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Experimental infections of *Plasmodium yoelii nigeriensis* in mice and rats, and hosts' reactions

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Summary

In investigations into the course of infection of *Plasmodium yoelii nigeriensis* in rats and mice, it was found that adult mice, baby mice and baby rats were susceptible to the infection, while adult rats were totally refractive. The infection was rapidly fatal in baby and adult mice but was maintained with a low parasitaemia and tolerated by baby rats until the animals were 7 weeks old, after which they eradicated the infection totally.

Although the adult rats did not exhibit any parasitaemia, there was a decrease in their haemoglobin and packed cell volume values. In adult mice, the haemoglobin and packed cell volume values were found to decrease with the increase in parasitaemia.

Résumé

Des investigations sur l'infestiosité du *Plasmodium yoelii nigeriensis* ont montré que les souris adultes et jeunes et les rats jeunes sont susceptibles alors que les rats adultes ne sont pas susceptibles. Neánmoins, tandisque l'infection est rapidement fatale chez les souris adultes et jeunes la imune infectious est supportée avec une parasitémie minimale chez les rats jeunes jusqu'à l'âge de sept semaines suivant lequel l'infection est complètement éliminée.

Malgré que les rats adultes n'avaient pas démontré une parasitémie il y avait une baisse de la concentration de l'hémoglobine et de l'hématie. Dans les souris adultes le taux de l'hémoglobine et de l'hématie était trouvé ebaisse en proportion universe que la parasitémie.

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Introduction

In routine passage of stock *Plasmodium yoelii nigeriensis* into mice, it was observed that from day 8 post-infection the animals started to die as a result of the infection, depending on the dose of challenge parasite.

The course of infection for many species of malaria parasites has been studied. *Plasmodium chabaudi* was found to have a prepatent period of 48 h in the blood of Swiss TO mice, with any deaths occurring between days 8 and 17 [1]. In infections of *Aotus trivirgatus* with *Plasmodium falciparum*, the onset of parasitaemia was 5 days, with some deaths occurring within 15 days of onset of patency [2].

The previous study of Adler *et al.* [3] showed that rats are as equally susceptible as mice to *Plasmodium berghei* which produces a fatal infection in both animals. Palmer [4] and other researchers found that, with age, baby rats progressively lost the ability to become infected with *P. berghei*, fatalities being more prominent in the 4-week-old group than in the 6-week-old group. Others such as Cox [5] have found that although *P. berghei* was not fatal in adult rats, a positive level of parasitaemia was recorded.

At the time of the routine passaging, described above, for *P. yoelii nigeriensis* into mice, the animal population of our Animal House was grossly inadequate and it was thought that keeping the parasites in rats might achieve a longer interval between the deaths of the animals, and thereby be a saving on the number of experimental animals used. It was found, however, upon challenging the adult rats with viable parasites that they failed to pick up the infection.

Since neither the rats nor the mice involved in the routine passaging had previously been

exposed to malaria and both were being fed the same diet, the possibility of an acquired immunity or a deficiency in diet could be ruled out.

It was therefore decided to study the course of infection of *P. yoelii nigeriensis* in mice and rats, the objectives of the study being:

(a) to study the course of *P. yoelii nigeriensis* infection in rats and mice;
(b) to study the effect of the parasite on rats and mice.

Materials and methods

Plasmodium yoelii nigeriensis, 12-week-old (adult) albino rats and mice, and 4-week-old (baby) rats and mice were used for these experiments.

Twelve males and 12 females of each type were obtained and the normal blood values (haemoglobin (Hb) and packed cell volume (PCV)) were measured.

The animals were infected intraperitoneally with 960 parasitized red blood cells using the method described by Oyerinde [6]. The central group consisted of four uninfected males and four uninfected females for each type of animal.

At 24-h intervals after inoculation of parasites, all animals were bled, and stained blood films were examined daily for estimation of parasitaemia. Blood was also collected and daily values of Hb and PCV were obtained.

Because of the difficulty in obtaining enough blood from the baby animals, only parasitaemia values were obtained in these cases.

Results

The course of P. yoelii nigeriensis *infection in rats and mice*

Adult mice. Microscopic examination of the blood of adult mice revealed the parasites in the erythrocytes 2 days after infection, with an average parasitaemia of 1.6%. In other words, the parasite had a prepatent period of 48 h (Table 1, Fig. 1).

The average parasitaemia of both the male and female mice increased progressively until the seventh day when the peak parasitaemia of 25.9% was reached (Table 1), and on day 8 the first mortality was recorded. It is interesting to note that the peak parasitaemia remained approximately constant for 2 days (days 8–10). During this period, 75% of the infected mice died. Subsequently, although there was a gradual decrease in the average parasitaemia, a further 17% of the mice died, bringing the total death rate to 91.7%.

It is important to note that 8.3% of the mice recovered spontaneously from the infection even though a parasitaemia of between 17 and 22% has previously been recorded for the mice.

Baby mice. Forty-eight hours after infection all infected baby mice were positive, with an average parasite count of 3.5%. It remained at approximately this level for the next 24 h. Within the next 3 days (patent days 3–5), the parasitaemia rose sharply to the peak of 15.2%. Surprisingly, no death was recorded among the animals on this day, but on the following day, 66.7% of the animals died and the average parasitaemia of the surviving animals dropped to 10% (Fig. 2). The parasitaemia continued in a downward trend to day 10 after infection when all the animals died (Table 1, Fig. 2). None of the animals exhibited the spontaneous recovery as reported for the adult mice.

It is worthy of mention that all the female baby mice and four male baby mice died on day 8 after injection. The remaining eight male baby mice survived until day 10 of the experiment, when they all died.

Adult rats. Adult rats were found to be completely refractive to the parasite throughout the 2-week experiment. In other words, the parasitaemia was nil. None of the adult rats died during the period of the experiment.

Baby rats. In the baby rats, unlike the adults, the parasite established itself with a prepatency of 48 h. The parasitaemia reached a maximum of 5.3% and remained at this level for 3 weeks until finally lost by the animal (Table 1 and Fig. 3).

Because the course of the infection was different in adult and baby rats, 5–9-week-old rats were infected with the same number of parasites in order to determine the age of transition from susceptibility to resistance to the infection.

The results of this experiment showed that rats were susceptible to *P. yoelii nigeriensis*

Table 1. The average parasitaemia* in mice and rats infected with *Plasmodium yoelii nigeriensis*

Days after infection	Adult mice			Baby mice			Adult rats			Baby rats		
	Males	Females	Males and females	Males	Females	Males and females	Males	Females	Males and females	Males	Females	Males and females
1	0	0	0	0	0	0	0	0	0	0	0	0
2	1.30	1.80	1.60	2.50	4.50	3.50	0	0	0	0.03	0.04	0.04
3	6.50	6.60	6.60	3.80	3.40	3.60	0	0	0	0.67	0.52	0.60
4	7.20	7.90	7.10	5.10	6.90	6.0	0	0	0	1.60	1.60	1.60
5	18.20	16.30	17.30	7.60	10.50	9.10	0	0	0	3.20	3.0	3.10
6	22.10	21.30	21.70	14.60	15.90	15.20	0	0	0	4.60	4.60	4.60
7	25.50	26.20	25.90	10.0	†	10.0	0	0	0	5.30	5.30	5.30
8	24.10	20.10	22.10	10.0		10.0	0	0	0	5.40	5.30	5.40
9	22.60	21.80	22.20	6.0		6.0	0	0	0	5.40	5.20	5.30
10	18.50	22.0	20.30				0	0	0			
11	16.0	18.10	17.10				0	0	0			
12	13.50	16.30	14.90				0	0	0			

*All values are percentages.
†All the female baby mice were dead.

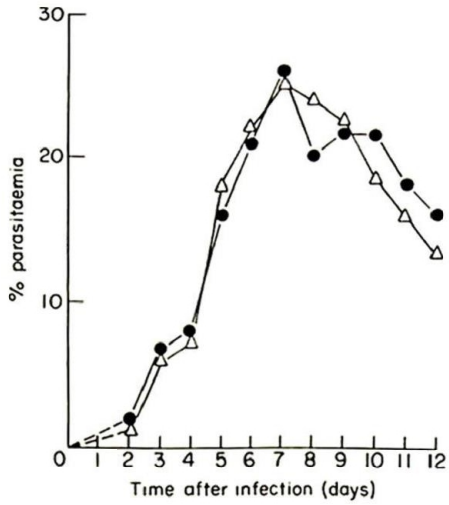

Fig. 1. The average parasitaemia in female (●) and male (△) adult mice infected with *Plasmodium yoelii nigeriensis*.

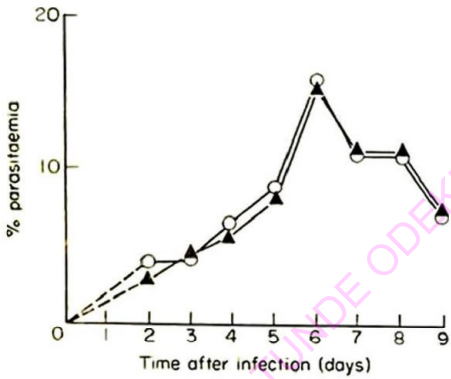

Fig. 2. The average parasitaemia of female (○) and male (▲) baby mice infected with *Plasmodium yoelii nigeriensis*.

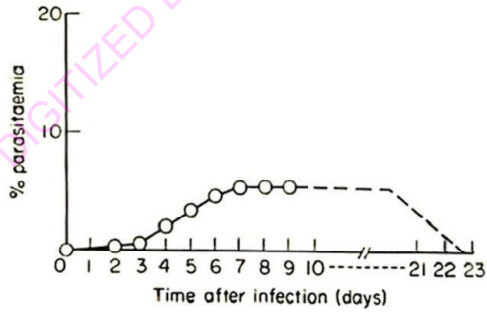

Fig. 3. The average parasitaemia of baby rats infected with *Plasmodium yoelii nigeriensis*.

infection up to the age of 6 weeks. From the age of 7 weeks, they were totally refractive to the infection.

However, when 'runts of the litter', that is, those babies that were at a disadvantage during feeding and hence were stunted in growth, became infected they remained susceptible until they were 8 weeks old and became resistant thereafter.

A comparison of the course of infection in adult mice and baby rats shows some interesting results:

(a) the prepatency of the parasite in the 4-, 5- and 6-week-old baby rats was the same as in the adult mice, i.e. 2 days;
(b) in the baby rats, the initial parasitaemia decreased as the age of the animal increased until adulthood when the parasitaemia became nil;
(c) the duration of the infection before the rats became refractive to the parasite decreased as the age of the rats increased; and
(d) no mortality was recorded among any age group of the rats studied.

Effect of Plasmodium yoelii nigeriensis *on rats and mice*

The effect or pathogenesis of the parasite was based on the decrease of the Hb and PCV values as well as the rate of mortality recorded among the infected animals.

Because of the experimental problem of obtaining enough blood from baby mice and baby rats for the Hb and PCV determinations, only adult animals were used for this study.

Adult mice. The Hb and PCV values of adult mice were found to decrease as the parasitaemia increased. In male mice, the Hb value fell from an average of 13.76 g/100 ml to 3.8 g/100 ml, a decrease of 72.3%, 8 days after infection; while in the female, there was a decrease of 59.7% during the same period (Table 2). It was observed that the male mice could withstand a decrease of 8.56 g/100 ml, i.e. 62.2% of the Hb value, and still survive. Below this level, the mortality rate progessively increased with a further drop in Hb value (Fig. 4). A similar result was recorded for the PCV value in the male mice (Fig. 5, Table 3).

Table 2. The average haemoglobin reading* of adult mice infected with *Plasmodium yoelii nigeriensis*

Sex of animals	Days after infection								
	0	1	2	3	4	5	6	7	8
Males	13.76	12.7	11.2	11.1	8.7	7.2	6.0	5.2	3.8
Females	13.66	12.7	11.6	10.5	8.1	6.9	5.8	5.4	5.1
Males and females	13.71	12.7	11.4	10.8	8.4	7.0	5.9	5.3	4.5

*All values are g/100 ml of blood.

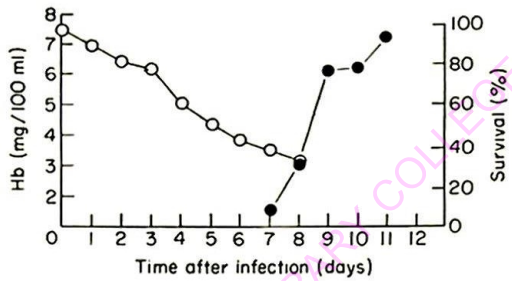

Fig. 4. The average haemoglobin reading (○) and survival rate (●) of adult male and female mice after infection with *Plasmodium yoelii nigeriensis*.

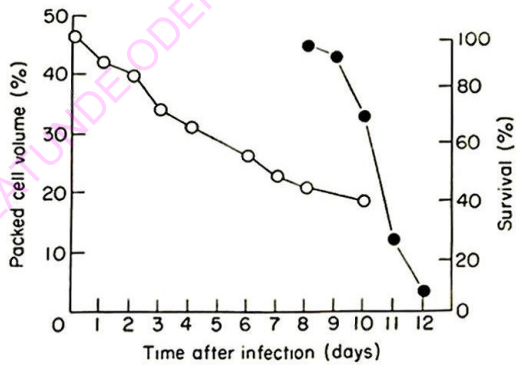

Fig. 5. The average packed cell volume readings (○) and survival rate (●) in adult male and female mice infected with *Plasmodium yoelii nigeriensis*.

Adult rats. The Hb and PCV values of the adult rats were found to decrease slightly, after infection from day 3–6 and then rose again from day 7 onwards returning to normal by day 9 (Figs 6 and 7, Tables 4 and 5).

In male rats, the Hb value dropped from 13.3 g/100 ml to 10.1 g/100 ml, a 24.1% drop, while in female rats, the drop was from 13.1 g/100 ml to 9.9 g/100 ml, a 24.5% decrease (Table 5, Fig. 7).

The PCV in male rats dropped from 48.1% to 40.6%, a decrease of 7.5%, while in female

Table 3. Average packed cell volume readings* of mice infected with *Plasmodium yoelii nigeriensis*

Sex of animals	Days after infection								
	0	1	2	3	4	5	6	7	8
Males	44.2	40.2	38.2	32.0	29.0	26.0	22.0	21.0	17.0
Females	48.0	43.5	42.0	35.0	32.0	26.7	23.0	21.0	20.0
Males and females	46.1	41.9	40.1	33.5	30.5	26.4	22.5	21.0	18.5

*All values are percentages.

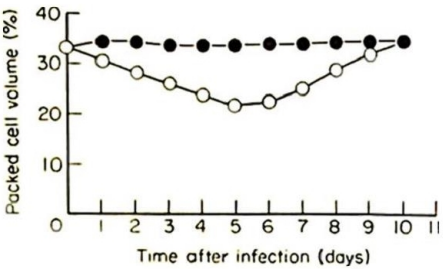

Fig. 6. The average packed cell volume readings in adult rats uninfected (●) and infected (○) with *Plasmodium yoelii nigeriensis*.

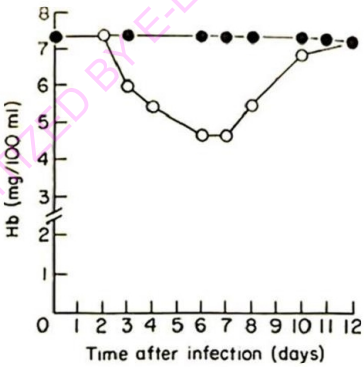

Fig. 7. The average haemoglobin reading in adult rats uninfected (●) and infected (○) with *Plasmodium yoelii nigeriensis*.

rats, the drop was from 40.6% to 33%, a decrease of 7.6% (Table 4, Fig. 6).

Control animals. The blood readings for the control animals remained constant and mortality was zero throughout the period of the experiments.

Discussion

It is obvious from the results that albino mice and baby rats are susceptible to infections with *P. yoelii nigeriensis*, while adult rats are not. The infection is, however, only fatal in mice.

The results showed that once the infection became patent, the parasitaemia increased sharply to reach a peak, following which the infection remained constant. Thereafter, the parasitaemia started to decrease, a decrease which culminated in the recovery of 8% of infected mice and 100% of infected baby rats.

Weiss [7] found that in Swiss mice there was a mortality peak at 6–7 days after patency at a low parasitaemia of 6–8%. The remaining animals started to die approximately 2 weeks later at 50–60% parasitaemia. In the present study, most of the experimental animals died within the peak period, with parasitaemia ranging between 20% and 27%, exceptional cases dying at 18% and 33%.

The host's reaction against the parasite gave rise to the following pattern of infection: an initial absence of host response in mice, leading

Table 4. Average packed cell volume readings* in adult rats infected with *Plasmodium yoelii nigeriensis*

Sex of animals	Days after infection								
	0	1	2	3	4	5	6	7	8
Males	48.1	47.5	45.0	42.4	40.1	40.6	41.6	47.8	48.9
Females	40.6	39.8	36.4	32.8	32.0	32.1	33.0	40.0	40.1
Males and females	44.4	43.7	40.7	37.6	36.1	36.4	37.3	43.9	44.5

*All values are percentages.

Table 5. Haemoglobin readings* of adult rats infected with *Plasmodium yoelii nigeriensis*

Sex of animals	Days after infection								
	0	1	2	3	4	5	6	7	8
Males	13.0	13.3	11.9	11.2	10.2	10.1	11.0	12.7	13.3
Females	13.1	13.1	11.3	10.8	10.0	9.9	11.2	12.4	13.0
Males and females	13.2	13.2	11.6	11.0	10.1	10.0	11.1	12.6	13.2

*All values are g/100 ml of blood.

to 100% susceptibility to the infection. The absence of the host's reaction would account for the rapid increase in the numbers of the parasites during which phase of parasite development, the host's reaction developed. At the peak parasitaemia, an equilibrium was established between the parasite's multiplication rate and the rate of host's reaction against the parasite. This would explain the constant parasitaemia between the fifth and eighth patent day.

The continued decrease in the number of parasites appears to coincide with the increased activity of the host's reaction, which was able to completely eliminate the parasites in 8% of the infected mice.

A similar course of infection for *P. yoelii nigeriensis* was observed in baby and adult mice, except that the peak of infection was lower and reacted sooner in baby mice than in the adults.

While the average peak was 22.6% on day 8 in adults, it was 15.9% on day 7 in baby mice. It is also of interest that adult mice were able to accommodate the infection for a much longer period (13 days) than the babies (10 days), when 8% of the adults threw off the infection. None of the baby mice recovered spontaneously. Since both groups of mice were not previously exposed to the parasite, the only plausible explanation for the different courses of infection is that of age. In other words, adult mice are capable of accommodating a higher level of parasitaemia for a longer period of time and sustain a lower mortality than baby or younger mice.

Although the mortality rate among the female baby mice is higher than that recorded for the male baby mice, it is difficult, just on the basis of this result, to attribute the phenomenon solely to sex, more so when the literature is silent on a possible correlation. A categorical

statement on such a relationship requires further experimentation on the subject.

Brown and Hills [8] reported that inbred August rats eradicated *P. berghei* infections within 12–15 weeks, and after this quickly eradicated challenge doses of the infection. Although adult rats in the present study failed to pick up the infection even though they had never previously been exposed to *P. yoelii nigeriensis*, a drop in the Hb and PCV value was recorded between days 1 and 7. This indicated a physiological response of the host to the presence of the parasites.

That the parasites eventually failed to appear in the peripheral blood of the adult rat might have been due to more than one factor. It could have been achieved by the phagocytic action of the white blood cells in the host, a conclusion that has been suggested by workers such as Taliafero [9], or even by the mounting of an immune response to the parasites by the host, as advocated by Weinbaum *et al.* [10].

Baby rats were found to be susceptible to *P. yoelii nigeriensis* infections which were sustained at a virtually stable level of parasitaemia until 7–8 weeks, depending on the stage of development, and then lost. In those generally referred to as the 'runts of the litter', the infection is lost at about 8 weeks of age, probably because they reach adult weight and maturity later.

It would appear that the immune mechanism present in the adult rats develops with age, since the babies were able to resist the infection on reaching adulthood.

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