</tr>
<tr>
<td></td>
<td>61%</td>
<td>33%</td>
<td>76%</td>
<td>70%</td>
<td>59%</td>
</tr>
<tr>
<td></td>
<td>682</td>
<td>529</td>
<td>284</td>
<td>608</td>
<td>2103</td>
</tr>
<tr>
<td></td>
<td>223</td>
<td>48</td>
<td>140</td>
<td>302</td>
<td>713</td>
</tr>
<tr>
<td></td>
<td>33%</td>
<td>9%</td>
<td>49%</td>
<td>50%</td>
<td>34%</td>
</tr>
</tbody>
</table>

Taking account of the totals only, the difference between the two groups seems to be negligible. But the quarterly figures separately show

Diagram No. 3 A and 3 B.

Quarterly rate of survival in anopheles caught in stables:

Diagram 3 A. At the moment sporozoites make their appearance in the salivary glands.

Diagram 3 B. Four weeks after the infecting meal.

Black columns: heavily infected anopheles.
White columns: slightly infected anopheles.

a considerable difference in the rate of survival of the two groups four weeks after the infecting meal. The heavily infected group lags much behind in the first and third quarter, in the fourth only (Oct.—Dec.) it keeps up with the slightly infected group. This difference, however, is
not yet apparent at the time of first apparition of salivary sporozoites. if anything the heavily infected group is slightly in the advantage.

So we conclude that there exists a greater mortality in heavily infected anopheles than in slightly infected ones. Whatever the cause may be of this increased mortality it is acting only during the unfavourable season of February—August and it affects the mosquitoes suffering from a salivary infection. Intestinal infection alone, however severe, does no apparent harm.

Conclusions.

These results, confirming JAMES, NICOL and SHUTE's obtained in England, and agreeing with observations in Holland on the presence of infected anopheles in human dwellings during autumn, emphasize the epidemiological importance of the autumnal mosquitoes by showing that the scarcity of summer infections in anopheles is not to be explained by stabular attraction only, but likewise by the short span of life of the reawakened wintergeneration and the subsequent summergenerations.

The comparison of anopheles bred in the laboratory and caught in nature confirms JAMES' views on the special importance as malarialvectors of anopheles protected from the dangers of an outdoor life.

The comparison of heavily and slightly infected anopheles, moreover, shows the menace to anopheline existence in spring and summer to be particularly serious for heavily infected mosquitoes.

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Botany — Rektifikation zu meiner Arbeit „Speichel-, Pankreas- und Aspergillusamylase (Taka-Diastase) als Gemisch zweier Arten von Amylasen. Von G. GIESBERGER. (Communicated by Prof. F. A. F. C. WENT)

(Communicated at the meeting of February 23, 1935.)