Medicine. — *Further investigations on "healthy" human carriers of Plasmodium vivax in North-Holland.* By N. H. SWELLENGREBEL, A. DE BUCK and H. KRAAN. (From SWELLENGREBEL's Laboratory in the Department of Tropical Hygiene of the Royal Colonial Institute at Amsterdam.) (Communicated by Prof. W. A. P. SCHÜFFNER.)

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In our paper on the transmission of malaria in North-Holland [1]) we showed that "healthy" parasite carriers are a more important source of anopheline infection than persons actually suffering from malaria and seeking medical assistance as a consequence. The term "healthy" serves no other purpose than to make it clear that these persons are carrying tertian parasites without consulting the physician or taking medicine.

In the present paper we shall try to answer the following questions:

1. Are the parasites in the carriers' blood sufficiently numerous to infect anopheles? The field test yields an answer to the affirmative, but can this be corroborated by the experiment?

2. When writing on healthy carriers we had mainly children in view (persons under 16). What about adults, are they of equal importance in this respect?

1. *Experimental evidence that carriers with few parasites in their peripheral circulation do infect anopheles.*

Anopheles (*maculipennis atroparvus*, North-Holland strain) were allowed once to take their fill on G. P. I.-patients infected with the Madagascar strain of *Plasmodium vivax*, at a time they had entered upon the afebrile period following the regulation series of paroxysms.

The results can be tabulated as follows (Table 1). With their help we can now assess the importance, as a source of anopheline infection, of parasite carriers found in nature.

In 1936 we found in Uitgeest and Wormerveer 103 "healthy" human carriers with the following parasite count:

I:	1	with	50	parasites	per	100	leucocytes,	1	with	male gametocytes.
II:	7	„	12—25	„	„	„	„	3	„	„ „
III:	4	„	6— 9	„	„	„	„	1	„	„ „
IV:	10	„	1— 5	„	„	„	„	3	„	„ „
V:	22	„	1— 5	„	„	1000	„	5	„	„ „
VI:	59	„	1— 5	„	„	6000	„	1	„	„ „

[1]) Bulletin trimestriel de l'organisation d'hygiène de la Soc. d. Nations, Vol. V, no. 2, June 1936, pp. 360—369.

As far as the number of parasites goes, the "infecting power" of the healthy carriers of group I—IV may be gauged by the result of experiments 2*a*, 2*b*, 3, 4, 4*a*, 5, 6, 6*a* and 7. These experiments show that such carriers may infect 53—90 pct. of the anopheles which bite them *once*, even if male gametocytes are too scarce to be detected within a reasonable time on the day of infection, so long as these gametocytes can be found at some earlier date (compare the successful experiments 3 and 6*a* with the unsuccessful ones 4, 4*a* and 5 [1])).

In the same way the "infecting power" of the healthy carriers of group V and VI may be assessed by the result of experiments 1 and 2. These experiments show that carriers with very few parasites may still infect anopheles which bite them *once*; but the resulting rate of mosquito-infection is a low one. The provision with regard to male gametocytes is the same as in the preceding paragraph.

Experiments, 2, 2*a* and 2*b* are of special interest. They refer to the same carrier who remained afebrile since January 6. On January 12, the parasite-count being 1 in 1000, he infected 3 anopheles out of 81. On January 19 the parasite-count rose to 15 in 100 and again, on January 22, to 56 in 100. On January 19 this carrier infected anopheles up to 63 pct. His parasite-relapse was not accompanied by a clinical relapse. Consequently, under natural conditions, no physician nor medicines would have interfered with the process of anopheline infection. Provided one and the same batch of anopheles is staying in the carrier's house feeding off and on all the time, some of them are bound to bite the parasite carrier at the moment his parasites are numerous and then they will become heavily infected, just like batch 2 would have done if it had continued to feed on the carrier as long as batches 2*a* and 2*b*.

Elsewhere [2]) we have said that the essential condition for anopheles to become heavily infected by feeding on healthy carriers is to feed repeatedly on him, i.e. to stay with him in the same house for a considerable time. We can now specify this condition by adding that this statement does not mean that a carrier with few parasites cannot infect anopheles at one sitting but that he can do so at several. It means that repeated feedings are required to allow anopheles to avail themselves of the increase of the parasites in the carrier which is due to appear at some time or another. We know this requirement is met by the conditions existing in North-Holland houses in late summer and early autumn.

[1]) N°. 5 is not unsuccessful, but the rate of anopheline infection is much too low compared with no. 3 and 6*a*. The administration of myosalvarsan has obscured the gametocyte findings. The counterpart of this experiment, not recorded in the list, is the carrier with a parasite-count of 1 in 300, without male gamet., on the day 30 anoph. took his blood. None became infected, although male gamet. were present on earlier dates. But the patient had been given neosalvarsan on several occasions.

[2]) Second international congress for microbiology, Section 5, July 28, 1936.

TABLE 1. Result of experimental infection of anopheles with afebrile parasite-carriers.

Number of experiment and initials of carrier	Date of last fever paroxysm	Last date of finding male gametocytes before anopheles were infected	Anopheline infection					Remarks
			Date of infection	Number of parasites in carrier	Date of dissection	Number of anoph. dissected	Number found infected and number of oocysts	
1. Bl.	April 19	April 15 : 3 ♂ per 100 leuc.	April 27	1 parasite per 6000 leucocytes. No ♂	May 4	42	1 infected 1 oocyst	
2. Br.	Jan. 6	Jan. 4 : 2 ♂ per 100 leuc.	Jan. 12	2 parasites per 2000 leucocytes. No ♂	Jan. 18	81	3 infected 2, 1 and 1 oocysts	
2*a*. Id.	Id.	Id.	Jan. 19	29 parasites per 200 leucocytes. No ♂	Jan. 23 and later	30	19 infected. Average number of oocysts 16	Same carrier as no. 2; had no more fever, notwithstanding rise in parasites
2*b*. Id.	Id.	Id.	Jan. 22	112 parasites per 200 leucocytes. 1 ♂ per 200 leucocytes	Used for infecting patients. Consequently rate of infection not established exactly			
3. P.	Aug. 8	Aug. 10 : 1 ♂ per 200 leuc.	Aug. 12	5 parasites per 500 leucocytes. No ♂	Aug. 17	52	47 infected. Average number of oocysts 6	

TABLE 1. (*Continued*)

4. Hr.	May 3	♂ always absent	May 5	3 parasites per 200 leucocytes. No ♂	May 11	26	None	
4*a*. Id.	June 3	♂ always absent	June 25	3 parasites per 100 leucocytes. No ♂	June 30	45	None	Same carrier as no. 4 after relapse on June 1 and 3
5. Zw.	Jan. 26	♂ always absent	Febr. 18	10 parasites per 200 leucocytes. No ♂	Febr. 25	40	1 infected 1 oocyst	carrier had myosalvarsan injected on Jan. 25 (100 mg.), Febr. 1 (75 mg.) and Febr. 11 (100 mg.)
6. M.	June 10	June 15 : 1 ♂ per 200 leuc.	June 17	18 parasites per 200 leucocytes. No ♂	June 23	10	8 infected. Average number of oocysts 24	
6*a*. Id.	July 7	July 7 : 1 ♂ per 100 leuc.	July 17	18 parasites per 300 leucocytes. 1 ♂ per 3000 leucocytes	July 22	38	26 infected. Average number of oocysts 6	Same carrier as no. 6 after relapse from June 27 till July 7
7. C.	May 13	May 8 : 10 ♂ per 100 leuc.	May 18	51 parasites per 100 leucocytes. 2 ♂ per 100 leucocytes	Used for infecting patients. Consequently rate of infection not established exactly.			

2. *"Healthy" parasite carriers among adults and children.*

31 families comprising 80 adults and 184 children have been examined in spring and early autumn of 1936.

In that year 16 adults and 62 children had malaria [1]), an incidence of 20 and 34 pct. respectively. The parasite carriers numbered 24 or 30 pct. among the adults and 79 or 43 pct. among the children.

The 24 adult carriers had more malaria in 1936 than the other adults, viz. 8 of them (33 pct.), whereas 8 only out of 56 non-carriers had malaria (14 pct.).

In children conditions are different: 28 out of 79 carriers had malaria (35 pct.) and 34 out of 105 non-carriers (32 pct.).

Consequently the carriers among the children are not in a worse position than the non-carriers, but the carriers among the adults are.

The following table (2) shows the number of parasites and the presence of male gametocytes in the "healthy" carriers under observation.

TABLE 2. Number parasites and presence of male gametocytes in "healthy" carriers.

	Number of carriers who had had malaria in 1936 and who were carrying the following number of parasites						Number of carriers who had had no malaria in 1936 and who were carrying the following number of parasites					
	1 or more per 100 leucocytes		1—5 per 1000 leucocytes		less than 1 per 1000 leucocytes		1 or more per 100 leucocytes		1—5 per 1000 leucocytes		less than 1 per 1000 leucocytes	
	all	with male gam.	all	with male gam.	all	with male gam.	all	with male gam.	all	with male gam.	all	with male gam.
24 adult-carriers	5	2	1	0	2	0	1	0	4	1	11	1
79 children-carriers	6	3	5	0	17	0	10	3	12	4	29	0

Taking separately the carriers with 1 parasite or more per 100 leucocytes (i.e. the carriers which were shown in section 1 to be able to cause a heavy anopheline infection) and calling them *"good carriers"* we find:

1. 6 good carriers in 62 children who had malaria in 1936 (10 pct.) and 10 good carriers in 122 children who had not (8 pct.), i.e. the good carriers are about equally numerous in both groups.

2. 5 good carriers in 16 adults who had malaria in 1936 (31 pct.) and

[1]) The term "malaria" means malaria fever.

1 good carriers in 64 adults who had not (1½ pct.), i.e. nearly all good carriers are to be found among the adults who had malaria in the course of the year.

3. 3 carriers of male gametocytes in 62 children who had malaria in 1936 (5 pct.) and 7 in 122 children who had not (6 pct.), i.e. the gametocyte carriers are about equally numerous in both groups.

4. 2 carriers of male gametocytes in 16 adults who had malaria in 1936 (12 pct.) and 2 in 64 adults who had not (3 pct.), i.e. the gametocyte carriers are four times more numerous among the adults who had malaria in the course of the year than among those who had not.

Whatever may be the explanation of this difference between adults and children, the broad fact remains that adult carriers likely to cause a heavy anopheline infection, judging by the number of their parasites (see section 1) or by the presence of male gametocytes, are more liable than children to become conspicuous by an attack of malaria.

So far as that goes we may conclude that children carriers are more important as a source of anopheline infection than adult carriers. But there can exist no doubt that adults sometimes are very important in this respect. Last year, in Wormerveer we met with the following case which is well appropriate to illustrate this statement:

In family Kr. the four adult members (there are no children) had malaria: H. on July 30, M. on August 31, W. on Sept. 9, K. on Oct. 8. They were all treated with a 7 days' course of atebrin, 0.3 grammes a day, and had no relapses. Infected anopheles were found on October 8 (17 infected out of 77, 16 sporozoite carriers), November 2 (8 infected out of 25, 6 sporozoite carriers) and December 8 (4 infected out of 11, 3 sporozoite carriers, the majority of the sporozoites degenerated). The source of anopheline infection was M., who had had no more malaria after his atebrin cure, but who nevertheless was found to carry 22 parasites, comprising 1 male gametocyte per 500 leucocytes on October 30. On that same date W. was carrying 3 parasites per 6000 leucocytes, H. and K. had no parasites.

Conclusions.

1. Healthy carriers with one parasite per 100 leucocytes can infect 60 pct., or more, of the anopheles which fed on them only once, even if no male gametocytes could be detected on the day of infection, provided gametocytes had been found at some earlier date and no salvarsan had been given. The same applies to healthy carriers with one parasite per 1000 leucocytes or less, who can likewise infect anopheles, but at a much lower rate.

2. Anopheles sharing a house with a human parasite carrier for a long time, are bound sooner or later to acquire the infection if they continue

to feed. For they are always on the spot to catch the first opportunity offered by a temporary rise in the number of the carriers' parasites, especially if this rise is not accompanied by any marked febrile reaction.

3. Children are in a better position to infect anopheles than adults, because "good carriers" (with 1 parasite per 100 leucocytes or more) are as numerous in children who had no malaria in the course of the year as in those who had. In adults, on the contrary, nearly all "good carriers" are found among those who had malaria in the course of the year and so these adult carriers are more likely to be found out.

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