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# TRANSMISSION BLOCKING IMMUNITY AND INFECTIVITY OF HUMAN POPULATIONS TO MOSQUITOES DURING MALARIA TRANSMISSION

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1) Measurement of the Infectious Reservoir in the Human Population

a) Parasite prevalences in the study villages.

Three vilages in the Madang study area have been selected for intensive study: Butelgut, Mebat and Maraga. These villages represent the range of variation observed in this area in mosquito biting density and prevalence of the three vector species Anopheles farauti, An.koliensis and An.punctulatus. The average biting rates (all species) during 1984-1985 were 67 bites per person per night in Butelgut, 155 in Mebat and 488 in Maraga (Burkot et al, in press). The mosquito population comprised mainly An.punctulatus in Butelgut, An.koliensis in Mebat and An.farauti in Maraga.

Six parasitological surveys at three-six month intervals have been carried out in these villages, at which blood films were made for determination of the rates of infection with Plasmodium falciparum, P.falciparum gametocytes, P.vivax, P.vivax gametocytes, P.malariae and P.ovale. Average prevalence for each age group for each of these species is given in Tables 1-3. The peak prevalence of P.falciparum was observed in the 5-9 years age group in Butelgut and Mebat but in the 10-19 years age group in Maraga. P.falciparum gametocyte rates peaked in all cases in the 0-4 year-olds. P.vivax rates (asexuals and gametocytes) peaked in the 5-9 years olds. Plasmodium falciparum gametocyte rates in all age groups were significantly higher in Butelgut (4.0%) and Mebat (3.6%) than in Maraga (2.2%) ($p<0.05$). In the 0-4 years age group, the P.falciparum gametocyte rate was significantly higher in Butelgut than in Maraga. The prevalence of P.vivax infections was

significantly higher in Mebat than the other two villages; Mebat also had the highest P.vivax gametocyte rate of 5.4%. The proportion of infections of either species with gametocytes was lower in Maraga than in the other two villages. Thus in Maraga 3.3% of P.falciparum infections had gametocytes, while in Butelgut and Mebat the percentages were 10.9% and 10.3% respectively. For P.vivax, 18.8% of infections in Maraga had gametocytes whilst in Butelgut and Mebat gametocytes were present in 40.0% and 36.2% of infections.

b) Direct measurement of infectivity to mosquitoes of individuals in the human population.

Random feeding of batches of laboratory reared Anopheles farauti was conducted at the parasitological surveys in the three study villages when the supply of mosquitoes permitted. In addition random feeds were performed in the villages of Uram, Bahor, Sah and Buksak. Anopheles farauti has not proved to be an avid membrane feeder and thus direct feeds were performed where possible if individuals gave their consent. Otherwise membrane feeds were performed with fingerprick blood samples. The results of the random feeds at the village surveys are given in Table 4. On average, 6.4% of feeds gave rise to an infection in the mosquito.

Because of the difficulty of feeding on the under five year-olds, who represent the age-group with the highest rate of P.falciparum gametocyte infections, mosquitoes were transported to Goroka, in the Papua New Guinea Highlands, to perform feeds on gametocyte

carriers presenting to the Goroka Hospital. Since malaria transmission does not occur in Goroka, these patients are adults who have acquired their infections in a coastal area. Blood samples were obtained from consenting persons and offered to An.farauti by membrane feeding. Results of these feeds are shown in Table 5. In Goroka half of the P.falciparum gametocyte carriers were infectious and one third of the P.vivax cases. Serum samples have been obtained from these individuals for later assessment of transmission-blocking immunity. In Madang, 28.6% of P.vivax cases were infectious, which is not significantly different from the proportion of 33.3% observed in Goroka.

Even when a person was infectious, not all mosquitoes in a batch became infected. Table 6 shows the P.falciparum carriers from both Madang and Goroka ranked in order of density, with the percent of mosquitoes infected and the average number of oocysts observed in each case. Table 7 gives similar information for P.vivax, although in this case only the feeds on infectious persons are given.

From the data on random feeds given in Table 4, and the age of the persons on whom feeds were performed, the proportion of each age group who are infectious can be determined (Table 8). From this data and the demographic profile of the study villages (Table 9), the proportion of the overall population who are infectious may be estimated by multiplying the proportion of persons infectious in a particular age group by the proportion of the population which that age group represents. By this method it is estimated that 5.4% of the population are infectious (for all malaria species).

c) Measurement of the infectious reservoir from wild caught mosquitoes.

The infectious reservoir is defined as the proportion of mosquito feeds taken on humans which pick up an infection. Recently Dr. Allan Saul of the Queensland Institute of Medical Research has developed a model which uses infection rates in wild-caught mosquitoes to estimate mosquito survival rate and the reservoir of infection (Saul et al, submitted). Data from mosquitoes caught either biting or blood-fed in resting catches may be used as input to the model, as long as the human blood index is also known.

Mosquitoes have been collected in the study villages and their infection rates (delayed oocyst rates and sporozoite rates) determined. Human blood indices from indoor and outdoor resting collections have been measured (Burkot et al, submitted). Although the mosquito collections have been completed, the data are still being analysed using the model of Saul et al for estimation of the infectious reservoir, and results of the analysis will be given in the next progress report.

2) Immunity and parasite antigenicity in relation to infectivity of the human population to mosquitoes

a) Naturally occurring serum factors which affect infectivity of gametocytes to mosquitoes.

To assess the effect of transmission blocking activity in serum from P.falciparum gametocyte carriers, blood containing gametocytes is fed to mosquitoes in the presence of autologous serum or washed and resuspended in non-immune serum. Sera may also be tested against P.falciparum gametocytes grown in culture. In Madang it has not proved possible to perform membrane feeds involving washing and resuspension of blood from gametocyte carriers, because of the extreme youth of such carriers and their low frequency in the population. Several experiments were however performed in Goroka. The results are given in Table 10. There was evidence for the presence of transmission-blocking activity in serum in two cases: 54 (P.vivax) and 36 (P.falciparum). In the other cases, resuspension of infected blood in non-immune serum did not result in non-infectious cases becoming infectious.

In order to test the effect of sera from individuals in Madang on P.falciparum gametocytes grown in culture, sera from a cross-sectional sample of individuals of all ages living in two villages in endemic areas (Agan and Tau) were taken to Dr. Richard Carter's laboratory at the National Institutes of Health. These studies were originally planned to take place at the Queensland Institute of Medical Research, but due to delay in the start of the grant, Dr. Carter had already returned to the N.I.H.. The sera

were combined with gametocytes of strain NF54 and fed to An.freeborni mosquitoes. Controls consisted of U.S.A. sera included in each experiment. Transmission blocking activity was assessed by the reduction in average number of oocysts on the mosquito stomachs at dissection 9 days after feeding. Results of one series of feeding experiments are shown in Fig 1. Each point represents one test of each serum. Forty-two sera have been tested so far, and nine of these consistently reduced infectivity to less than 5% of control.

When the sera were reacted with 125-I-surface labelled gametes, many (although not all) of the blocking sera immunoprecipitated a 230 KD gamete surface protein, and one reacted with the 230 KD and 48/45 KD proteins. A negative correlation was observed between the total c.p.m. immunoprecipitated and the average oocysts per gut (mean of two experiments). The data obtained in one series of immunoprecipitations is shown in Fig. 2. The relationship is described by the regression line $y = 106.8 - 23.76\ x$ ($t = 5.47$, $p<0.01$). Reaction of sera with particular proteins was investigated by counting the radioactivity associated with particular bands cut out of the polyacrylamide gels. Because of the differing intensity of infection in experiments not performed concurrently, results from such experiments cannot be combined. However, infectivity in the presence of different sera can be ranked, and Spearmann's rank correlation coefficient showed a strong negative correlation between infectivity and counts associated with the 230 KD protein (rho = -0.384, $p<0.01$) but not with the 48/45 KD doublet (rho = -0.142, $p=0.17$).

## REFERENCES

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Table 1: Average parasite prevalence by age in Butelgut.

| | Age Group | | | | |
|---|---|---|---|---|---|
| Species | 0-4 | 5-9 | 10-19 | over 20 | Total |
| P.falciparum: | | | | | |
| asexuals | 41.8 | 50.6 | 46.9 | 24.3 | 36.6 |
| gametocytes | 9.0 | 6.7 | 3.4 | 2.0 | 4.0 |
| P.vivax: | | | | | |
| asexuals | 10.4 | 22.5 | 7.6 | 4.2 | 9.1 |
| gametocytes | 9.5 | 13.0 | 2.0 | 0.0 | 3.6 |
| P.malariae: | 0.0 | 1.1 | 2.1 | 0.0 | 0.7 |
| P.ovale: | 0.0 | 1.1 | 0.7 | 0.0 | 0.4 |
| Overall: | 49.3 | 65.2 | 51.0 | 29.1 | 43.1 |
| N : | 67 | 89 | 145 | 251 | 552 |

Table 2: Average parasite prevalence by age in Mebat.

| Species | Age group 0-4 | 5-9 | 10-19 | over 20 | Total |
|---|---|---|---|---|---|
| P.falciparum: | | | | | |
| asexuals | 45.6 | 52.0 | 30.8 | 22.5 | 34.3 |
| gametocytes | 7.8 | 5.0 | 1.9 | 2.0 | 3.6 |
| P.vivax: | | | | | |
| asexuals | 22.2 | 26.0 | 10.6 | 8.5 | 14.9 |
| gametocytes | 10.5 | 11.4 | 2.8 | 1.4 | 5.4 |
| P.malariae: | 1.1 | 10.0 | 4.8 | 2.5 | 4.2 |
| P.ovale: | 1.1 | 0.0 | 0.0 | 0.0 | 0.2 |
| Overall: | 56.7 | 69.0 | 42.3 | 33.3 | 46.6 |
| N : | 90 | 100 | 104 | 204 | 498 |

Table 3: Average parasite prevalence by age in Maraga.

| | Age group | | | | |
|---|---|---|---|---|---|
| Species | 0-4 | 5-9 | 10-19 | over 20 | Total |
| P.falciparum: | | | | | |
| asexuals | 28.8 | 42.6 | 44.3 | 17.6 | 30.5 |
| gametocytes | 5.8 | 1.1 | 1.9 | 1.1 | 2.2 |
| P.vivax: | | | | | |
| asexuals | 8.7 | 25.5 | 11.3 | 1.1 | 9.6 |
| gametocytes | 1.4 | 4.6 | 1.4 | 0.8 | 1.8 |
| P.malariae: | 1.0 | 3.2 | 0.9 | 0.5 | 1.2 |
| P.ovale: | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Overall: | 33.7 | 62.8 | 53.8 | 19.3 | 38.1 |
| N : | 104 | 94 | 106 | 187 | 491 |

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Table 4: Mosquito feeds on individuals living in Madang.

| Slide result | Total feeds | Total feeds, >=5 mosq.* | No. pos feeds | % persons infectious |
|---|---|---|---|---|
| Negative | 151 | 103 | 0 | |
| Pf asex | 78 | 46 | 0 | |
| Total, Neg + Pf asex | 229 | 149 | 0 | 0.0 |
| Pf asex + Pfg | 7 | 4 | 1 | |
| Pfg only | 2 | 0 | 0 | |
| Total Pfg | 9 | 4 | 1 | 25.0 |
| Pv only | 32 | 21 | 7 | |
| Pf asex + Pv | 20 | 14 | 3 | |
| Total Pv | 52 | 35 | 10 | 28.6 |
| Pm only | 15 | 10 | 2 | |
| Pf asex + Pm | 7 | 4 | 0 | |
| Tot Pm | 22 | 14 | 2 | 14.3 |
| TOTAL | 312 | 202 | 13 | 6.4 |

*No. of feeds in which at least 5 mosquitoes were dissected.

Table 5: Mosquito feeds on patients attending Goroka Hospital.

| Slide result | Total feeds | Total feeds, >=5 mosq.* | No. pos feeds | % persons infectious |
|---|---|---|---|---|
| Pf asex + Pfg | 7 | 7 | 4 | |
| Pfg only | 1 | 1 | 0 | |
| Total Pfg | 8 | 8 | 4 | 50.0 |
| Pv only | 12 | 10 | 5 | |
| Pf asex + Pv | 6 | 5 | 0 | |
| Total Pv | 18 | 15 | 5 | 33.3 |
| TOTAL | 26 | 23 | 9 | 39.1 |

* No. of feeds in which at least 5 mosquitoes were dissected.

Table 6: Feeds on *P.falciparum* gametocyte carriers.

| ID No. | Location | Age | Density* | % inf | No.diss. | Mean oocysts |
|---|---|---|---|---|---|---|
| E/156-40 | Madang | 8 | 1/100 | 0.0 | 10 | |
| E/168-23 | " | 7 | 1/100 | 0.0 | 5 | |
| E/156-7 | " | 2 | 2/100 | 0.0 | 7 | |
| E/180-26 | " | 15 | 5/100 | 23.1 | 13 | 1.3 |
| JMS/G76 | Goroka | 35 | 6/100 | 11.1 | 18 | 3.5 |
| JMS/G13 | " | 25 | 12/100 | 63.6 | 11 | 3.0 |
| JMS/G231 | " | 33 | 16/100 | 0.0 | 33 | |
| JMS/G36 | " | 25 | 10/200 | 0.0 | 12 | |
| JMS/G49 | " | 39 | 10/200 | 0.0 | 10 | |
| JMS/G83 | " | 17 | 16/200 | 5.4 | 37 | 1.0 |
| JMS/G222 | " | 16 | 29/200 | 100.0 | 13 | 54.2 |
| JMS/G11 | " | 50 | 44/200 | 0.0 | 8 | |

* Density measured per 200 wbc or (for scanty infections) per 100 fields.

Table 7: Feeds on infectious P.vivax cases.

| ID No. | Location | Age | Density* | % inf | No.diss. | Mean oocysts |
|---|---|---|---|---|---|---|
| E/188-14 | Madang | 17 | 6/100 | 44.4 | 9 | 1.8 |
| E/188-26 | " | 54 | 11/100 | 75.0 | 4 | 1.3 |
| E/184-2 | " | 4 | 12/200 | 14.3 | 14 | 1.0 |
| E/188-212 | " | 9 | 12/200 | 33.3 | 6 | 2.0 |
| E/180-82 | " | 7 | 31/200 | 50.0 | 8 | 3.3 |
| LFN 72 | " | 12 | 62/200 | 75.0 | 4 | 4.0 |
| JMS/G54 | Goroka | 20 | 87/200 | 12.5 | 16 | 1.0 |
| JMS/G26 | " | 20 | 118/200 | 60.9 | 23 | 5.0 |
| JMS/G29 | " | 30 | 171/200 | 11.1 | 9 | 1.0 |
| JMS/G3 | " | 50 | 190/200 | 66.7 | 3 | 31.5 |
| JMS/G8 | " | 25 | 213/200 | 46.7 | 15 | 4.1 |
| LFQ 70 | Madang | 1 | H | 8.3 | 24 | 1.0 |
| LFQ 55 | Madang | 2 | VH | 3.8 | 52 | 1.0 |

*Density measured per 200 wbc or per 100 fields
H = Heavy (50-100 parasites per field)
VH = Very Heavy ( more than 100 parasites per field)

Table 8: Proportion of infectious feeds by age group.

| | Agegroup | | | |
|---|---|---|---|---|
| | 0-4 | 5-9 | 10-19 | over 20 |
| No. of feeds | 19 | 45 | 60 | 74 |
| No. infectious feeds: | | | | |
| *P.falciparum*: | 0 | 0 | 1 | 0 |
| *P.vivax*: | 3 | 3 | 2 | 0 |
| *P.malariae*: | 0 | 2 | 0 | 0 |
| Total: | 3 | 5 | 3 | 0 |
| Percent | 15.8 | 11.1 | 5.0 | 0.0 |

Table 9: Demographic profile of the study villages.

| Village | Agegroup 0-4 | 5-9 | 10-19 | over 20 | Total |
|---|---|---|---|---|---|
| Butelgut | 16 | 14 | 33 | 48 | 111 |
| Mebat | 36 | 54 | 80 | 123 | 293 |
| Maraga | 38 | 24 | 34 | 87 | 183 |
| Total | 90 | 92 | 147 | 258 | 587 |
| Percent | 15.3 | 15.7 | 25.0 | 44.0 | |

Table 10: Membrane feeds of washed and resuspended blood from patients attending Goroka Hospital.

| Species from: | Case No. | Percent of mosquitoes infected (no. dissected) | | |
|---|---|---|---|---|
| | | Whole Blood | Resuspended in Aust. serum | Resuspended in own plasma |
| P.VIVAX | 54 | 12.5 (16) | 3.8 (26) | 0.0 ( 9) |
| | 115 | 0.0 ( 7) | 0.0 (13) | 0.0 (13) |
| | 136 | 0.0 ( 6) | 0.0 (10) | 0.0 ( 2) |
| | 161 | 0.0 ( 9) | 0.0 (12) | 0.0 ( 5) |
| | 242 | 0.0 ( 8) | 0.0 (12) | 0.0 ( 5) |
| P.FALCIPARUM | 36 | 0.0 (12) | 16.7 (12) | 0.0 (15) |
| | 49 | 0.0 (10) | 0.0 (11) | 0.0 ( 5) |
| | 76 | n.d. | 0.0 (13) | 11.1 (18) |
| | 83 | 7.1 (14) | 5.3 (19) | 3.6 (28) |
| | 222 | 100.0 ( 8) | 72.7 (11) | 87.5 ( 8) |
| | 231 | 0.0 (23) | 0.0 (14) | 0.0 (15) |

Figure. 1

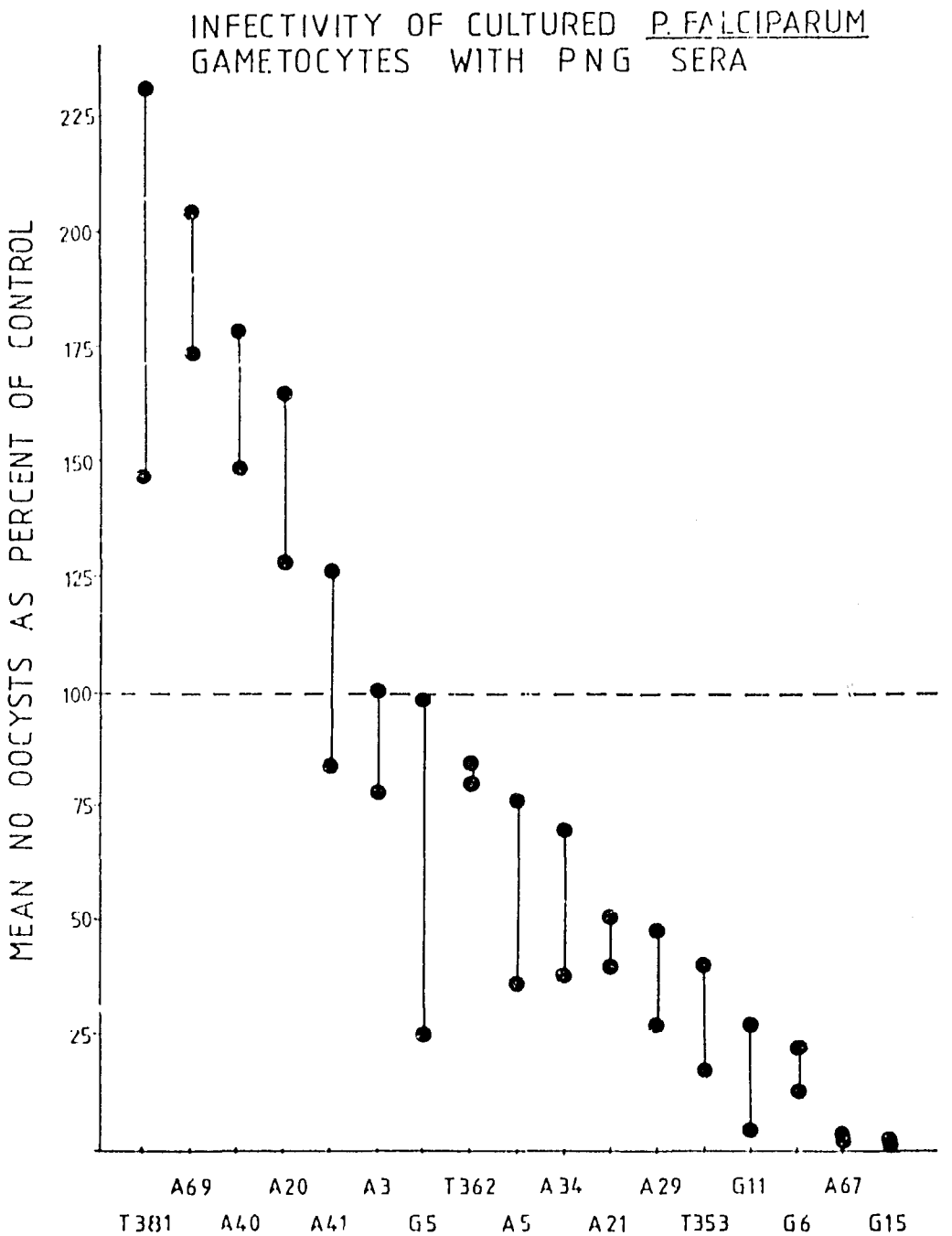
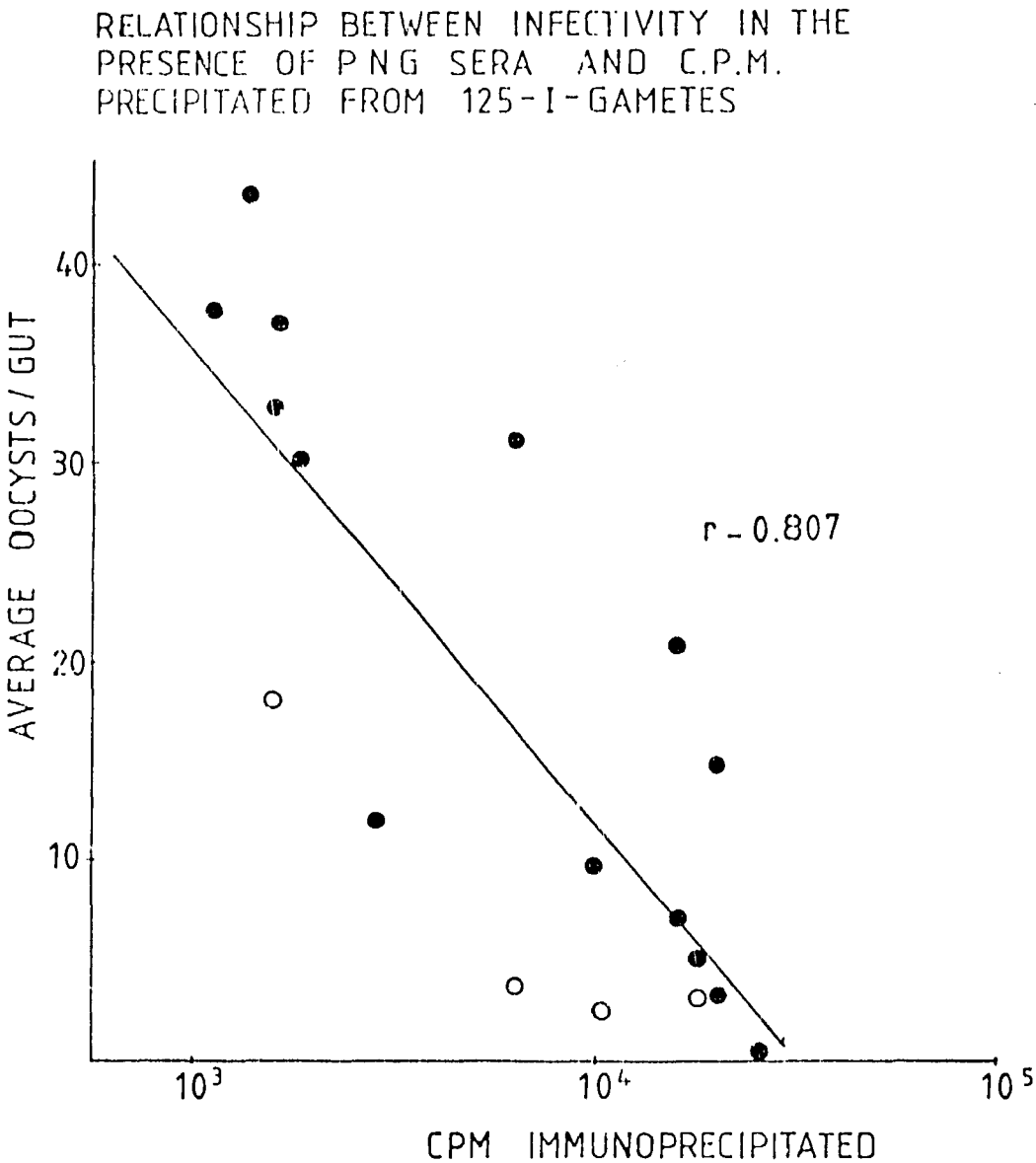

INFECTIVITY OF CULTURED P. FALCIPARUM
GAMETOCYTES WITH PNG SERA
MEAN NO OOCYSTS AS PERCENT OF CONTROL
225
200
175
150
125
100
75
50
25
T381
A69
A40
A20
A41
A3
G5
T362
A5
A34
A21
A29
T353
G11
G6
A67
G15

Figure. 2


RELATIONSHIP BETWEEN INFECTIVITY IN THE PRESENCE OF PNG SERA AND C.P.M. PRECIPITATED FROM 125-I-GAMETES
AVERAGE OOCYSTS / GUT
40
30
20
10
r - 0.807
$10^3$
$10^4$
$10^5$
CPM IMMUNOPRECIPITATED