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THE INDUCED INFECTION OF SEMI-IMMUNE AFRICANS WITH SPOROZOITES  
OF LAVERANIA FALCIPARA ( - PLASMODIUM FALCIPARUM) IN LIBERIA

(Preliminary report)

by

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Introduction

In an attempt to learn more about the fate of sporozoite infections with Laverania falcipara in a population in a hyper-endemic area, local Liberians living under constant risk to malaria were infected with sporozoites of L. falcipara in the laboratory and any subsequent infections studied.

Material and methods

The subjects for infection were: (a) Liberian volunteers living in villages near the Liberian Institute where the sporozoite rate in Anopheles gambiae at the time was 4-6%, and (b) Liberian volunteers living at Kpain in the centre of a malaria eradication pilot project (DDT spraying) where A. gambiae and A. funestus had been non-existent for two years. All local subjects were tested for sickle-cell trait.

Laboratory-bred A. gambiae were infected with L. falcipara by feeding on suitable gametocyte-carriers. In four series the gametocyte donors were local Liberians who had lived in the vicinity of the Liberian Institute for at least the past two years. In one series the gametocyte donor was a Liberian who had lived his whole life in Voinjama, Western Province, 240 miles by road from the Liberian Institute. A. gambiae were maintained on sugar water at room temperature (22°-30°C) after the infective blood meal.

When sporozoites were present in the salivary glands batches of 3 to 8 A. gambiae were fed on the subjects and the mosquitos dissected subsequently to determine the number of infected bites per subject. These ranged from 2 to 6 infective bites per subject usually with heavily-infected mosquitos. The subjects were infected in 5 series as follows:

- (a) Twenty-seven local (i.e. Harbel) adults infected with local (i.e. Harbel) strains and examined for parasites and symptoms daily for 40 days.
- (b) Twelve local children aged 8 months to 11 years infected with a local strain and examined for parasites and symptoms.
- (c) Twenty protected adults from Kpain infected with a Harbel strain and examined for parasites and symptoms daily for 40 days.
- (d) Eighteen local adults infected with a Voinjama strain and examined for parasites and symptoms daily for 40 days.
- (e) Eight local adults infected and reinfected 12 days later with the same local strain, subjected to various procedures designed to diminish immunity to malaria and examined daily for parasites and symptoms for 50 days with extra examinations as necessary.

In series 5, 4 subjects underwent surgery 18 days after the second infection, 2 for hernia and 2 for hydrocoele. The other 4 subjects were inoculated intravenously with TAB vaccine ( $5 \times 10^6$  killed E. typhosa, B. paratyphosus A, B. paratyphosus B organisms in proportion 4:1:1). 18 days after the second infection, and  $20 \times 10^6$  killed TAB organisms 20 days after the second infection. They were blood donors 21 days after the second infection.

Thick blood films were taken from all subjects daily after the day of infection. The films were stained with Giemsa and 100 fields examined for parasites. Parasite counts were made by taking white cell counts every two days during parasite patency and counting the proportion of parasites to white cells daily. Subjective symptoms

were recorded daily by a physician who was kept in ignorance of the parasitological findings. Some attempt was made by the physician to correlate subjective symptoms to malaria. If a reasonable correlation appeared to occur and parasitaemia exceeded 3000 parasites per  $\text{mm}^3$  or if frank malaria intervened subjects were given specific treatment.

### Results

#### Series (a). Local adults, local strains of parasites

Of 27 subjects 15 (56%) showed parasites during the period from the time of infection to 10 days after but 11 of these showed no parasites at 10 days after infection. Ten (37%) showed no parasites from 10 to 40 days after infection. Nine (33%) showed a parasitaemia not exceeding 500 parasites per  $\text{mm}^3$  without symptoms referable to malaria in the same period. Four (15%) showed a parasitaemia of between 500 and 3000 parasites per  $\text{mm}^3$ , one with definite symptoms referable to malaria and 3 without symptoms referable to malaria in the same period. Four (15%) showed parasitaemia in excess of 3000 parasites per  $\text{mm}^3$  with definite symptoms referable to malaria in the same period.

Of this series 4 showed the sickle-cell trait and 3 of the 4 showed parasitaemias in excess of 3000 parasites per  $\text{mm}^3$  with definite symptoms referable to malaria.

In the period 10 to 40 days after infection and excluding those subjects treated for malaria 10 showed no parasites, 1 showed parasites for 2 days, 2 for 5 days, 1 for 6 days, 2 for 7 days, 1 for 11 days, 1 for 12 days, 1 for 13 days, 1 for 14 days, 1 for 16 days and 1 for 22 days; average 5.8 days.

#### Series (b). Local children, local strains of parasites

Of 12 subjects 8 (67%) showed parasites in the period from the time of infection to 10 days after but 3 of these showed no parasites at 10 days after infection. None showed no parasites from 10 days after infection onwards. None showed a parasitaemia not exceeding 500 parasites per  $\text{mm}^3$ . One (8%) showed a parasitaemia between 500 and 3000 parasites per  $\text{mm}^3$  in the period 10 to 36 days after infection without symptoms referable to malaria. Eleven (92%) showed parasitaemias in excess of 3000 parasites per  $\text{mm}^3$  (up to 25 000 parasites per  $\text{mm}^3$ ) and all showed some symptoms and were given antimalarial treatment.

Series (c). Protected adults, local strain of parasites

Of 20 subjects 2 showed a low parasitaemia at the time of infection but none showed parasites 10 days after infection. Eighteen (90%) showed no parasitaemia in the period from 10 to 40 days after infection. One (5%) showed a parasitaemia during the same period which appeared for 2 days only and never exceeded 20 parasites per  $\text{mm}^3$  and 1 (5%) showed a parasitaemia in excess of 3000 parasites per  $\text{mm}^3$  (up to 21,000 parasites per  $\text{mm}^3$ ) with definite symptoms referable to malaria on the twenty-first and twenty-second days after infection and parasitaemia from 17 to 22 days after infection. Treatment was instituted on the twenty-second day after infection.

Series (d). Local adults, Voinjama strain of parasites

Of 18 subjects 3 (17%) showed parasites during the period from the day of infection to 10 days after but one of these showed no parasites at 10 days after infection. Eight (44%) showed no parasites from 10 to 40 days after infection. Four (22%) showed a parasitaemia not exceeding 500 parasites per  $\text{mm}^3$  without symptoms in the same period. Two (11%) showed a parasitaemia of from 500 to 3000 parasites per  $\text{mm}^3$  without symptoms referable to malaria in the same period. Four (22%) showed a parasitaemia in excess of 3000 parasites per  $\text{mm}^3$  in this period; 3 showed definite symptoms referable to malaria and required treatment while one showed symptoms probably referable to malaria and a parasitaemia of 4000 parasites per  $\text{mm}^3$  but did not require treatment.

In the period 10 to 40 days after infection and excluding those subjects treated for malaria 8 showed no parasites, 1 showed parasites for 1 day, 1 for 2 days, 2 for 4 days, 1 for 5 days, 1 for 7 days and 1 for 12 days; average 2.3 days.

Series (e). Local adults, local strain of parasites, surgery or intravenous TAB vaccine and blood donors

Of the 4 subjects undergoing subsequent surgery one showed a high parasitaemia (up to 6500 parasites per  $\text{mm}^3$ ) with some symptoms (untreated) for several days prior to surgery but showed no parasites on the night before and at surgery. One hour after surgery a few parasites were seen eight hours after surgery and at all times after up to 50 days after the first infection no parasites were seen. In another subject parasites were present at the time of the first infection but disappeared five days after the first infection. Subsequently parasites were seen only one hour after surgery when a few were present.

In the two other subjects low parasitaemias not exceeding 500 parasites per mm<sup>3</sup> were present for 5 and 10 days prior to surgery, persisted at the same low level through surgery and for 5 and 20 days after surgery. At no time did the parasitaemias exceed 500 parasites per mm<sup>3</sup>, show any sharp peaks or cause symptoms referable to malaria.

Of the 4 subjects undergoing intravenous TAB inoculation and bleeding only one showed a small rise in temperature following the inoculation of  $5 \times 10^6$  TAB organisms but all showed fever (up to 104°F) following the inoculation of  $20 \times 10^6$  TAB organisms. One subject showed a high parasitaemia (5800 parasites per mm<sup>3</sup>) with some symptoms (untreated) on the fourth to the eighth day after the first infection. No parasites were seen 10 days after infection and no asexual parasites were seen subsequently. One subject showed parasites only one hour after each TAB inoculation when a few parasites were present and at no other time. One subject first showed parasites 4 days before TAB inoculation, continued to show less than 300 parasites per mm<sup>3</sup> for 12 days covering the TAB inoculation and bleeding episodes and then became negative for the remainder of the observation period. No sharp peaks of parasitaemia occurred and no especial effect of the inoculation and bleeding episodes were observed.

The last subject showed a parasitaemia not exceeding 300 parasites per mm<sup>3</sup> for 3 to 12 days after the first infection and then became negative, commenced to show a low parasitaemia again 22 days after the first infection which rose steadily to 1100 parasites per mm<sup>3</sup> 33 days after the first infection. Days 30 to 33 after the first infection were the days of TAB inoculation and bleeding. The parasitaemia then dropped to a low level for several days but on the thirty-eighth day after the first infection it rose sharply to 2900 parasites per mm<sup>3</sup> with confused symptoms not directly referable to malaria. This parasitaemia dropped equally sharply to zero on the forty-first day. At this time antimalarial treatment was administered at the subject's request.

#### Pre-patency

Taking all local adult subjects who were a parasitic on the tenth to twelfth day after infection and who subsequently showed a parasitaemia, parasites were first seen on days 15 to 38 after infection. Two subjects first showed parasites on day 15 after infection, 4 on day 16, 3 on day 17, 1 on day 18, 3 on day 21, 2 on day 25, 1 on day 27, 1 on day 29, 1 on day 34 and 1 on day 38; average 21.6 days. Among children a parasitic on days 10 to 12 after infection parasites were first seen in individuals on days 13, 13, 13, 20 and 26. In 9 children a sharp rise in parasitaemia was noted on days 11, 14, 15, 15, 16, 17, 21, 24, 25 after infection; average 17.4 days.

### Peak parasitaemias

Among adult parasitized subjects in the period 12 to 40 days after infection peak parasitaemias varied from 1 to 21 000 parasites per  $\text{mm}^3$  when untreated; average 2621 parasites per  $\text{mm}^3$ . The peak parasitaemias causing some symptoms probably referable to malaria were 2890, 3980 and 6365 parasites per  $\text{mm}^3$ . The peak parasitaemias before treatment which caused definite symptoms referable to malaria were 1460, 3960, 5120, 7170, 10 270 and 21 000 parasites per  $\text{mm}^3$ . The average of parasitaemias causing symptoms was 6915 parasites per  $\text{mm}^3$ . Among children treatment usually intervened while parasitaemia was rising sharply however parasitaemias of 7440, 9140, 15 460 and 25 920 were recorded.

### Symptoms

This portion of the study was pursued seriously for the first 36 adult subjects and the 12 children and subsequently merely kept as a record. It was found that only in cases where distinct chills and rigours, fever and headache all occurred were the symptoms correlated to the parasitaemia. In all other cases confusion reigned supreme. Of symptoms with some connexion to malaria headache, body-pain, bone-ache, joint-ache, back-ache, fever, cold and chills, cough and poor appetite were the common subjective complaints individually or several together. Absolutely no correlation was found between this type of subjective complaint and the parasitaemia. Individuals remaining aparasitic displayed as many symptoms as those showing continuous or sporadic parasitaemia. Such parasitaemias did not elicit the above symptoms with any regularity and the relation between parasitaemia and the above symptoms was completely haphazard. One subject reported himself completely well only when showing parasitaemia and complained of many and varied symptoms possibly referable to malaria only when aparasitic.

Among 65 adult subjects infected 10 (15.4%) showed symptoms of malaria correlatable with parasitaemia. Three out of four of sickle-cell trait carriers showed symptoms of malaria correlatable with parasitaemia.

### Discussion

These experiments were originally designed to explore a number of aspects of L. falcipara infections in semi-immune Africans but the inadequate results to hand have allowed only a few indications on some of these aspects as applied to adults only.

In our series about 15% of adult semi-immune Africans who live in a hyper-endemic area displayed high parasitaemias with clinical symptoms following relatively heavy sporozoite infection. The remainder may or may not show detectable parasitaemias. As shock usually forces enough parasites temporarily into the peripheral circulation to be detected it seems probable that the large majority of this remainder is in a state of parasitaemia though a proportion may possess only an occult parasitaemia. The pre-patent period of parasitaemia averages about 21 days and is therefore appreciably longer than the pre-patency of sporozoite-induced L. falcipara infections in non-immunes (11 days according to Coatney et al. 1947, Kitchen 1949).

A strain of L. falcipara taken from Voinjama 240 miles by road from Harbel behaved no differently in Harbel semi-immunes than did a Harbel strain. There was no evidence of wide immunological variation between the strains which confirms the work done by Voller and Bray (in press) who were unable to measure any immunological differences between strains from these two localities using a measure based on the fluorescent antibody staining technique. We are unable therefore to support the hypothesis that a number of immunologically differing strains exist in relatively small areas and its commonly held practical consequence that a semi-immune who travels relatively short distances in Africa is particularly liable to a "foreign" malaria infection with symptoms. (See also Davey & Robertson, 1957.)

The results of infecting subjects under protection by eradication of the vectors for two years at Kpain surprised us. Eighteen out of 20 showed no parasitaemia and only one showed parasitaemia and symptoms. All subjects were employees of the malaria eradication pilot project and some, at least, had access to antimalarial drugs. On the other hand two showed parasites at the time of infection and three more had shown parasites within the previous year. We feel entitled to conclude that two years of a protection which does not destroy the parasites themselves in man does not drastically lower immunity to malaria in the majority of adults.

The results of the effect of surgical interference and TAB inoculation with bleeding on malaria infections are interesting. In this experiment we attempted to obtain two different effects - firstly a surgical shock as a poll of experienced medical opinion in the country showed plainly a belief that major surgery and parturition were common if not invariable causes of malarial relapse or recrudescence in hyper-endemic endemic areas - secondly a fever-producing septicaemia followed by an appreciable blood

loss as the poll also elicited the opinion that intercurrent infection especially if pyrexial or appreciable blood-loss following accidents also caused a malarial episode, In our series only two in eight showed a parasitaemia above 500 parasites per mm<sup>3</sup>, one however showed before surgery and only one after TAB inoculation and bleeding. In neither case did the parasitaemia cause clear-cut symptoms. Thus 25% of this series showed a higher parasitaemia with some symptoms possibly referable to malaria and none showed definite symptoms referable to malaria only. Following surgery or TAB inoculation and bleeding only 12.5% showed a higher parasitaemia with some symptoms which compares with 15.4% showing definite symptoms in all the other series or 19% in local adults infected with local strains.

We cannot say that we have found any evidence to support the claims that surgery, fever-producing shock, a septicaemia due to killed organisms and blood-loss contribute towards a recrudescence of malaria in semi-immune adults under the conditions of our experiment.

Our tabulation of subjective symptoms gave us to believe that there is no firm symptomatological basis for such supposed clinical entities as "low-grade malaria", "chronic malaria", "a touch of malaria" as complete explanations for a variety of subjective symptoms in semi-immunes, such symptoms frequently being those which can be suffered by true malaria cases at the prodromal stage. We hasten to add that such clinical entities may well exist but short of a succession of distinct symptoms of malaria (rigor, fever, headache and long-bone ache) we do not believe that a symptomatological entity exists which can be recognized and diagnosed as something less than frank malaria among semi-immunes. We are unable to believe therefore that "sub-clinical malaria" is a recognizable and diagnosable entity in the absence of knowledge of the parasitaemia. We remain doubtful if the clinical entity exists among semi-immunes when the known parasitaemia is low.

No feature of the individual infections studied distinguished them from all other infections seen in the field under natural conditions. No characteristics presented themselves which would distinguish the "fresh infection" from a recrudescence of an old infection in, for instance, the absence of a vector.

### Summary

Semi-immune adults were infected with sporozoites of L. falcipara. Local strains in local adults caused frank malaria in 19% of subjects. A strain from 240 miles away caused frank malaria in 22% of subjects. A local strain in adults living in a sprayed zone and protected for two years caused frank malaria in 5% of subjects.

The pre-patent period of parasitaemia in semi-immune adults was an average of 21.6 days.

No correlation was found between symptoms and parasitaemia except in cases of frank malaria.

Surgical interference or intravenous inoculation of TAB vaccine followed by blood donation failed to alter the course of induced infection in eight semi-immune subjects.

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REFERENCES

- Coatney, G. R., Cooper, W. C., Young, M. D. & McLendon, S. B. (1947) Amer. J. Hyg. 46, 84
- Davey, D. G. & Robertson, G. I. (1957) Trans. roy. Soc. trop. Med. Hyg. 51, 450
- Kitchen, S. F. (1949) Symptomatology: general considerations. In: Malariology, Ed. Boyd, M. F., W. B. Saunders & Co., Philadelphia
- Voller, A. & Bray, R. S., Fluorescent antibody staining as a measure of malarial antibody. Preliminary report, Proc. Soc. exp. Biol. (N.Y.), (in press)

TABLE 1

	Total Number	Number <sup>*</sup> aparasitic	Number <sup>*</sup> parasit- aemia below 500 per mm <sup>3</sup>	Number <sup>*</sup> parasit- aemia 500- 3000 per mm <sup>3</sup>	Number parasit- aemia above 3000 per mm <sup>3</sup>	Number frank malaria
Local subjects, local strain	27	10 (37%)	9 (33%)	4 (15%)	4 (15%)	5 (19%)
Protected subjects, local strain	20	18 (90%)	1 (5%)	0	1 (5%)	1 (5%)
Local subjects, Voinjama strain	18	8 (44%)	4 (22%)	2 (11%)	2 (11%)	4 (22%)
Total	65	36 (55%)	14 (22%)	6 (9%)	9 (14%)	10 (15%)

\* From the tenth to the fortieth day after sporozoite infection.

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