Pathology. — *Experimental malaria with protracted incubation.* By W. A. P. SCHÜFFNER, P. C. KORTEWEG and N. H. SWELLENGREBEL. (From the Institute of Tropical Hygiene, Amsterdam.)

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I. *Introduction.* By W. A. P. SCHÜFFNER.

1. *Korteweg's hypothesis.*

The manifestation of fever in malaria is generally held to be strictly controlled by the process of merogony of *Plasmodium* in the human host, as it is wholly dependent on the presence of a certain minimum number of parasites in the circulation. In *Pl. vivax* this minimum amounts to 20—50 parasites [1]) per cub. mm. It is reached 10—20 days after the infection, by each sporulating parasite, of 5—10 successive generations, yielding 10—20 merozoites every other day. Consequently, the length of the incubation of malaria is a function of the number of merozoites and of the period of the sporulation. As these two factors are fairly constant, the length ~~of~~ incubation can vary only within narrow limits.

As early as 1902 KORTEWEG's observations threw some doubt on the general applicability of this view. Among his patients some had never suffered from malaria and others a very long time ago. Among these groups of men he observed numerous cases of malaria in March—May, at the time of low anopheline density preceding the hatching of the first summergeneration. Considering his intimate knowledge of the whole population of his semi-rural practice, the careful record he kept of all his cases, and his invariable habit to check his diagnosis by bloodexamination, his statement that these spring-cases are primary ones (and no relapses, as was asserted by others) carry particular weight. His conclusion, *that these cases are due to infections acquired during the previous autumn,* merited more attention than it actually gained, except among the doctors of the Zaanregion (VAN DER HORST, 1903, and our late lamented malariologist SCHOO, 1905, among them), to whom this view became so familiar as to need no further proof.

2. *Confirmatory investigations.*

Apart from certain observations made during the war, where the interpretation is rendered doubtful owing to the possible application of quinine prophylaxis (FÜLLEBORN, 1924).

The investigation of this subject was taken up again by SWELLENGREBEL (1921, '22A, '24), showing *malarial infection in anopheles to be practically*

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[1]) The so-called "pyrogenic limit".

confined to autumn and winter (without a corresponding rise in the human malarial incidence) and to be *almost absent during the height of the malarial season.*

KORTEWEG (1921) supported his hypothesis by new evidence, showing that individuals, living in houses where malaria occurred in early autumn, run a greater risk of catching malaria before June 1st. than others, an observation confirmed by HONIG (1922).

The study of experimental malaria in man, showing that the injection of malarious-blood, during autumn and winter, causes a fever after an incubation of usual length, seemed clearly to disprove KORTEWEG's hypothesis. But JAMES and SHUTE (1926) showed, that this artificial transmission cannot be compared with the natural insect-transmission. For the latter frequently fails in autumn or winter, a circumstance which constitutes a serious obstacle to the introduction of insect-transmission in the practice of malarial treatment of paretics: The mental alienist naturally wishes his patients to catch malaria at a moment he judges to be the most propitious. If insect-transmission fails, he insists on the injection of malarious blood as a substitute. Unfortunately this attitude renders it very difficult to ascertain the subsequent history of paretics, who failed to develop malaria within the usual timelimits, after being subjected to insect-transmission in autumn or winter.

Nevertheless JAMES (1927) succeeded in tracing the history of such patients: one was bitten by infected Anopheles in November, two in December, the fourth one in February. *All four developed a typical malaria, but not before 6—9 months had passed.*

3. *Object of the present investigation.*

We deemed it of the highest importance to have these results confirmed. 1°. Because our psychiatrist and our venereologist, Prof. BOUMAN and Prof. MENDES DA COSTA had expressed their wish to have a regular supply of infected mosquitoes, as a means of treating special cases; 2°. To confirm KORTEWEG's hypothesis, because its further development, consequent upon SWELLENGREBEL's researches, had led already to practical applications in malarial control and, 3°. had proved, moreover, useful in explaining the phenomenon of "anophelism without malaria" in the Netherlands. (SWELLENGREBEL 1922B, SWELLENGREBEL, DE BUCK and SCHOUTE 1927—1928, VAN THIEL 1922).

II. *Autumnal transmission, of malaria with normal incubation, by numerous bites of infected mosquitoes.*

By P. C. KORTEWEG.

1. *Explanatory remarks.*

The first point mentioned at the end of the preceding paragraph was my principal consideration, as we could not hope the mental alienist to take an interest in malarial infection of paretics by way of insect-transmission, unless we succeeded in applying this method all the year round.

For my transmission experiments I made use of two lots of infected mosquitoes,

2. *Tabulated record of my transmission experiments.*

Patient. History of malaria	Bitten by mosquitoes of lot N⁰.	Number of times infected anopheles were given an opportunity to feed	Number of well-ascertained infecting bites	Dates on which they were inflicted	Date on which commenced the fever	Date on which parasites were first detected	Incubation; in days		Remarks
							maximum	minimum	
K. no history of malaria	2	14	5	Oct. 30—Nov. 5 1928	Nov. 11 1928	Nov. 12 1928	12	6	Male gametocytes on Nov. 23, 1928
W. History of malaria 7 years ago	2	41	9	Oct. 29—Nov. 6 1928	Nov. 14 1928	Nov. 9 1928	16	8	Spontaneous defervescence on Nov. 19. A few parasites remain till Dec. 17, 1928.
B. no history of malaria	2	24	13	Oct. 30—Nov. 3 1928	Nov. 21 1928	Nov. 24 1928	22	18	
C. History of malaria 7—10 years ago	2	45	8	Nov. 2—22 1928	no fever till Febr. 11 1929	Nov. 15 1928	—	—	Parasites found again on Nov. 22 and Dec. 10, 1928. Patient lost sight of since Febr. 11, 1929.
M. no history of malaria	3	38	12	Dec. 21 1928 Jan. 4 1929	Jan. 9 1929	Jan. 9 1929	19	5	

described as N°. 2 and N°. 3 in the next paragraph. In the following tabulated record, the expression "number of times infected Anopheles were given an opportunity to feed" means (as an instance) that, out of a lot of 10 anopheles , 3 were applied to the patient's skin for half an hour on the first, 3 on the second and 8 on the third day . The "number of well-ascertained infection bites" is the number of bites I myself saw inflicted by Anopheles, afterwards proved to be infected. This number is only a fraction of the number of infecting bites actually inflicted.

3. *Conclusion.*

These records show, that I succeeded four out of five times to cause a malarial fever to appear within the usual time-limits, after the bite of infected mosquitoes in autumn and winter. Considering, however, the heavy infection of the Anopheles I used (see next paragraph) and the number of bites they inflicted, this experiment cannot be regarded as a contradiction of my hypothesis on the autumnal origin of primary cases of malaria in spring.

III. *Autumnal transmission, of malaria with protracted incubation, by one or two bites of infected mosquitoes.* [1])

By N. H. SWELLENGREBEL, A. DE BUCK, C. E. DE MOOR, and J. M. H. SWELLENGREBEL—DE GRAAF.

1. *To imitate natural conditions, the infecting dose should be a light one.*

In Holland the average number of infected Anopheles present in every 4 houses where malaria occurred, during the whole time infected mosquitoes are most numerous (September—December), amounts to no more than three. (SWELLENGREBEL, 1924). Some of these do not even carry sporozoites at the time of examination. Granting that most of them would have done so in due course, it still follows that an average of less than one Anopheles is available for malarial transmission in houses, sheltering parasite carriers among the human inhabitants.

To give KORTEWEG's hypothesis a fair trial it is, consequently, necessary to effect autumnal transmission by one single bite of a sporozoite carrier. Even with this precaution, the heavy infection of our Anopheles (half of them carried more than 49 zygotes ; under natural conditions no more than one infected Anopheles in nine does so) and the fact that two of our subjects were bitten twice, were likely to influence unfavourably the result of our experiment.

2. *Data concerning the infected mosquitoes.*

The transmissions were effected with three lots of *A. maculipennis* (short-winged strain), infected with *Plasmodium vivax* (see table I).

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

TABLE I. *Details regarding the origin of the infected mosquitoes used in our experiments.*

N°. of Lot	Number of anopheles		Infecting bloodmeals ingested by the anopheles			Kind of food ingested by the mosquitoes on subsequent dates
	A. surviving at the moment of maturation of the sporozoites	B. carrying salivary sporozoites	A. number of meals	B. number of male gametes per 1000 leucocytes	C. Dates of the ingestion	
N°. 1	12	9	3	8—20	Oct. 11—13, 1928	Guineapig's blood only.
N°. 2	18	18	6	the same gamete carrier as in N°. 1.	Oct. 11—13 and 15—17, 1928	Human blood only.
N°. 3	7	5	3	7—10	Nov. 27—29, 1928	Guineapig's blood only.

Note: The human gamete-carrier infecting lot 1 and 2 had been infected, himself, by the injection of blood, containing plasmodia, which had been transmitted in this artificial way, without interruption, for 43 passages. The carrier, infecting lot 3, was KORTEWEG's patient "K", himself infected by insect-transmission.

The feeding of recently infected mosquitoes on guineapig's blood did not check the development of the parasite, a confirmation of OTTOLENGHI and BROTZU's results (1928). The number of infected Anopheles in lot 1 was less than in lot 2; but this is accounted for by the number of infecting meals in the latter being twice that in the former. It will be remarked that the bite of the mosquitoes of lot 1 invariably caused malaria after a prolonged incubation, those of lot 2 usually after an incubation of normal length. This might be explained by a lessened vitality of the sporozoites, developed in mosquitoes fed on non-human blood. But this explanation cannot be upheld; for our subject N°. 4 was also bitten by a mosquito of lot N°. 2 (fed on human blood exclusively). Moreover KORTEWEG's patient "M" was successfully infected (maximal incubation 21 days) by mosquitoes of lot 3, fed on guinea-pigs like lot 1.

3. *Data relative to the individuals infected in autumn, 1928.*

Owing to the difficulties encountered in this kind of experiments, when carried out with paretics, we employed a number of healthy subjects, who volunteered to be bitten by mosquitoes of lot N°. 1 (one also by a mosquito of lot N° 2).

They were:

1°. W. A. P. SCHÜFFNER; frequently acquired malaria while living in Sumatra. His last attack occurred in 1921. Lives in Holland since 1922. — 2°. P. C. KORTEWEG; suffered from simple tertian between 1880 and 1885 (acquired in Holland) but never since that time. — 3°. J. M. H. SWELLENGREBEL—DE GRAAF; had two attacks (subtertian and simple tertian) of malaria in Sumatra, 1917 and three (simple tertian) in Java, the last one in September 1918. Lives in Holland since 1920. — 4°. N. H. SWELLENGREBEL; had two attacks (simple- and subtertian) in Sumatra, 1917, one (simple tertian) in Java, 1918, the last one (subtertian) in Java, January 1919. Lives in Holland since 1920, but frequently visits highly malarious localities (the last time in October 1928); never caught malaria on these tours (N.B.: no prophylactic quinine). — 5°. A. DE BUCK; never had malaria, always lived in Holland. — 6°. C. E. DE MOOR; like N°. 5.

All these individuals inhabit localities in the province of North-Holland where malaria

is extremely rare; moreover, in the endemic regions of this province, malaria is very rare since 1923. Subject N°. 1 may have acquired an infection December 1928 in Cairo · N°. 4, October 1928 in Bulgaria. But if the malaria they developed this summer ows its origin to these hypothetical infections and not to the real ones they acquired experimentally, this fact needs not alter our conclusions.

We conclude, accordingly, that the attacks of malaria from which these individuals suffered in the summer of 1929, were caused by the bite of infected Anopheles in the autumn of 1928.

4. *Record of transmissions.*

Every Anopheles, biting one of our subjects, was dissected immediately afterwards to ascertain the extent of the infection of the salivary glands. The results of our experiments are collected in Table II.

TABLE II. *Tabulated record of our transmission experiments.*

Serial number of subject	Number of bites inflicted by infected anopheles	Origin and degree of salivary infection of biting anopheles	Date of infecting bite	Fever occurring on:	Parasites detected on:	Incubation	Remarks (Parasites 7 : 1000 means 7 Parasites per 1000 leucocytes
1	Once	Lot 1. Heavily infected	Oct. 30, 1928	June 26, 28, 1929	June 28, 1929	7 months 27 days	Tertian fever. Parasites 7 : 1000.
2	Once (without drawing blood).	Lot 1. moderately infected	Oct. 31. 1928	July 6—9 1929	July 6—10, 1929	8 months 6 days	Quotidian remittent fever [1] Parasites 54—580 : 1000.
3	Twice	Lot 1. heavily infected	Nov. 1, 1928	July 15, 17, 1929	July 17, 18, 1929	8 months 15 days	Tertian fever. Parasites 132—204 : 1000.
4	Twice	Lot 1. heavily infected Lot 2. heavily infected	Oct. 30, 1928 Dec. 5, 1928	July 20—26, 1929 —	July 20—26, 1929 —	8 months 18 days (7 months 16 days)	Male gametes on July 23, 25, in small numbers; Parasites 2—124 : 1000. Quotidian intermittent fever.
5 [2]	Once	Lot 1. heavily infected	Nov. 7, 1928	July 23—26, 1929	July 23, 25, 26, 1929	8 months 15 days	Quotidian remittent fever changing into tertian intermittent. Parasites 1—2 : 1000.
6	Once	Lot 1. moderately infected	Nov. 2, 1928	Aug. 2—4, 1929	Aug. 3, 5, 1929	8 months 30 days	Quotidian remittent fever. On Aug. 4th complete intermission. Parasites very few to 39 : 1000.

1) KORTEWEG's (1924) "initial malarial fever".

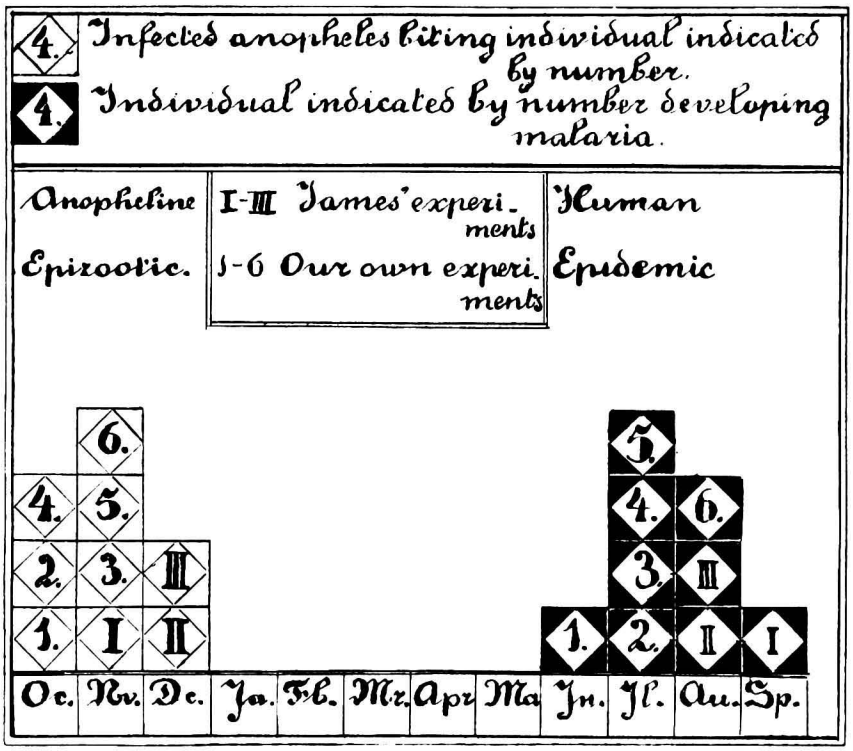
2) This case is remarkable for the number of parasites remaining well below the pyrogenic limit for the greater part of the fever period, although the successive paroxysms ran up to 38°.4, 40°.0 and 40°.3.

5. *Precautions against overlooking slight attacks.*

During the three weeks following the infecting bites, temperatures were taken thrice a day and thick films examined at frequent intervals. Later on, all our subjects closely watched their state of health; temperatures were taken and bloodfilms examined on the slightest provocation. In no case parasites were detected previous to the attacks mentioned in Table I. Only N°. 1 showed a pigmented leucocyte (but no parasites) on May 13, 1929. Consequently, the attacks of fever from which our subjects suffered cannot be taken as relapses of slight undetected attacks, occurring shortly after the infecting bite or later on [1]).

6. *Summary.*

The accompanying diagram, showing the combined result of JAMES' (except for the case infected in February [2])) and our own experiments,



may serve as a graphical summary of both. It is a model on a small scale of a summer-epidemic of human malaria, arising from an autumnal epizootic

[1]) This does not imply, that primary cases with prolonged incubation can be identified with those showing an incubation of normal length. The appearance of male gametocytes in N°. 4 suggests that these two are not the same.

[2]) Although there are still infected mosquitoes, human infections in Holland are unlikely to occur in February. Experiments to infect mosquitoes in that month were much less successful than in autumn (4 infected ones on 36 Anopheles, no sporozoite-carriers), owing to the high mortality consequent upon a precocious development of the eggs.

of anopheline malaria. The model is not a perfect one, as the maximum of the "epidemic" should have fallen in May or June instead of July. This suggests, that the majority of autumnal infections are acquired in September and October, not in November and December [1]).

On the other hand our experiments demonstrate, that autumnal anopheline infection not only accounts for malaria occurring before, but also after the first of June [2]). This evidence suggests, that the more important part of the annual malarial epidemic, i.e. the portion occurring before the second half of July, is due to infections acquired during the preceding autumn.

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[1]) This is in striking accordance with KORTEWEG's hypothesis, but somewhat at variance with the monthly prevalence of infected Anopheles, which is higher, on an average, in November and December, viz. 5 %, than in September and October, viz. 4 % (SWELLENGREBEL 1924).

[2]) This date (marking the hatching of the first summer generation of Anopheles) was formerly considered as a critical one, the beginning of the renewed activity of Anopheles as malarial vectors, consequent upon their increased numbers. At present we know (SWELLENGREBEL, DE BUCK and SCHOUTE 1929), that the Anopheline density, which reaches a minimum in May, does not markedly increase till the end of July (hatching of the second summergeneration).

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