The total number of males collected was nearly equal to that of females (128 : 174) and the peak time of collection was almost identical to that of females. This observation indicates that male mosquitos are attracted to a human bait as much as females are, possibly by visual factors such as the contrast of dark and light (Fay, 1968; Sippell & Brown, 1953) or by chemical factors.

ACKNOWLEDGEMENTS

We wish to express our thanks to the staff of the WHO *Aedes* Research Unit, Bangkok, for their constant assistance during the long-term field study. We are also indebted to Dr Sujarti Jatanasen who gave us valuable suggestions in the performance of this study.

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Protective Immunity between Malaria Parasites and Piroplasms in Mice*

by F. E. G. Cox[1]

Immunity to either malaria or piroplasmosis is usually regarded as being both species- and strain-specific (see Targett, 1968). However, in recent years there have been a number of reports which suggest that this is not wholly true. In mice, for example, it has been shown that immunity to *Plasmodium chabaudi* confers a strong resistance to the virulent parasite *P. vinckei* (Cox & Voller, 1966; Nussenzweig, Yoeli & Most, 1966; Yoeli et al., 1966; and others) and that immunity to the piroplasm *Babesia microti* also holds against *B. rodhaini* (Cox & Young, 1969). These results in themselves could be explained on the basis of the morphological similarities which exist between *P. chabaudi* and *P. vinckei* on the one hand and *B. microti* and *B. rodhaini* on the other, but two subsequent and independent studies have produced results which indicate that this explanation is too simple. Cox & Milar (1968) showed that mice which had recovered from infections with *P. chabaudi* were immune to challenge with *B. rodhaini* and Cox (1968) reported, in a preliminary note, that mice which had recovered from infections with *B. microti* were resistant to challenge with *P. vinckei* or *P. chabaudi* and that the immunity was reciprocal. This paper presents the results of a study on the patterns of cross-immunity between six intra-erythrocytic protozoa in mice—namely, *P. vinckei*, *P. chabaudi*, *P. berghei berghei*, *P. berghei yoelii*, *B. rodhaini* and *B. microti*. The antigenic relationships of these same parasites are considered in another paper (Cox & Turner, 1970).

* This investigation received financial support from the World Health Organization and the Wellcome Trust.

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Materials and Methods

The following strains of parasites were used: *P. vinckei* (Katanga 52), *P. chabaudi* (54X), *P. berghei*

TABLE 1
CROSS-IMMUNITY BETWEEN SIX INTRA-ERYTHROCTYIC PROTOZOA IN MICE[a]

Immunizing infection	Challenge infection					
	P. vinckei	*P. chabaudi*	*P. b. berghei*	*P. b. yoelii*	*B. rodhaini*	*B. microti*
P. vinckei	12/12	14/20	1/15	10/10	12/12	10/11
P. chabaudi	25/30	16/16	0/24	4/12	13/22	19/19
P. b. berghei	17/20	4/9	8/10	7/12	10/15	11/16
P. b. yoelii	7/12	4/12	4/12	10/10	9/12	7/12
B. rodhaini	16/17	6/15	0/11	0/11	12/12	12/12
B. microti	18/21	18/24	2/30	0/15	20/20	10/10

[a] As measured by no. of animals protected/no. challenged. For criteria used to determine protection, see text.

berghei (173K), *P. berghei yoelii* (RCA 17X) *Babesia rodhaini* (Antwerp) and *B. microti* (King's 67). All these parasites were maintained by serial passage of infected blood in Alsever's solution every 7 days. The inocula used never exceeded 1×10^6 parasitized red blood cells. The mice in which the parasites were maintained, and which were used for the experiments described in this paper, were female TO Swiss. All the strains of parasites and mice were free from *Eperythrozoon coccoides* and *Haemobartonella muris*.

The experimental procedure was to infect mice with one or other of the six parasites, using $1 \times 10^6 - 1 \times 10^5$ parasites, and to check the progress of the infection by taking regular blood films. Mice recovered naturally from *P. chabaudi*, *P. berghei yoelii* and *B. microti* infections but had to be treated with drugs to prevent their dying from the other infections. *P. vinckei* infections were cured with a single dose of chloroquine phosphate at dosages of 10 mg/100 g of body weight on the fifth day after infection. *P. berghei berghei* infections were treated in the same way except that several doses of the drug had to be given at intervals of 3–4 days and some mice received 6 treatments. Infections with *B. rodhaini* were cured with a single injection of amicarbalide isethionate (Diampron, May & Baker Ltd) at dosages of 2 mg/100 g of body weight on the fifth day after infection. All the mice which had recovered from infections were kept until no parasites were apparent in the peripheral blood. They were challenged with approximately 1×10^6 parasitized red blood cells containing the homologous or heterologous strain some time later, up to a maximum of 26 weeks. A preliminary series of experiments showed that the inoculation of uninfected blood or treatment with the drugs chloroquine or amicarbalide isethionate had no effect on the challenge infections, and thereafter the controls used were normal, uninfected mice. Blood films were taken daily and the parasitaemias in immunized and control animals were compared. The six parasites provided 36 combinations of immunizing and challenge infections and, because of the great number of animals which would have been involved, not all were examined in the same detail. The actual numbers of animals used are shown in Table 1. The criteria of immunity used were reduced parasitaemias during the first 7 days of the infection and survival for 30 days in the case of *P. vinckei*, *P. berghei berghei* and *B. rodhaini*. In practice, no animal which survived this 30-day period died later from the infection.

Results

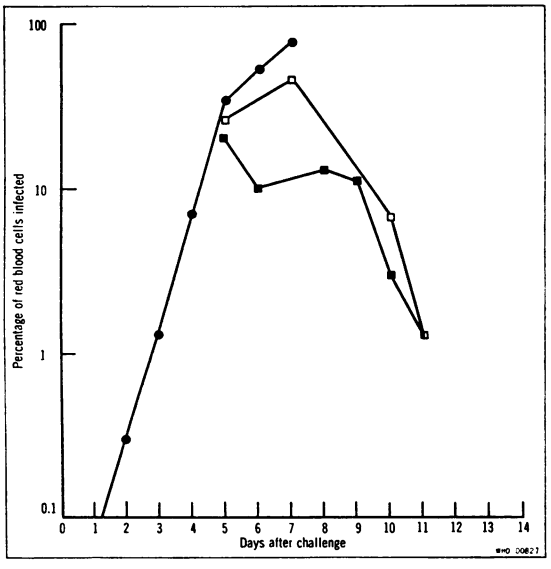
The results are summarized in Table 1. These results showed that there was a considerable degree of heterologous immunity between the parasites used and the actual patterns of parasitaemia after challenge are described below.

Challenge with P. vinckei

The results obtained after challenge with *P. vinckei*, and the pattern of infection, are shown in Fig. 1. In control mice, parasites appeared in the blood on the day after infection, 2% of the red blood cells being infected after 3.25 days. The parasitaemias increased in a near-logarithmic manner until the

FIG. 1

PATTERNS OF PARASITAEMIA IN MICE INFECTED WITH *P. VINCKEI*, IN MICE WHICH HAD RECOVERED FROM INFECTIONS OF *P. B. BERGHEI* AND WHICH BECAME INFECTED ON CHALLENGE WITH *P. VINCKEI*, AND IN MICE WHICH HAD RECOVERED FROM INFECTIONS OF *P. B. YOELII* AND WHICH BECAME INFECTED ON CHALLENGE WITH *P. VINCKEI* [a]



P. vinckei, controls (12)
P. b. berghei challenged with *P. vinckei* (17)
P. b. yoelii challenged with *P. vinckei* (12)

[a] For sake of clarity, some of the points have been omitted.

mice died on the seventh day after infection, with parasitaemias of between 70% and 80%.

P. vinckei *challenged with* P. vinckei. A batch of 12 mice was initially infected with *P. vinckei* and challenged with the homologous parasite 7–10 weeks later. No infections occurred.

P. chabaudi *challenged with* P. vinckei. A batch of 30 mice was initially infected with *P. chabaudi* and challenged with *P. vinckei* 7–10 weeks later; infections developed in 12 of them. In 5 mice the parasitaemias were at a low level and transient, 2 mice developed high parasitaemias but recovered while 5 died 7–10 days after challenge. In the animals which died, the parasitaemias were similar to those in control mice.

P. b. berghei *challenged with* P. vinckei. A batch of 20 mice was initially infected with *P. berghei berghei* and challenged with *P. vinckei* 4–9 weeks later; infections developed in 17 mice. The parasitaemias rose in exactly the same way as in control animals for the first 5 days after infection, but thereafter declined in 14 mice and rose in the other 3 which died 7, 8 and 9 days after challenge.

P. b. yoelii *challenged with* P. vinckei. A batch of 12 mice was initially infected with *P. berghei yoelii* and challenged with *P. vinckei* 5–13 weeks later. Infections developed in all the mice. In 7 mice the parasitaemias rose in the same way as in control animals for the first 5 days of the infection and then more slowly until day 7; thereafter the parasitaemias declined. In the remaining 5 mice the parasitaemias did not decline and the mice died on days 6, 7 (2 mice), 13 and 14 after challenge.

B. rodhaini *challenged with* P. vinckei. A batch of 17 mice was initially infected with *B. rodhaini* and challenged with *P. vinckei* 4–6 weeks later. Infections developed in 8 mice; in 6 the parasitaemias were low and transient and in 1 mouse the parasitaemia rose to a high peak 7 days after infection, and then fell. The remaining mouse died 7 days after challenge.

B. microti *challenged with* P. vinckei. A batch of 21 mice was initially infected with *B. microti* and challenged with *P. vinckei* 6–14 weeks later; infections developed in 12 of them. In 7 mice the parasitaemias were at a low level and transient, in 2 the infections rose as in the controls until the fifth day after infection and then declined; 3 mice died on the seventh, tenth and twelfth days after challenge with parasitaemias resembling those in the control animals.

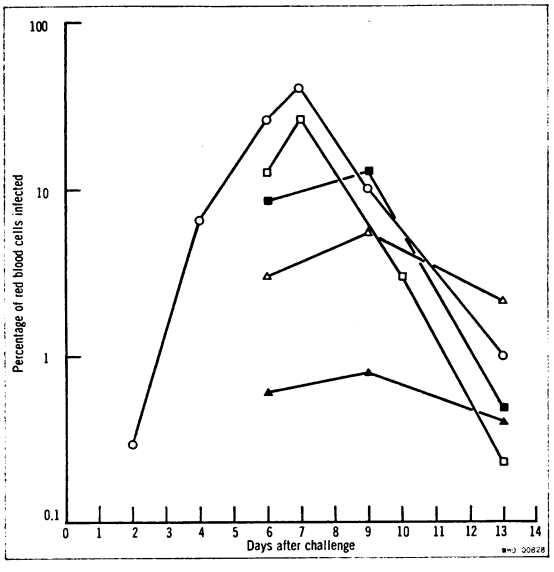
Challenge with P. chabaudi

The results obtained after challenge with *P. chabaudi* and the patterns of infection are shown in Fig. 2. In control mice, parasites appeared in the blood on the day after infection, 2% of the red blood cells being infected after 3.25 days. The parasitaemias increased in a near-logarithmic manner until the fourth day after infection and then continued to rise less rapidly to reach peaks in which about 40% of the red blood cells were infected on the seventh day after infection. Thereafter, the parasitaemias gradually declined and parasites disappeared from the blood by the twentieth day after infection.

P. chabaudi *challenged with* P. chabaudi. A batch of 16 mice was initially infected with *P. chabaudi* and challenged with the homologous parasite 6–13

FIG. 2

PATTERNS OF PARASITAEMIA IN MICE INFECTED WITH *P. CHABAUDI*, IN MICE WHICH HAD RECOVERED FROM INFECTIONS OF *P. B. BERGHEI* AND WHICH BECAME INFECTED ON CHALLENGE WITH *P. CHABAUDI*, IN MICE WHICH HAD RECOVERED FROM INFECTIONS OF *P. B. YOELII* AND WHICH BECAME INFECTED ON CHALLENGE WITH *P. CHABAUDI*, IN MICE WHICH HAD RECOVERED FROM INFECTIONS OF *B. RODHAINI* AND WHICH BECAME INFECTED ON CHALLENGE WITH *P. CHABAUDI* AND IN MICE WHICH HAD RECOVERED FROM INFECTIONS OF *B. MICROTI* AND WHICH BECAME INFECTED ON CHALLENGE WITH *P. CHABAUDI* [a]



P. chabaudi, controls (12)
P. b. berghei challenged with *P. chabaudi* (8)
P. b. yoelii challenged with *P. chabaudi* (12)
B. rodhaini challenged with *P. chabaudi* (14)
B. microti challenged with *P. chabaudi* (22)

[a] For sake of clarity, some of the points have been omitted.

weeks later. Infections developed in 2 mice; in both, the parasitaemias were low and transient.

P. vinckei *challenged with* P. chabaudi. A batch of 20 mice was initially infected with *P. vinckei* and challenged with *P. chabaudi* 4–10 weeks later and infections developed in 16. In 9 mice the parasitaemias were low and transient, in 6 the parasitaemias were similar to those in the control animals and in 1 mouse the peak of parasitaemia was delayed until the twelfth day after challenge.

P. b. berghei *challenged with* P. chabaudi. A batch of 9 mice was initially infected with *P. b. berghei* and challenged with *P. chabaudi* 5–17 weeks later. Infections developed in 8 of them. In 3 mice, the parasitaemias were very low, in 1 mouse the parasitaemia was higher, but lower than in the controls, and in 4 the parasitaemias approached those in the control animals.

P. yoelii *challenged with* P. chabaudi. A batch of 12 mice was initially infected with *P. b. yoelii* and challenged with *P. chabaudi* 5–10 weeks later. Infections developed in all the mice. In 4 mice the parasitaemias were low but in the remainder approached those in the control animals; 2 mice died on days 7 and 9 after challenge.

B. rodhaini *challenged with* P. chabaudi. A batch of 15 mice was initially infected with *B. rodhaini* and challenged with *P. chabaudi* 4–10 weeks later. Infections developed in 14 mice; in 5 animals the parasitaemias were considerably lower than in the control animals; in 8 of the remaining mice the parasitaemias were similar to those in control animals and in 1 mouse the peak parasitaemia was similar, but delayed until the tenth day after challenge.

B. microti *challenged with* P. chabaudi. A batch of 24 mice was initially infected with *B. microti* and challenged with *P. chabaudi* 6–12 weeks later, infections developed in 22. In 18 mice, the parasitaemias were considerably lower than in control animals and the peaks of parasitaemia were delayed until the ninth day after challenge (see Fig. 2). In the 6 remaining mice the parasitaemias did not differ significantly from those in the control animals.

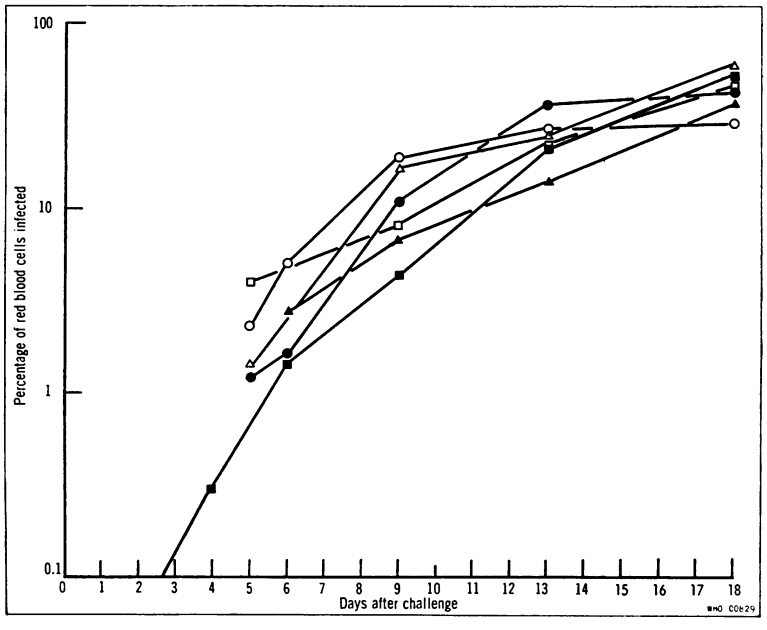
Challenge with P. b. berghei

The results obtained after challenge with *P. b. berghei* and the patterns of infection are shown in Fig. 3. In control mice, parasites appeared in the blood on the second day after infection, 2% of the red blood cells being infected after 6 days. The parasitaemias increased until about 50% of the red blood cells were infected 18 days after infection and thereafter oscillated about this level until the mice died between the twentieth and thirtieth days after infection.

P. b. berghei *challenged with* P. b. berghei. A batch of 10 mice was initially infected with *P. b. berghei* and challenged with the homologous parasite 3–6 weeks later; infections developed in 5. In 3 mice the parasitaemias were low and transient but in the 2 remaining mice they approached the levels seen in the control animals.

FIG. 3

PATTERNS OF PARASITAEMIA IN MICE INFECTED WITH *P. B. BERGHEI*, IN MICE WHICH HAD RECOVERED FROM INFECTIONS OF *P. VINCKEI* AND WHICH BECAME INFECTED ON CHALLENGE WITH *P. B. BERGHEI*, IN MICE WHICH HAD RECOVERED FROM INFECTIONS OF *P. CHABAUDI* AND WHICH BECAME INFECTED ON CHALLENGE WITH *P. B. BERGHEI*, IN MICE WHICH HAD RECOVERED FROM INFECTIONS OF *P. B. YOELII* AND WHICH BECAME INFECTED ON CHALLENGE WITH *P. B. BERGHEI*, IN MICE WHICH HAD RECOVERED FROM INFECTIONS OF *B. RODHAINI* AND WHICH BECAME INFECTED ON CHALLENGE WITH *P. B. BERGHEI* AND IN MICE WHICH HAD RECOVERED FROM INFECTIONS OF *B. MICROTI* AND WHICH BECAME INFECTED ON CHALLENGE WITH *P. B. BERGHEI* [a]



P. b. berghei, controls (12)
P. vinckei challenged with *P. b. berghei* (15)
P. chabaudi challenged with *P. b. berghei* (24)
P. b. yoelii challenged with *P. b. berghei* (12)
B. rodhaini challenged with *P. b. berghei* (11)
B. microti challenged with *P. b. berghei* (30)

[a] For sake of clarity, some of the points have been omitted.

P. vinckei *challenged with* P. b. berghei. A batch of 15 mice was initially infected with *P. vinckei* and challenged with *P. b. berghei* 6–9 weeks later; infections developed in all mice. In 14, the parasitaemias were higher than in control animals and all these mice died (see Fig. 3).

P. chabaudi *challenged with* P. b. berghei. A batch of 24 mice was initially infected with *P. chabaudi* and challenged with *P. b. berghei* 4–13 weeks later; infections developed in all mice. In 6, the levels of parasitaemia were slightly lower than in control animals but in the remaining 18 the levels were higher (see Fig. 3).

P. b. yoelii *challenged with* P. b. berghei. A batch of 12 mice was initially infected with *P. b. yoelii* and challenged with *P. b. berghei* 5–9 weeks later. Infections developed in all mice; in 4, the parasitaemias were low and transient and in 8 they did not differ from those in control animals (see Fig. 3).

B. rodhaini *challenged with* P. b. berghei. A batch of 11 mice was initially infected with *B. rodhaini* and challenged with *P. b. berghei* 3–10 weeks later; infections developed in all mice. The levels of parasitaemia were similar to those in control animals and all the mice challenged died between the twelfth and twenty-sixth days after infection (see Fig. 3).

B. microti *challenged with* P. b. berghei. A batch of 30 mice was initially infected with *B. microti* and challenged with *P. b. berghei* 4–14 weeks later; infections developed in all cases. In 28 mice, the

levels of parasitaemia were as high as, or higher than, in control animals and the mice died between the twelfth and thirty-first days after challenge (see Fig. 3). In 2 mice the levels of parasitaemia were lower than in control animals, not rising above 3%; these mice did not die.

Challenge with P. b. yoelii

The results obtained after challenge with *P. b. yoelii* and the patterns of infection are shown in Fig. 4. In control mice, parasites appeared in the blood on the second day after infection, 2% of the red blood cells being infected after 6 days. The parasitaemias increased until about 14% of the red blood cells were infected 12 days after infection, and thereafter declined, disappearing altogether by the seventeenth day.

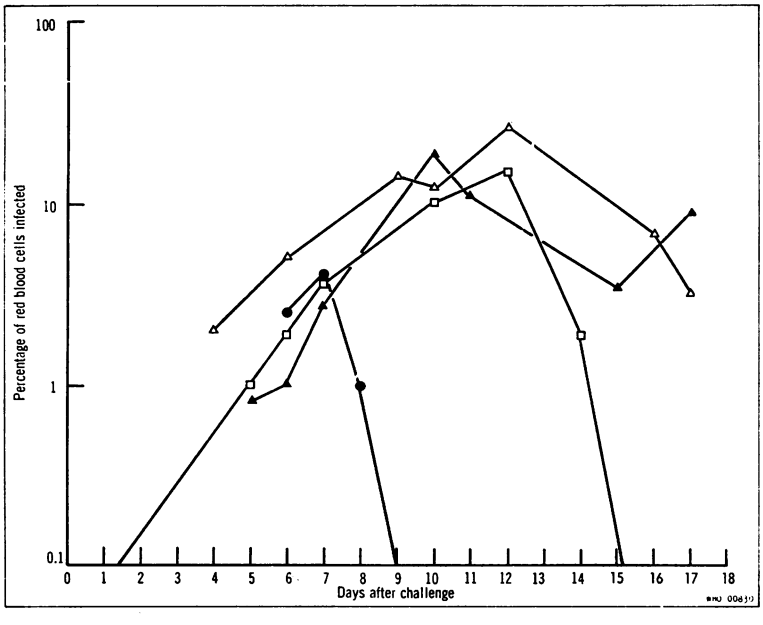
P. b. yoelii *challenged with* P. b. yoelii. A batch of 10 mice was initially infected with *P. b. yoelii* and challenged with the homologous strain 5–10 weeks later. No infections developed.

P. vinckei *challenged with* P. b. yoelii. A batch of 10 mice was initially infected with *P. vinckei* and challenged with *P. b. yoelii* 5 weeks later. Infections developed in all cases (see Fig. 4). The parasitaemias rose in exactly the same way as in control animals for the first 7 days after challenge to reach a peak of 4% but thereafter declined, disappearing altogether by the eleventh day after challenge.

P. chabaudi *challenged with* P. b. yoelii. A batch of 12 mice was initially infected with *P. chabaudi* and challenged 3–26 weeks later with *P. b. yoelii*. Infections developed in all animals. In 4 mice the levels of parasitaemia were low and transient, in 6 they

FIG. 4

PATTERNS OF PARASITAEMIA IN MICE INFECTED WITH *P. B. YOELII*, IN MICE WHICH HAD RECOVERED FROM INFECTIONS WITH *P. VINCKEI* AND WHICH BECAME INFECTED ON CHALLENGE WITH *P. B. YOELII*, IN MICE WHICH HAD RECOVERED FROM INFECTIONS OF *B. RODHAINI* AND WHICH BECAME INFECTED ON CHALLENGE WITH *P. B. YOELII* AND IN MICE WHICH HAD RECOVERED FROM INFECTIONS OF *B. MICROTI* AND WHICH BECAME INFECTED ON CHALLENGE WITH *P. B. YOELII* [a]

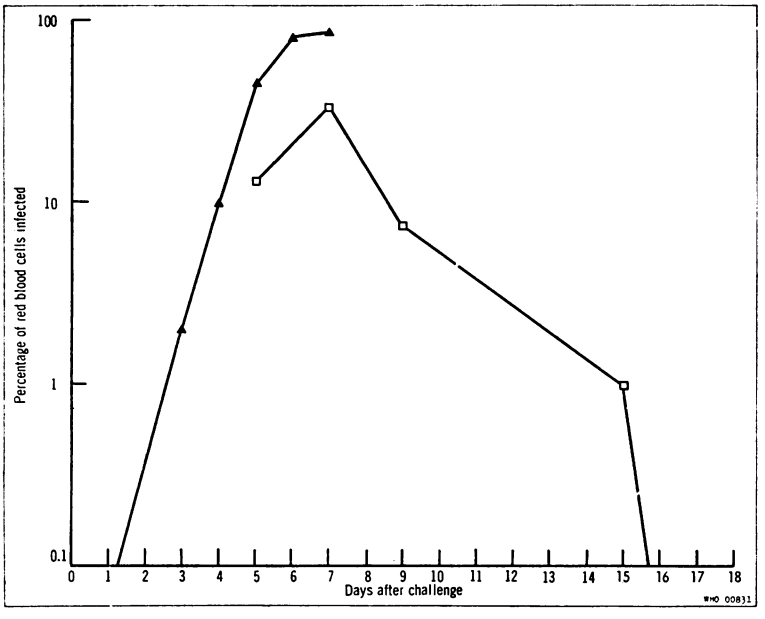


P. b. yoelii, controls (12)
P. vinckei challenged with *P. b. yoelii* (10)
B. rodhaini challenged with *P. b. yoelii* (11)
B. microti challenged with *P. b. yoelii* (15)

[a] For sake of clarity, some of the points have been omitted.

FIG. 5

PATTERNS OF PARASITAEMIA IN MICE INFECTED WITH *B. RODHAINI* AND IN MICE WHICH HAD RECOVERED FROM INFECTIONS OF *P. B. YOELII* AND WHICH BECAME INFECTED ON CHALLENGE WITH *B. RODHAINI* [a]



▲—▲ *B. rodhaini*, controls
□—□ *P. b. yoelii* challenged with *B. rodhaini*

[a] For sake of clarity, some of the points have been omitted.

resembled those in the control animals and in 2 they resembled those in control animals except that the peaks of parasitaemia occurred about 5 days later.

P. b. berghei *challenged with* P. b. yoelii. A batch of 12 mice was initially infected with *P. b. berghei* and challenged 3–13 weeks later with *P. b. yoelii*. Infections developed in 6 mice, in 1 the parasitaemia never rose above 2% while in the remaining 5 the parasitaemias resembled those in the control animals.

B. rodhaini *challenged with* P. b. yoelii. A batch of 11 mice was initially infected with *B. rodhaini* and challenged 4–11 weeks later with *P. b. yoelii*. All became infected (see Fig. 4). The parasitaemias rose in the same way as in control animals for the first 10 days after challenge, then fell slowly to rise again until the eighteenth day after challenge, and thereafter declined.

B. microti *challenged with* P. b. yoelii. A batch of 15 mice was initially infected with *B. microti* and challenged with *P. b. yoelii* 8–21 weeks later; all became infected (see Fig. 4). The parasitaemias rose in the same way as in control animals except that they were somewhat higher, reaching peaks on the twelfth day after challenge. Thereafter, the parasitaemias declined and disappeared by the twenty-second day after challenge.

Challenge with B. rodhaini

The results obtained after challenge with *B. rodhaini* and the patterns of infection are shown in Fig. 5. In control mice, parasites appeared in the blood on the day after infection, 2% of the red blood cells being infected after 3 days. The parasitaemias increased in a near-logarithmic manner until the mice died on the 7th–8th day after infection with parasitaemias between 70% and 80%.

B. rodhaini *challenged with* B. rodhaini. A batch of 12 mice was initially infected with *B. rodhaini* and challenged with the homologous parasite 6 weeks

later. Infections developed in 6 mice but the levels of parasitaemia never rose above 0.1%.

P. vinckei *challenged with* B. rodhaini. A batch of 12 mice was initially infected with *P. vinckei* and challenged with *B. rodhaini* 3–6 weeks later; infections developed in 8. The parasitaemias were low and transient.

P. chabaudi *challenged with* B. rodhaini. A batch of 22 mice was initially infected with *P. chabaudi* and challenged with *B. rodhaini* 7–23 weeks later; infections developed in 16. In 6 mice the infections were low and transient, in 1 the parasitaemia rose to the same level as in the control group but the animal recovered, and in 9 the parasitaemias were similar to those in the control group and the mice died between the sixth and tenth days after challenge.

P. b. berghei *challenged with* B. rodhaini. A batch of 15 mice was initially infected with *P. b. berghei* and challenged with *B. rodhaini* 4–6 weeks later and infections developed in 11. In 2 mice the parasitaemias were low and transient, in 2 others the parasitaemias were originally low but gradually rose, reaching about 50% 25 days after challenge, and then suddenly declined. In 2 mice the parasitaemias were similar to those in the control group but the mice recovered; the 5 remaining mice all had parasitaemias resembling those in the control group and died on the sixth, seventh, eighth, ninth and twenty-first days after challenge.

P. b. yoelii *challenged with* B. rodhaini. A batch of 12 mice was initially infected with *P. berghei yoelii* and challenged with *B. rodhaini* 5–10 weeks later; all developed infections (see Fig. 5). In 2 mice, the parasitaemias were low and transient and in the remainder the parasitaemias were similar to those in the control group until the fourth day after challenge when they ceased to rise as rapidly and reached peaks in which 30% of the red blood cells were infected on the seventh day. Thereafter, the parasitaemias began to decline and parasites had disappeared from the blood by the seventeenth day. In all, 3 mice died between the sixth and eighth day after challenge.

B. microti *challenged with* B. rodhaini. A batch of 20 mice was initially infected with *B. microti* and challenged with *B. rodhaini* 4–9 weeks later; infections developed in 14. In 12 of these mice the parasitaemias were low and never reached a level of more than 2%; in the 2 remaining mice the parasitaemias rose to about 40% 7 days after infection and then declined.

Challenge with Babesia microti

The results obtained after challenge and the pattern of infection are shown in Fig. 6. In control mice, parasites appeared in the blood on the second day after infection, 2% of the red blood cells being infected after 3.5 days. The parasitaemias increased to peaks in which about 55% of the red blood cells were infected on the eleventh day after infection and then slowly declined until no parasites were seen in the blood 25 days after infection.

B. microti *challenged with* B. microti. A batch of 10 mice was initially infected with *B. microti* and challenged with the homologous parasite 7 weeks later. Infections developed in 7 mice but did not rise above a level of 0.1%.

P. vinckei *challenged with* B. microti. A batch of 11 mice was initially infected with *P. vinckei* and challenged with *B. microti* 4–10 weeks later; infections developed in 3 but in 2 only an occasional parasite was seen.

P. chabaudi *challenged with* B. microti. A batch of 19 mice was initially infected with *P. chabaudi* and challenged with *B. microti* 5–11 weeks later. Infections developed in 8 mice but only an occasional parasite was seen and in 1 mouse there was no patent parasitaemia until the twenty-seventh day after infection.

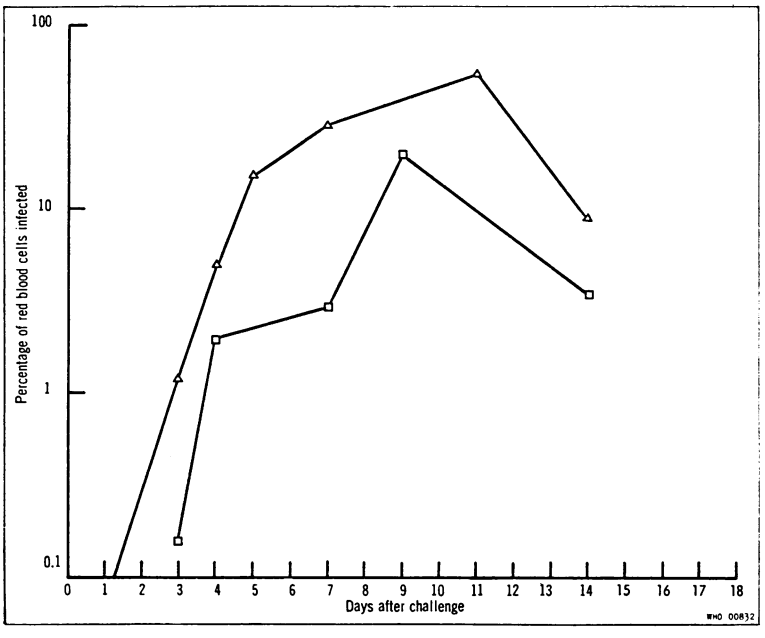
P. b. berghei *challenged with* B. microti. A batch of 16 mice was initially infected with *P. b. berghei* and challenged with *B. microti* 6–7 weeks later; infections developed in 11. In 6 mice the parasitaemias were low and transient, in 1 mouse the parasitaemia rose to a peak 17 days after infection and then declined, and in 2 the infections resembled those in the control group; 2 mice died from the infection 21 and 25 days after infection.

P. b. yoelii *challenged with* B. microti. A batch of 12 mice was initially infected with *P. b. yoelii* and challenged 5–7 weeks later with *B. microti*; all became infected (see Fig. 6). In 4 mice, the parasitaemias were low and transient, in 3 they rose to low peaks of about 10% on day 10 and in 5 the parasitaemias resembled those in the control group; 2 mice died on the eleventh day after challenge.

B. rodhaini *challenged with* B. microti. A batch of 12 mice was initially infected with *B. rodhaini* and challenged with *B. microti* 7 weeks later. Infections developed in 9 mice but in no case did the parasitaemia rise above a level of 1%.

FIG. 6

PATTERNS OF PARASITAEMIA IN MICE INFECTED WITH *B. MICROTI* AND IN MICE WHICH HAD RECOVERED FROM INFECTIONS WITH *P. B. YOELII* AND WHICH BECAME INFECTED ON CHALLENGE WITH *B. MICROTI* [a]



△—△ *B. microti*, controls
□—□ *P. b. yoelii* challenged with *B. microti*

[a] For sake of clarity, some of the points have been omitted.

Attempted immunization with serum from infected mice

In order to eliminate the possibility that some factor present in the serum of infected animals might be immunizing the mice against challenge infections, blood was taken from mice at the height of parasitaemia, filtered through a 0.45-μm filter and injected into uninfected mice. This procedure was repeated for all parasites and in no case did it result in any protection on subsequent challenge with the homologous, or a heterologous, species.

Discussion

The 6 parasites used in this investigation were chosen for the following reasons. Firstly, they had all been maintained in mice for some time and always gave rise to reproducible infections in these animals and their use avoided any complications due to changes which might have occurred on transfer from one host to another. Secondly, they represented three comparable pairs of morphologically similar parasites, each of which gave rise to a different kind of infection; one virulent and one benign. Thus *P. vinckei* and *P. chabaudi* were morphologically similar although *P. vinckei* killed mice in about 7 days while animals infected with *P. chabaudi* nearly always recovered. Similarly, *P. b. berghei* and *P. b. yoelii*, and *B. rodhaini* and *B. microti*, constituted morphological pairs in which the first-named was the virulent form. These six parasites, then, provided an opportunity to study homologous immunity and various degrees of heterologous immunity ranging from what amounted to intraspecific immunity through intrageneric to suprageneric immunity. In order to obtain as much information as possible from a relatively small number of animals, the mice were challenged randomly after recovery in such a way that infections with various parasites could be compared. This accounts for the variation in the periods between infection and challenge. The shortcomings of this approach were appreciated but

limited accommodation for the animals permitted no other possibility. It appeared, however, that the period between the initial infection and challenge was irrelevant in the context of the present study.

This investigation occupied about a year and during this time no signs of *E. coccoides* or *H. muris* were apparent, despite continual surveillance and routine splenectomies. Similarly, the inoculation of uninfected blood, or the serum from infected animals, gave no protection to challenge whatever and it is concluded that the results obtained were not due to contamination with some other infective organism. This conclusion is confirmed by the duration of the immunity observed which exceeds the short-lived resistance induced by such organisms as *Eperythrozoon* (see Voller & Bidwell, 1968).

The results indicate that immunity to the homologous parasite is strong and thus confirm previous observations (Cox, 1966; Cox & Voller, 1966; and Cox & Young, 1969). A considerable degree of heterologous immunity also exists and in most cases this extends to the heterologous member of the pair and to other species and genera. A number of observers have recorded immunity between *P. chabaudi* and *P. vinckei* (Cox & Voller, 1966; Nussenzweig et al., 1966; Yoeli et al., 1966; and others). Immunity has also been recorded between *P. vinckei* and *P. chabaudi* (Cox & Voller, 1966) and between *B. microti* and *B. rodhaini* and *vice versa* (Cox & Young, 1969). Several workers, including Cox & Voller (1966), Nussenzweig et al. (1966), Yoeli et al. (1966) and Cox & Milar (1968), have reported the absence of immunity between *P. chabaudi* and *P. b. berghei*, while Demina et al. (1969) have reported the absence of immunity between *P. b. yoelii* and *P. b. berghei*. Apart from the preliminary note by Cox (1968), only Cox & Milar (1968) have reported suprageneric immunity; they showed that mice which had recovered from infections with *P. chabaudi* were immune to challenge with *B. rodhaini*.

TABLE 2

PERCENTAGE OF MICE PROTECTED AGAINST CHALLENGE WITH HETEROLOGOUS PARASITES

Challenge infection	Percentage of mice protected	
	All heterologous strains	Heterologous strains, excluding morphologically similar forms
P. vinckei	83	83
P. chabaudi	58	53
P. b. berghei	8	4
P. b. yoelii	35	29
B. rodhaini	79	72
B. microti	84	81

TABLE 3

PERCENTAGE OF MICE PROTECTED BY HETEROLOGOUS PARASITES

Challenge infection	Percentage of mice protected	
	All heterologous strains	Heterologous strains, excluding morphologically similar forms
P. vinckei	69	69
P. chabaudi	57	47
P. b. berghei	68	70
P. b. yoelii	52	56
B. rodhaini	52	41
B. microti	53	42

The degree of heterologous immunity is best seen by reference to Tables 1, 2 and 3, from which it is apparent that recovery from all six parasites results in some degree of immunity to homologous strains, heterologous, but similar, strains, and to heterologous species. The results are best analysed by considering the challenge infections. All six parasites protected the majority of mice against *P. vinckei*, which was a particularly easy infection to study since all the control animals died. The most unexpected result was the high degree of protection afforded by the two piroplasms. Another interesting result was seen in mice which had recovered from infections with *P. b. berghei*. As has been pointed out above, previous observations (Cox & Voller, 1966) failed to reveal any immunity between *P. b. berghei* and *P. vinckei* and the different results obtained in the present study are probably due to the use of different lines of the same strain, and to a difference in technique. In order to maintain parity between all the parasites used, the inocula were maintained at 1×10^6 or less, which is smaller than the challenge dose formerly used. Fig. 1 clearly shows that in mice which had recovered from *P. b. berghei* or *P. b. yoelii* infections the parasitaemias rose in exactly

the same way as in control animals until the fifth day after challenge when they began to decline. It seems probable that the process of immunity elicited by the other parasites is different from that elicited by *P. b. berghei* or *P. b. yoelii* since in the others the challenge infection never really became established, suggesting an immune mechanism operating from the time of challenge, whereas after *P. b. berghei* or *P. b. yoelii* infections the immune mechanism only became manifest on the fifth day after challenge. In the earlier experiments (Cox & Voller, 1966), the higher challenge doses, and possibly also the mice being male, never allowed this late immune response to occur.

All six parasites also protected some mice at least against *P. chabaudi* but protection was least in mice previously infected with *P. b. berghei* or *P. b. yoelii*. These particular results are in general agreement with those reported previously. As far as initial infections with piroplasms are concerned, the degree of protection afforded against *P. chabaudi* was less than that against *P. vinckei*. Fig. 2 shows that the patterns of parasitaemia following challenge with *P. chabaudi* are basically similar, although the intensity of the infections varies; this suggests a similarity in the immune mechanism.

There was practically no heterologous immunity against *P. b. berghei*, only *P. b. yoelii* being protective at all. The absence of immunity afforded by *P. vinckei* infection is in agreement with the observations of Cox & Voller (1966) and a similar lack of immunity following infections with *P. chabaudi* was recorded by Cox & Voller (1966), Nussenzweig et al. (1966), Yoeli et al. (1966) and Cox & Milar (1968). Cox & Milar (1968) noted a reduction in the level of parasitaemia in some mice, as in the present study. Demina et al. (1969) also observed a lack of immunity between *P. b. yoelii* and *P. b. berghei*. Fig. 3 shows that far from protecting against *P. b. berghei*, previous infections with *P. vinckei*, *P. chabaudi*, *B. rodhaini* and *B. microti* resulted in higher parasitaemias than in control animals.

In mice previously infected with *P. vinckei* or *P. chabaudi*, heterologous immunity to *P. b. yoelii* was more marked than that to *P. b. berghei* but less so than that to other parasites. In mice which had recovered from infections with *P. vinckei* the challenge infections rose unchecked until the seventh day, when the parasitaemias began to decline, suggesting an immune mechanism that became effective late after challenge. No immunity was elicited by the piroplasms *B. rodhaini* or *B. microti* which actually enhanced the challenge infections, as may be seen by reference to Fig. 4.

There was a considerable degree of heterologous immunity against *B. rodhaini* and the greatest protection was afforded by *P. vinckei*. This immunity was as strong as, if not stronger than, the homologous immunity. The least immunity was afforded by *P. b. berghei*. In mice which had recovered from infections with *P. b. yoelii* the immunity became apparent about 5 days after challenge, as may be seen by reference to Fig. 5.

Heterologous immunity to *B. microti* and to *B. rodhaini* was similar; a very strong immunity resulted from infection with *P. vinckei* and the least immunity followed infections with *P. b. berghei*. After infections with *P. b. yoelii* the patterns of parasitaemia after challenge were similar but the levels were lower than in control animals (see Fig. 6).

In summarizing these results, it is apparent that the greatest degree of heterologous immunity exists against *P. vinckei*, *B. rodhaini* and *B. microti*, less against *P. chabaudi* and *P. b. yoelii* and least against *P. b. berghei*. As a working hypothesis, it is suggested that a certain degree of homologous and heterologous immunity is induced by all these parasites and that the breakdown of this immunity is dependent on the ability of the parasite to evade this immune response. This ability may well be correlated with the degree of antigenic variation exhibited by the parasites. *P. vinckei* and *B. rodhaini* are both virulent and kill their hosts within about 7 days. They have both been maintained in mice for a number of years and are now probably stable variants. *B. microti* has been carefully passaged at regular intervals since its isolation over 2 years ago and is probably also a stable variant. *P. chabaudi* and *P. b. yoelii* have relatively long periods of patency, and thus every opportunity to undergo antigenic variation, but the infections are overcome within a month, suggesting a limited range of antigenic potential. *P. b. berghei* is different; the course of infection, under the conditions of this investigation, tends to be long and the parasitaemia may remain at an elevated level for over 30 days before the mouse eventually dies. This suggests an active process of antigenic variation in which the parasite eventually defeats the immune response of the host. It is suggested that it is this capacity for antigenic variation that enables *P. b. berghei* to survive both homologous and heterologous immunity. The fact that immunity induced by *P. b. berghei* against *P. vinckei*, *B. rodhaini* and *B. microti* is not reciprocal suggests that immunity is

a function of the parasite and not an inadequacy on the part of the host's immune response. Cox & Milar (1968) have suggested that antigens released into the plasma of infected animals provide the basis of the heterologous immunity between *P. chabaudi* and *B. rodhaini* and that the lack of immunity between *P. chabaudi* and *P. b. berghei* is due to the selection of " immunity resistant " strains. Serum-soluble antigens were not studied in this investigation but the antigenic nature of the six parasites used has been studied by means of a fluorescent-antibody technique (Cox & Turner, 1970). The results obtained showed that all the parasites possessed antigens in common and the presence of these common antigens could form a basis for heterologous immunity. However, the actual levels of antibody titres, which correspond with the degree of antigenic similarity, cannot be correlated with the degree of protective immunity. The conclusion must therefore be that common antigens, either bound or serum-soluble, enable the host to " recognize " the invading parasites and to react against them. This would account for both the failure of challenge infections to establish and for the decline of infections after they had taken a course similar to the control infections for about 5 days. Antigenic variation, or the " selection of immunity resistant strains ", if this is different, must account for failures of the immune response. Heterologous immunity cannot be dismissed as a non-specific (and therefore non-immunological) reaction because if this were the case the same degree of " immunity " should have been induced by all parasites to all challenge infections. The absence of immunity in some cases emphasizes the specificity of the immune response.

There are several significant features of these results. Firstly, there is the heterologous immunity itself and its possible implications for the study of homologous immunity. Secondly, there is the possibility that rodents collected in the field may be resistant to an infection because of prior exposure to another, unrelated, one. Finally, there must exist the possibility that humans exposed to the piroplasms of rodents or other animals may thereby acquire an immunity to malaria.

ACKNOWLEDGEMENTS

I wish to thank Professor L. J. Bruce-Chwatt for his interest and encouragement.

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