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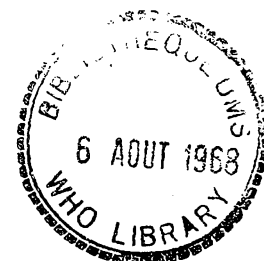
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## IMMUNOLOGICAL STUDIES WITH SIMIAN MALARIAS

II. HETEROLOGOUS IMMUNITY IN THE "PLASMODIUM CYNOMOLGI" GROUP<sup>1</sup>

by

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## 1. INTRODUCTION

The technique of superinfection with homologous or heterologous species or strains of Plasmodia has been utilized for over 40 years in malaria research. In many instances, the experimental procedures and the results therefrom were on a qualitative rather than a quantitative basis. The influence of length and intensity of previous parasite experience, the time between initial infection and superinfection, and the number of parasites contained in the challenge inoculum are parameters that must be considered in evaluating data acquired from superinfection procedures.

The purpose of the present study was to determine whether rhesus monkeys, Macaca mulatta, which had experienced infections with Plasmodium cynomolgi bastianellii of varying duration and intensity and were currently chronically infected would respond differently from control subjects when challenged with P. cynomolgi bastianellii, P. cynomolgi cynomolgi or P. cynomolgi ceylonensis.

## 2. MATERIALS AND METHODS

The methodology discussed by Voller & Rossan (1968) is applicable in the present study. The strain of P. cynomolgi ceylonensis (Dissanaike et al., 1965) was obtained through the courtesy of Professor P. C. C. Garnham. P. cynomolgi cynomolgi designated by Schmidt et al. (1961) as the "M" strain, has been maintained since 1946, first at the Christ Hospital Institute of Medical Research, and later at the National Center for Primate Biology, by serial trophozoite passage at approximately 30-day intervals.

All infections, primary or superinfection, were initiated by the intravenous inoculation of  $5 \times 10^5$  trophozoites derived by the dilution of citrated blood from a donor monkey. Parasitaemias were monitored by blood films for 28 consecutive days following superinfection.

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### 3. RESULTS

A total of 35 monkeys, 27 of which were chronically infected with P. cynomolgi bastianellii, comprised the experimental subjects. The parameters of cumulative parasitaemias and the duration of infection, prior to superinfection, are indicated in Table 1. The monkeys in Groups A to D were essentially comparable with respect to total parasite burdens prior to the present challenge. The monkeys in Groups E and F had been infected for 34 days when the parasitaemias were then eliminated with chloroquine. The animals were rechallenged 25 days after the termination of chloroquine treatment.

The mean group parasitaemias observed after superinfection are depicted in Figs. 1 to 3, and the parameters of peak and cumulative parasitaemias are summarized in Table 2. As there was no marked difference after superinfection between the animals that had received two or three previous infections, these were treated as one group for subsequent analysis. The nine monkeys in Group D constituted chronic infection controls.

Homologous superinfection with P. cynomolgi bastianellii (Group A) did not produce higher parasitaemias than were observed in the unchallenged animals with chronic infections during the same observation period. Immunity was evident when the peak and cumulative parasitaemias of the subjects of Group A were compared to those of the controls. The challenge parasites of P. cynomolgi bastianellii were isolated several passages distant to those used to initiate the original infections.

Monkeys challenged with P. cynomolgi cynomolgi evidenced parasite burdens, after superinfection, considerably less than those in the control animals. Three monkeys had parasitaemias greater than the other challenged animals in the same group and those challenged with the homologous P. cynomolgi bastianellii.

Those subjects superinfected with P. cynomolgi ceylonensis experienced as intense an infection as the controls when measured by the initial peak parasitaemia. The cumulative parasitaemia over the 28-day observation period was less than in the controls, due to a more rapid reduction of parasite levels in the superinfected animals than in the controls (Fig. 3).

The monkeys (Group E), which were challenged with the homologous sub-species after one initial infection cured 25 days earlier evidenced both higher initial parasite peaks and cumulative parasitaemias than did those monkeys in Group A which had homologous challenge superimposed on chronic infections but still had significantly lower infections post-challenge than did the controls.

The monkeys challenged with P. cynomolgi ceylonensis after a single infection with P. cynomolgi bastianellii lasting 34 days cured 25 days earlier showed no evidence of immunity when their parasitaemias were compared with those of the controls (Fig. 4).

### 4. DISCUSSION

Superinfection as a technique has had a long history in malaria studies and a full review of its applications will not be attempted but reference will be made to those studies relating to the present work.

Gingrich (1932) carried out what is still the most extensive study of this type on the avian malaria parasites and he found that a latent infection of any strain of one species normally conferred protection against all other strains of that species and sometimes to another species. In general, the more virulent species protected against challenge with the less virulent species but not vice versa. He also emphasized that the immunity was not due to a generalized activation of non-specific factors. More recently, Dhanapala (1965) found that two strains of P. gallinaceum were cross-immune, as were two strains of P. jextanucleare; however, there was no cross-protection between the species.

FIG. 1 RESULTS OF SUPERINFECTION WITH *P. cynomolgi bastianellii*  
ON MONKEYS WITH CHRONIC *P. cynomolgi bastianellii*

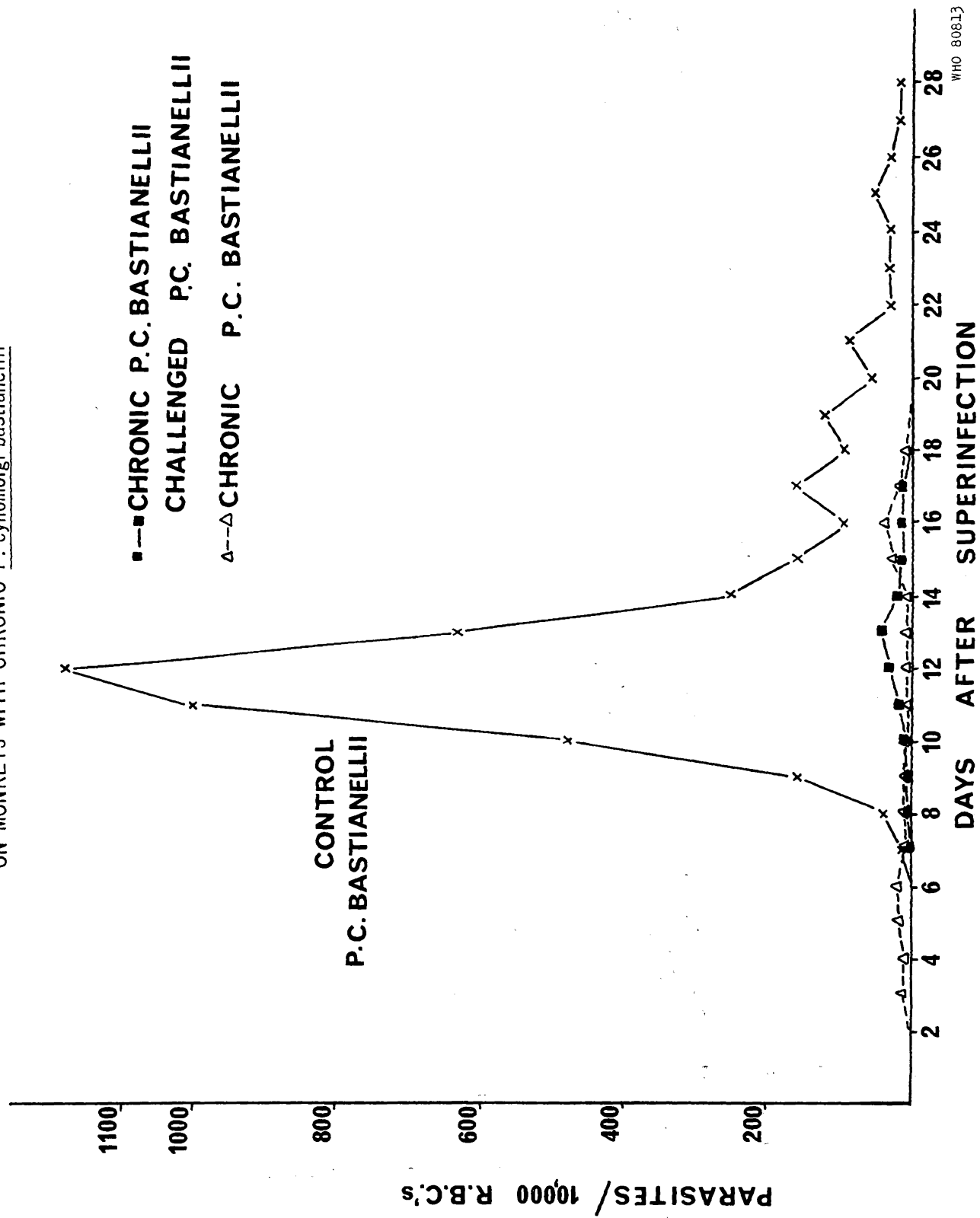


FIG. 2 RESULTS OF SUPERINFECTION WITH *P. cynomolgi cynomolgi*  
ON MONKEYS WITH CHRONIC INFECTIONS OF *P. cynomolgi bastianellii*

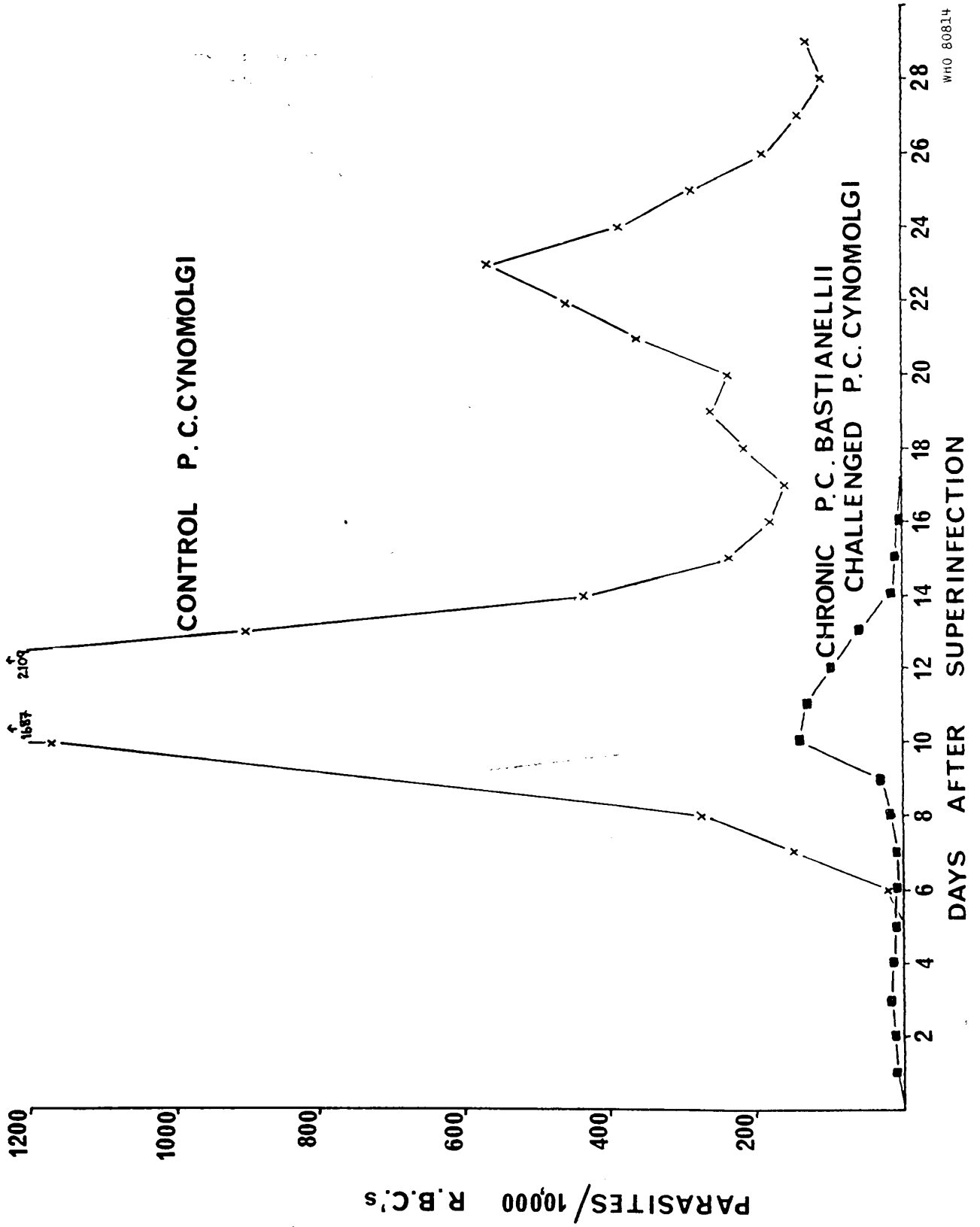


FIG. 3 RESULTS OF SUPERINFECTION WITH *P. cynomolgi* *ceylonensis*  
ON MONKEYS WITH CHRONIC INFECTIONS OF *P. cynomolgi* *bastianellii*

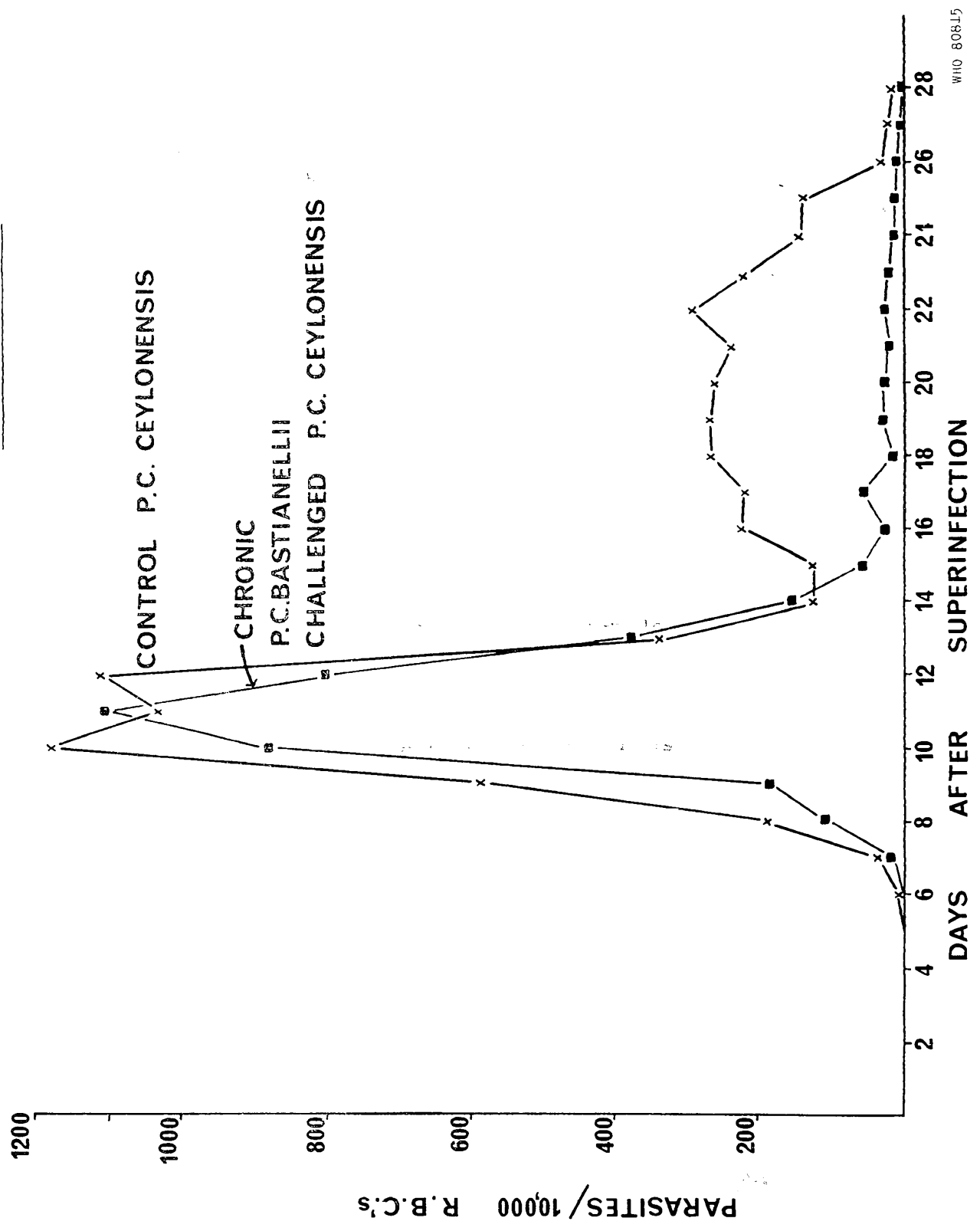
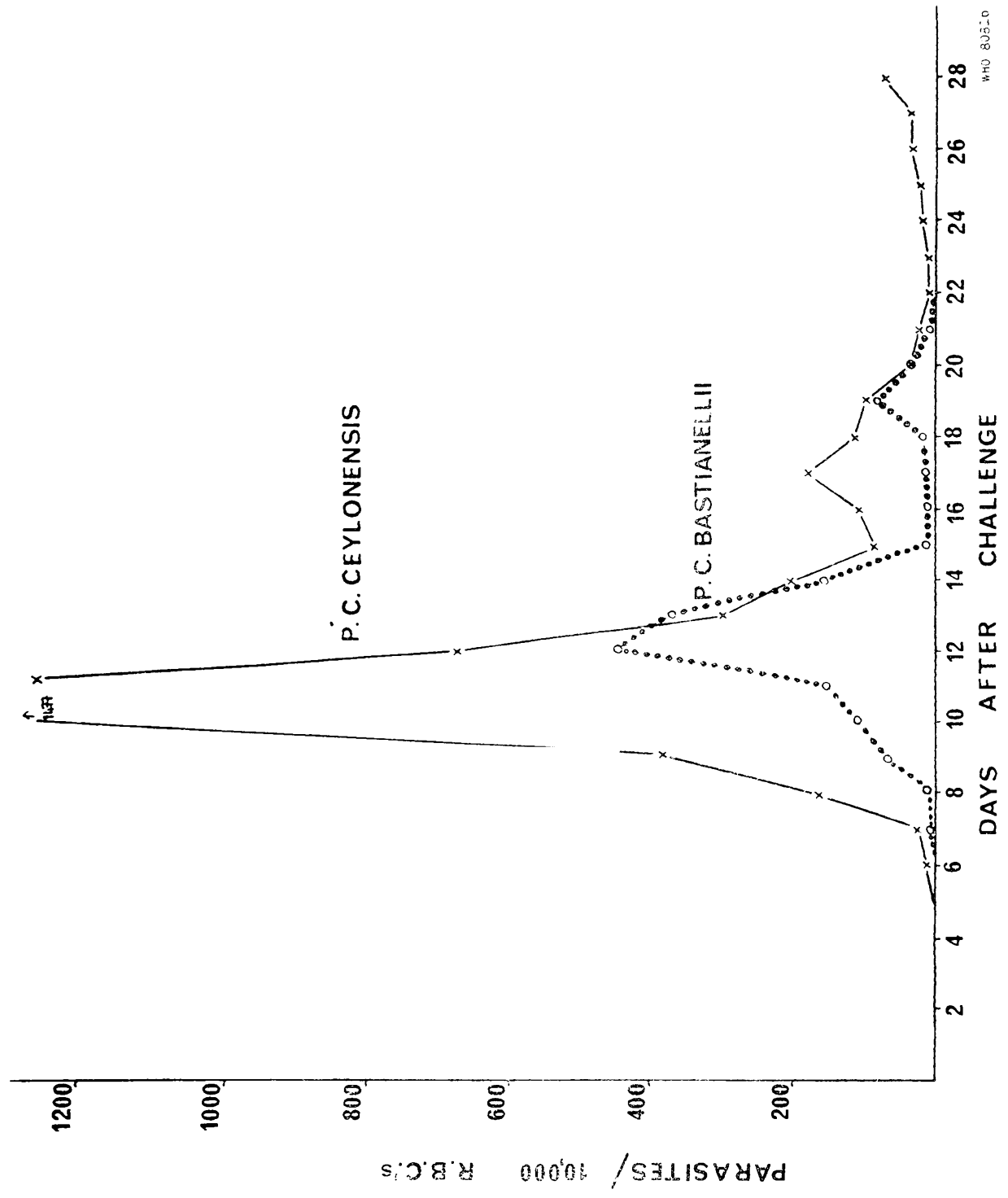


FIG. 4 RESULTS OF CHALLENGE OF MONKEYS WITH *P. cynomolgi bastianellii* ON *P. cynomolgi ceylonensis*  
 25 DAYS AFTER CURE OF ONE INFECTION OF *P. cynomolgi bastianellii*



Because of the suitability of rodents as laboratory animals, their malaria parasites have been frequently utilized in immunological studies. Soon after the discovery of P. berghei and P. vinckei Rodhain (1954) showed there was no cross-immunity between them, although a strong homologous immunity can be obtained (Corradetti, 1955). More recently, P. chabaudi has been shown to protect against P. vinckei but not against P. berghei (Nussenzweig et al., 1966; Cox & Voller, 1966). Whether this represents a species cross-immunity or whether P. vinckei and P. chabaudi are merely strains of one species is a matter of contention at the present time. There is preliminary evidence (Voller, unpublished) that strains of P. berghei excite immunity which is most effective against the homologous strain and less so against heterologous strains.

The human malarias have naturally attracted more attention although the experimental manipulations are of necessity more limited. From an early time it was generally recognized that adults, native to malaria endemic areas, were little affected by malaria which caused severe illness and was frequently fatal in recently-arrived adults, as well as in children native to the area.

The advent of malaria therapy centres and the not infrequent necessity for more than one malaria exposure in certain patients opened the way for an experimental approach to the question of immunity in human malaria. Yorke & Macfie (1924) observed that there was immunity to homologous challenge in the case of P. vivax and this finding was in due course extended by other workers to P. falciparum, P. ovale and P. malariae.

It was soon recognized that there could be a different result depending on whether the challenge was made with the homologous strain or with one from a separate isolation or different geographical region (Boyd & Stratman-Thomas, 1935; Ciuca, et al., 1934; Sinton, et al., 1939). Jeffery (1966) summarizing his extensive work in this field concluded that in the case of P. vivax the introduction of a heterologous strain even after several challenges with another strain, resulted in an infection comparable to that of the first homologous challenge. Homologous challenges with P. falciparum gave lower peak parasitaemias than challenge with the heterologous strains. Both P. malariae and P. ovale produced much lower parasitaemias than controls in heterologous and homologous challenges.

In the field of simian malaria the phenomenon of acquired resistance was first recognized by Blanchard & Langeron (1912) but the detailed studies of Mulligan & Sinton (1933) provided the base lines for future work. Using superinfection as an indicator for immunity, they concluded that for P. knowlesi the immunity was strain specific in so far as the parasitaemia consequent upon challenge was only reduced in intensity when the homologous strain was used for the challenge. When heterologous strains were used for challenge little modification of the initial attack occurred although some tolerance to the clinical effects of the superinfection were evident. There "strains" referred to isolations from different monkeys but no definite data were available to indicate whether the monkeys came from the same area.

The effective immunity against superinfection with the homologous strain of P. cynomolgi which we have observed is comparable to that reported by Shortt, et al. (1938) for the same species.

That the maintenance of functional immunity to malaria depends on the continuing infection at a patent or sub-patent level is shown by the contrast between the results of homologous challenge in cured animals and those with a chronic infection. Even a homologous variant challenge given seven weeks after cure (see Voller & Rossan, 1968) results in greater parasitaemia than the homologous species (but probably heterologous variant) superinfection imposed on animals with a chronic infection.

Boyd & Stratman-Thomas (1933) isolated several strains of P. vivax from within 25 miles (40 km) of each other and they found that chronic infection of any one conferred immunity only against the homologous challenge. In view of this it is not surprising that a chronic infection of P. cynomolgi bastianellii has little effect on a challenge with P. cynomolgi ceylonensis (Voller, et al., 1966) since these two parasites were originally isolated from different hosts in Malaya and Ceylon respectively.

The high degree of protection conferred upon monkeys by chronic P. cynomolgi bastianellii to challenge with P. cynomolgi cynomolgi was rather unexpected and probably indicates a very close relationship between these parasites. The differentiation of these sub-species is based upon differences in the morphology of the exo-erythrocytic schizont, the length of the sporogonic cycle and the difference in susceptibility of some species of mosquitos to the parasites (Garnham, 1966). However, recent comparative studies on susceptibility and course of infection in the insect hosts (Bennett, et al., 1966) emphasized the similarity of P. cynomolgi cynomolgi and P. cynomolgi bastianellii. Both probably originated from a similar geographical area, the east coast of Malaya, and from the same host Macaca irus. It is possible that the apparent lack of cross-immunity between P. cynomolgi cynomolgi and P. cynomolgi bastianellii observed by Garnham (1959) was due to the lengthy period which had elapsed between the homologous and heterologous challenges.

The studies described in this and the previous paper indicate that the immunity associated with a continuing infection is much more effective than that following cure, and has a wider spectrum than the variant specific immunity present even a short time after cure. Support is given for the theory that immunologically distant geographical strains or sub-species can occur within a single species of malaria parasite.

#### SUMMARY

Superinfection tests showed that animals with current chronic P. cynomolgi bastianellii infections were immune to challenge with P. cynomolgi bastianellii, and were also protected to a high degree against challenge with P. cynomolgi cynomolgi. In contrast P. cynomolgi ceylonensis challenge resulted in severe infections in the animals with chronic P. cynomolgi bastianellii. The immunity was shown to decrease rapidly following cure.

#### RESUME

La présente étude visait à établir si des singes rhésus, Macaca mulatta, préalablement infectés plus ou moins longtemps et plus ou moins intensément par Plasmodium cynomolgi bastianellii, et chroniquement infectés au moment de l'expérience, réagissaient ou non comme les témoins à une épreuve par P. cynomolgi bastianellii, P. cynomolgi cynomolgi ou P. cynomolgi ceylonensis.

Les infections primaires et les surinfections avaient toutes été provoquées par l'injection intraveineuse de  $5 \times 10^5$  trophozoïtes provenant d'une dilution du sang citraté d'un singe donneur. La parasitémie était suivie sur des étalements de sang pratiqués quotidiennement pendant 28 jours après la surinfection.

Les épreuves de surinfection ont révélé qu'au cours des infections chroniques à P. cynomolgi bastianellii, les animaux se montrent immuns à l'épreuve par P. cynomolgi bastianellii ainsi que très protégés contre l'épreuve par P. cynomolgi cynomolgi. Au contraire, l'épreuve par P. cynomolgi ceylonensis provoquait des infections sévères. L'immunité déclinait rapidement après guérison.

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TABLE 1. PARASITOLOGICAL EXPERIENCE OF MACACA MULATTA WITH PLASMODIUM CYNOMOLGI BASTIANELLII PRIOR TO SUPERINFECTION

Group **	Number of monkeys	Duration of initial challenge (days)	Parasitaemias *							
			Cumulative		Duration of second challenge (days)	Cumulative		Duration of current infection (days)	Cumulative	
			Mean	Range		Mean	Range		Mean	Range
A	4 2	34 28,30	4775 4061	3021-5893 3380,4741	27-30 -	1116 -	37-3264 -	62 62	738 327	372-1237 223,431
B	4 2	34 28	5139 2923	3298-7323 2808,3047	27-30 -	955 -	246-1522 -	62 62	425 183	255-795 106,260
C	4 2	34 28,30	4425 3040	1732-9552 3016,3064	27-30 -	866 -	220-1489 -	62 62	799 1471	659-1118 1340,1601
D	6 3	34 28,30	5415 5601	4183-8022 3071-7744	27-30 -	1421 -	603-1930 -	62 62	862 642	354-1651 235-1294
E	3	34	3869	3659-4223	-	-	-	-	-	-
F	3	34	7256	5753-9596	-	-	-	-	-	-

\* Expressed as numbers of parasites per  $10^4$  erythrocytes.

\*\* See Table 2 for species of plasmodium used for superinfection.

TABLE 2. PARASITAEMIAS AFTER SUPERINFECTION OF MACACA MULATTA BEARING CHRONIC INFECTIONS OF PLASMODIUM CYNOMOLGI BASTIANELLII

Group	Superinfection with sub-species of <u>Plasmodium cynomolgi</u>	Number of monkeys	Parasitaemias *			
			Peak		Cumulative	
			Mean	Range	Mean	Range
A	<u>bastianellii</u>	6	59	14-155	173	28-403
B	<u>cynomolgi</u>	6	169	5-450	532	15-1484
C	<u>ceylonensis</u>	6	1318	744-2070	4037	1872-6148
D	-	9	53	12-143	150	22-383
E **	<u>bastianellii</u>	3	485	380-640	1545	1133-2181
F **	<u>ceylonensis</u>	3	1511	1377-1765	5322	3377-7146
Control	<u>bastianellii</u>	3	1131	915-1445	4756	4096-6061
Control	<u>cynomolgi</u>	3	2213	1568-2865	10 656	7337-12 413
Control	<u>ceylonensis</u>	3	1353	1020-1836	7074	4663-8831

\* Expressed as numbers of parasites per  $10^4$  erythrocytes.

\*\* Groups E and F were rechallenged 25 days after termination of their previous P. cynomolgi bastianellii infection so did not have a chronic infection at time of challenge.

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